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Radical Scavenging and Antioxidant Activities of Isocoumarins and a Phthalide from the Endophytic Fungus *Colletotrichum* sp.

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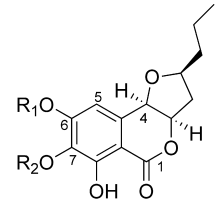
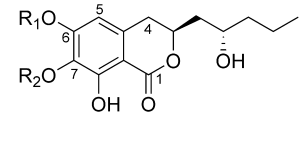
Five known isocoumarins, monocerin (**1**), derivative **2**, and fusarentin derivatives **3**−**5**, and a new phthalide (**6**) were isolated from the endophytic fungus *Colletotrichum* sp. **2** selectively exhibited cytotoxic activity toward the HepG2 cell line. Compounds **2** and **4** scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (IC_{50} values of 23.4 and 16.4 μM, respectively) and inhibited superoxide anion radical formation (IC_{50} values of 52.6 and 4.3 μM, respectively). The C-7 hydroxyl group in **2** and **4** might be important for radical scavenging activities. Isocoumarins **1**−**3** and phthalide **6** showed potent antioxidant activity.

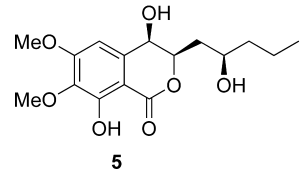
Endophytic fungi are a rich source of bioactive compounds.[1,2] Particular endophytic fungi can produce pharmaceutically important substances, for example, paclitaxel (Taxol),[3,4] podophyllotoxin,[5] camptothecin,[6] and hypericin.[7] As part of our ongoing research on bioactive compounds from fungal endophytes, we report herein the isolation and identification of five known isocoumarin derivatives (**1**−**5**) and a new phthalide (**6**) from the endophytic fungus *Colletotrichum* sp. CRI535-02, together with the results of their cytotoxic, radical scavenging, and antioxidant activities. Natural phthalides are found in plants and fungi and have a broad spectrum of biological activities.[8] The phthalide skeleton is also a common motif in drugs and drug candidates, and several hundred phthalide derivatives are in preclinical development worldwide.[9] Due to their pharmaceutical importance, phthalides have been intensively studied.[10] Natural isocoumarins have been reported to exhibit broad biological activities including antimicrobial,[11,12] antimosquito,[12] antioxidant,[13] antibacterial, and cytotoxic activities.[14] Natural phthalides, largely isolated from plants and fungi, have been reported to exhibit a variety of biological activities including inhibition of Akt1 biochemical activity,[15] cytotoxicity,[16] inhibition of superoxide generation and elastase release,[17] serotonergic,[18] antiproliferative,[19] and progesterone-like[20] activities, and inhibition of lipopolysaccharide-induced TNF-alpha production and TNF-alpha-mediated NF-kappaB activation.[21]

Extraction of the culture filtrate of *Colletotrichum* sp. CRI535-02 and fractionation of the resulting extract using gel permeation chromatography and reversed-phase HPLC yielded the known isocoumarins monocerin (**1**), its demethylated derivative **2**, fusarentin 6,7-dimethyl ether (**3**), fusarentin 6-methyl ether (**4**), and fusarentin derivative **5**, as well as the new phthalide **6**. Structure elucidation of the known metabolites **1**−**5** was based on a detailed analysis of their spectroscopic data and by comparing it with those reported in the literature.[22−26]

Compound **6** was isolated as yellow oil. The ESITOFMS spectrum of **6** showed a pseudomolecular ion peak at *m/z* 309.1339 $[M + H]^+$ (calcd for $C_{16}H_{21}O_6$, 309.1338), thus establishing a molecular formula (M) of $C_{16}H_{20}O_6$. The IR spectrum of **6** exhibited an absorption band at 1730 cm^{-1}, a characteristic of phthalide carbonyl esters, whose absorption bands are typically found at 1730−1739 cm^{-1}.[17] The IR absorption band at 1711 cm^{-1}, together with the ^{13}C NMR resonance at δ_C 209.8, indicated the presence of a ketone carbonyl in **6**. The ^1H NMR spectrum of **6** showed signals of an sp^2 methine (δ_H 6.49), an oxygenated sp^3 methine (δ_H 5.41), two methoxy groups (δ_H 3.94 and 3.90), four methylene groups (δ_H 1.60−2.71), and methyl protons (δ_H 0.91). Analyses of ^{13}C NMR, HMQC, and DEPT spectra revealed that **6** contained 16 carbons, attributable to three methyl (two *O*-CH_3), four methylene, two methine (one sp^2 and one oxygenated), and seven nonprotonated carbons. The $^1H-^1H$ COSY spectrum of **6** allowed the assignment of partial structures H-3/H_2-8/H_2-9 and H_2-11/H_2-12/H_3-13. The HMBC spectrum exhibited correlations from H_2-8, H_2-9, H_2-11, and H_2-12 to the C-10 ketone, establishing the structure of the 3-hexanone side chain in **6**. A downfield shift (δ_H 5.41; δ_C 80.9) of an oxygenated sp^3 methine implied either an ester or ether linkage; the HMBC spectrum showed a correlation from H-3 to the C-1 carbonyl ester, indicating the presence of an ester in **6**, i.e., a five-membered-ring lactone. The HMBC correlations from H-3 to C-1 and C-3a; H-8 to C-3a; and H-4 to C-3 and C-7a indicated that a five-membered-ring lactone was attached to an aromatic unit, leading to the construction of a phthalide skeleton (1(3*H*)-isobenzofuranone) of **6**. The HMBC correlations from H-4 to C-3, C-5, C-6, and C-7a; 5-OCH_3 to C-5 and C-6; and 6-OCH_3 to C-6, as well as the NOESY correlation between H-4 and 5-OCH_3, unambiguously assigned the positions of H-4, 5-OCH_3, and 6-OCH_3







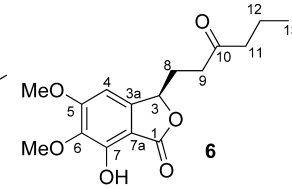


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Table 1. Cytotoxic Activity of Compounds **1**−**6**

	cytotoxic activity (IC_{50}, μM)			
compound	HuCCA-1	HepG2	A549	MOLT-3
1	>162.1	>162.1	>162.1	153.0
2	>169.8	23.7	>169.8	124.3
3	>161.1	120.8	>161.1	115.3
4	ND[a]	ND	ND	>168.7
5	ND	ND	ND	>153.2
6	>162.1	>162.1	>162.1	147.8
doxorubicin	0.67	0.29	0.34	ND
etoposide	ND	15.8	ND	0.03

[a] ND = not determined.

Table 2. Radical Scavenging, Antioxidant, and Aromatase Inhibitory Activities of **1**−**6**

	radical scavenging and antioxidant activities (IC_{50}, μM)					
	DPPH[a]	XXO[b]	IXO[c]	HL-60[b]	ORAC (unit)[d]	inhibition of aromatase (IC_{50}, μM)[e]
1	>324	>648	>648	>130	10.8	>16.2
2	23.4	52.6	>679	>136	11.5	>16.9
3	>322	>644	>644	>128	14.4	>16.1
4	16.4	4.3	>675	>135	1.4	>16.8
5	>306	352.4	>612	>122	1.4	>15.3
6	>324	>648	>648	>130	2.4	>16.2

[a] DPPH = scavenging 2,2-diphenyl-1-picrylhydrazyl free radicals. Ascorbic acid was used as the reference compound (IC_{50} value 21.2 μM). [b] XXO = inhibition of superoxide anion radical formation by xanthine/xanthine oxidase. HL-60 = inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced superoxide anion radical generation in differentiated HL-60 cells. Superoxide dismutase was used as a positive control (scavenging 100% of the radicals). [c] IXO = inhibition of xanthine oxidase. Allopurinol is the reference inhibitor (IC_{50} value 3.0 μM). [d] ORAC (oxygen radical absorbance capacity against ROO·). Results were expressed as ORAC units, where 1 ORAC unit equals the net protection of β-phycoerythrin produced by 1 μM Trolox. [e] Ketoconazole was a positive control (IC_{50} value 2.4 μM).

in **6**. The ^{13}C NMR resonance at δ_C 148.9 placed the remaining hydroxyl group at C-7. On the basis of these spectroscopic data, a planar structure of **6** was proposed and named colletotrialide. In general, the 3*S*-phthalides exhibit negative optical rotation, while 3*R*-derivatives have positive rotation.[17,27,28] Colletotrialide (**6**) was dextrorotatory ($[\alpha]^{28}_D$ +16.8), and C-3 was proposed as *R*.

Four cancer cell lines, HuCCA-1 (human lung cholangiocarcinoma), HepG2 (human hepatocellular liver carcinoma), A549 (human lung carcinoma), and MOLT-3 (acute lymphoblastic leukemia), were used for the evaluation of cytotoxic activity. Isocoumarins **1**−**5** were weakly cytotoxic (IC_{50} 115.3−153.0 μM) or inactive against the cell lines tested (Table 1). However, it is interesting to note that the cytotoxic activity toward the HepG2 cell line of monocerin derivative **2** (IC_{50} 23.7 μM) was the same order of magnitude as that of a standard anticancer drug, etoposide (IC_{50} 15.8 μM), and was at least 5 times higher than that toward HuCCA-1, A549, and MOLT-3 cancer cells (Table 1). The isolated natural substances **1**−**6** were also evaluated for potential cancer chemopreventive properties by measuring radical scavenging, antioxidant activity, and aromatase (CYP19) inhibition (Table 2). Monocerin derivative **2** and fusarentin derivative **4** could scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (IC_{50} values of 23.4 and 16.4 μM, respectively) and inhibit superoxide anion radical formation in the xanthine/xanthine oxidase (XXO) assay (IC_{50} values of 52.6 and 4.3 μM, respectively) (Table 2). Ascorbic acid was the reference compound (IC_{50} value 21.2 μM). On the basis of these radical scavenging properties, compounds **2** and **4** might be potential candidates for cancer chemoprevention. While isocoumarins **1** and **3** did not show radical scavenging activities, their desmethyl derivatives **2** and **4** exhibited such activities. On the basis of this observation, it is possible that the C-7 hydroxyl group in **2** and **4** may be important for radical scavenging activities. None of the tested compounds could suppress superoxide anion generation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in differentiated HL-60 human promyelocytic leukemia cells (Table 2). Isocoumarins **1**−**3** showed excellent ORAC antioxidant activity with 10.8−14.4 ORAC units, and phthalide **6** exhibited 2.4 units (Table 2), while none of the compounds tested inhibited aromatase enzyme (at 15.3−16.9 μM) (Table 2). Fusarentin derivatives were previously reported to have insecticidal activity,[26] while monocerin derivatives exhibited antifungal, antibacterial, and antialgal activities[22] and antimalarial activity.[29]

Experimental Section

General Experimental Procedures. Optical rotations were measured at the sodium D line (590 nm) on a JASCO DIP-370 digital polarimeter. UV−vis spectra were obtained using a Shimadzu UV-1700 PharmaSpec spectrophotometer. FTIR data were obtained using a universal attenuated total reflectance (UATR) attachment on a Perkin-Elmer Spectrum One spectrometer. ^1H, ^{13}C, and 2D NMR spectra were recorded on a Bruker AVANCE 600 NMR spectrometer (operating at 600 MHz for ^1H and 150 MHz for ^{13}C). ESI-TOF MS spectra were obtained from a Bruker MicroTOF$_{LC}$ spectrometer.

Fungal Material and Identification. *Colletotrichum* sp. CRI535-02 was isolated following the method described previously[30] from *Piper ornatum*, which was collected from the Tai Rom Yen National Park, Surat Thani Province. The fungus was identified on the basis of both the morphological characteristics and the analysis of DNA sequences of the ITS1-5.8S-ITS2 rRNA gene region (Supporting Information). On the basis of macroscopic and microscopic morphological characteristics and phylogenetic analyses of the ITS1-5.8S-ITS2 sequences, the fungus was identified as *Colletotrichum* sp. CRI535-02, which is potentially a new species. The DNA sequence of the ITS1-5.8S-ITS2 rRNA gene region of the CRI535-02 fungus has been submitted to GenBank with the accession number of HM357614. A culture of CRI535-02 has been deposited at the Chulabhorn Research Institute (CRI), Bangkok, Thailand.

Extraction and Isolation. *Colletotrichum* sp. CRI535-02 was cultured in 250 mL of potato dextrose broth in a 1 L Erlenmeyer flask under static conditions for 21 days. The culture (5 L) was filtered to broth and mycelia. The culture broth was extracted with EtOAc (equal volume, five times) to yield an EtOAc crude extract (1.61 g). The broth extract was subjected to Sephadex LH-20 column chromatography (CC) eluted with MeOH to give 23 fractions (F1−F23). F13 (119.3 mg) was further separated by reversed-phase C_{18} HPLC, eluted with a mixture of MeOH/H_2O (50:50), to yield 16.6 mg of monocerin (**1**) and 26.2 mg of **2**. Fractions F10 and F11 were combined and separated by Sephadex LH-20 CC, eluted with MeOH, to give 10 fractions (FA1−FA10). FA3 was purified by reversed-phase C_{18} HPLC (MeOH/H_2O, 35:65), affording compound **5** (11.4 mg) and colletotrialide (**6**, 9.4 mg). Fractions FA5, FA6, and FA7 were combined and then rechromatographed by Sephadex LH-20 CC, eluted with MeOH, to give 12 fractions (FB1−FB12). Fraction FB3 (20.9 mg) and fraction FB4 (58.0 mg) were individually separated by reversed-phase C_{18} HPLC (MeOH/H_2O, 50:50), yielding compounds **3** (4.4 mg) and **4** (10.0 mg), respectively. Moreover, fraction FB5 (74.5 mg) was further separated by reversed-phase C_{18} HPLC (MeOH/H_2O, 50:50) to afford compounds **1** (26.3 mg) and **2** (8.2 mg).

Colletotrialide (6): yellow, viscous oil; $[\alpha]^{28}_D$ +16.8 (*c* 0.47, in MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.46), 260 (4.07), 293.5 (3.57) nm; IR ν_{max} 3406, 2935, 1730, 1711, 1607, 1465, 1364, 1316, 1258, 1133, 1098, 1007, 930, 826 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.49 (1H, s, H-4), 5.41 (1H, d, J = 6.7 Hz, H-3), 3.94 (3H, s, 5-OC*H*$_3$), 3.90 (3H, s, 6-OC*H*$_3$), 2.71 (1H, ddd, J = 18.1, 7.6, 7.6 Hz, H-9a), 2.52 (1H, ddd, J = 18.2, 7.7, 5.1 Hz, H-9b), 2.42 (1H, m, H-8a), 1.83 (1H, m, H-8b), 2.39 (2H, q, J = 7.4 Hz, CH_2-11), 1.60 (2H, sextet, J = 7.3 Hz, H_2-12), 0.91 (3H, t, J = 7.4 Hz, H_3-13); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.8 (C, C-10), 171.0 (C, C-1), 159.9 (C, C-5), 148.9 (C, C-7), 145.5 (C, C-3a), 136.2 (C, C-6), 105.4 (C, C-7a), 97.0 (CH, C-4), 80.9 (CH, C-3), 60.9 (CH_3, 6-O*C*H$_3$), 56.6 (CH_3, 5-O*C*H$_3$), 45.0 (CH_2, C-11), 37.2 (CH_2, C-9), 28.5 (CH_2, C-8), 17.3 (CH_2, C-12), 13.7 (CH_3, C-13); ESITOFMS *m*/*z* 309.1339 $[M + H]^+$ (calcd for $C_{16}H_{21}O_6$, 309.1338).

Cytotoxicity Assay. Cytotoxic activity for HepG2, HuCCA-1, and A549 cancer cell lines was evaluated with the MTT assay,[31] while that

for the MOLT-3 cell line was assessed using the XTT assay.[32] Doxorubicin and etoposide were the reference drugs (Table 1).

Scavenging 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radicals. Scavenging of DPPH free radicals was determined photometrically as described by Gerhauser et al.[33] Scavenging potential was compared with a solvent control (0% radical scavenging) and ascorbic acid (250 μM final concentration, 100% radical scavenging, used as a blank). Ascorbic acid was used as the reference compound, showing a half-maximal scavenging concentration (IC_{50} value) at 21.2 μM.

Inhibition of Superoxide Anion Radical Formation by Xanthine/Xanthine Oxidase (XXO Assay). The XXO assay was performed following the method essentially described by Gerhauser et al.[33] Superoxide dismutase (30 U) was used as a positive control, and it could scavenge 100% of the radials formed. The IC_{50} value of the compound tested represents the half-maximal scavenging concentration (50% inhibition of the radicals formed). Allopurinol, the reference compound, inhibited xanthine oxidase (IXO) with an IC_{50} value of 3.0 μM. Inhibition of superoxide anion radical formation was measured only when the tested compounds did not inhibit xanthine oxidase.

Inhibition of 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)-Induced Superoxide Anion Radical Generation in Differentiated HL-60 Cells (HL-60 Assay). TPA-induced superoxide anion radical formation was detected in differentiated HL-60 human promyelocytic leukemia cells by photometric determination of cytochrome *c* reduction, following the method previously described by Gerhauser et al.[33] Superoxide dismutase (12 U) was used as a positive control (scavenging 100% of the radicals). IC_{50} value is the concentration exhibiting 50% inhibition of superoxide anion radical formation. Only the test samples with cell viability more than 50% were considered for the calculation of scavenging potential.

Measurement of Oxygen Radical Absorbance Capacity (ORAC). Peroxyl radical absorbance capacity of compounds was tested in a modified ORAC assay previously described by Gerhauser et al.[33] Antioxidant potential of the test compounds (1 μM) was compared with that of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue. Results were expressed as ORAC units, where 1 ORAC unit equals the net protection of β-phycoerythrin produced by 1 μM Trolox. Scavenging capacities of >1 ORAC unit were considered as positive.

Inhibition of Aromatase (CYP19). Inhibition of aromatase was assayed following the method described by Stresser et al.[34] Ketoconazole was a positive control, exhibiting an IC_{50} value of 2.4 μM.

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Supporting Information Available: ^1H, ^{13}C, and 2D NMR spectra of **6** and identification of *Colletotrichum* sp. CRI535-02. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gunatilaka, A. A. L. *J. Nat. Prod.* **2006**, *69*, 509–526.
(2) Tan, R. X.; Zou, W. X. *Nat. Prod. Rep.* **2001**, *18*, 448–459.
(3) Stierle, A.; Strobel, G.; Stierle, D.; Grothaus, P.; Bignami, G. *J. Nat. Prod.* **1995**, *58*, 1315–1324.
(4) Shrestha, K.; Strobel, G. A.; Shrivastava, S. P.; Gewali, M. B. *Planta Med.* **2001**, *67*, 374–376.
(5) Eyberger, A. L.; Dondapati, R.; Porter, J. R. *J. Nat. Prod.* **2006**, *69*, 1121–1124.
(6) Puri, S. C.; Verma, V.; Amna, T.; Qazi, G. N.; Spiteller, M. *J. Nat. Prod.* **2005**, *68*, 1717–1719.
(7) Kusari, S.; Lamshöft, M.; Zühlke, S.; Spiteller, M. *J. Nat. Prod.* **2008**, *71*, 159–162.
(8) (a) Kitajima, J.; Ishikawa, T.; Satoh, M. *Phytochemistry* **2003**, *64*, 1003–1011. (b) Li, Y. H.; Peng, S. L.; Zhou, Y.; Yu, K. B.; Ding, L. S. *Planta Med.* **2006**, *72*, 652–656. (c) Beck, J. J.; Chou, S. C. *J. Nat. Prod.* **2007**, *70*, 891–900. (d) Lee, T. F.; Lin, Y. L.; Huang, Y. T. *Planta Med.* **2007**, *73*, 527–534.
(9) Faigl, F.; Thurner, A.; Molnr, B.; Simig, G.; Volk, B. *Org. Process Res. Dev.* **2010**, *14*, 617–622.
(10) (a) Huang, L. L.; Xu, M. H.; Lin, G. Q. *J. Am. Chem. Soc.* **2006**, *128*, 5624–5625. (b) Zhang, B.; Xu, M. H.; Lin, G. Q. *Org. Lett.* **2009**, *11*, 4712–4715. (c) Phan, D. H.; Kim, B.; Dong, V. M. *J. Am. Chem. Soc.* **2009**, *131*, 15608–15609. (d) Zhang, H.; Zhang, S.; Liu, L.; Luo, G.; Duan, W.; Wang, W. *J. Org. Chem.* **2010**, *75*, 368–374. (e) Xing, C. H.; Liao, Y. X.; He, P.; Hu, Q. S. *Chem. Commun.* **2010**, *46*, 3010–3012. (f) Ye, Z.; Lv, G.; Wang, W.; Zhang, M.; Cheng, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 3671–3674. (g) Willis, M. C. *Angew. Chem., Int. Ed.* **2010**, *49*, 6026–6027.
(11) Tabopda, T. K.; Fotso, G. W.; Ngoupayo, J.; Mitaine-Offer, A. C.; Ngadjui, B. T.; Lacaille-Dubois, M. A. *Planta Med.* **2009**, *75*, 1258–1261.
(12) Kihampa, C.; Nkunya, M. H.; Joseph, C. C.; Magesa, S. M.; Hassanali, A.; Heydenreich, M.; Kleinpeter, E. *Phytochemistry* **2009**, *70*, 1233–1238.
(13) Devienne, K. F.; Cálgaro-Helena, A. F.; Dorta, D. J.; Prado, I. M.; Raddi, M. S.; Vilegas, W.; Uyemura, S. A.; Santos, A. C.; Curti, C. *Phytochemistry* **2007**, *68*, 1075–1080.
(14) Whyte, A. C.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod.* **1996**, *59*, 765–769.
(15) Mullady, E. L.; Millett, W. P.; Yoo, H. D.; Weiskopf, A. S.; Chen, J.; Ditullio, D.; Knight-Connoni, V.; Hughes, D. E.; Pierceall, W. E. *J. Nat. Prod.* **2004**, *67*, 2086–2089.
(16) Prachyawarakorn, V.; Mahidol, C.; Sureram, S.; Sangpetsiripan, S.; Wiyakrutta, S.; Ruchirawat, S.; Kittakoop, P. *Planta Med.* **2008**, *74*, 69–72.
(17) Chou, T. H.; Chen, I. S.; Hwang, T. L.; Wang, T. C.; Lee, T. H.; Cheng, L. Y.; Chang, Y. C.; Cho, J. Y.; Chen, J. J. *J. Nat. Prod.* **2008**, *71*, 1692–1695.
(18) Deng, S.; Chen, S. N.; Yao, P.; Nikolic, D.; van Breemen, R. B.; Bolton, J. L.; Fong, H. H.; Farnsworth, N. R.; Pauli, G. F. *J. Nat. Prod.* **2006**, *69*, 536–541.
(19) Lee, T. F.; Lin, Y. L.; Huang, Y. T. *Planta Med.* **2007**, *73*, 527–534.
(20) Lim, L. S.; Shen, P.; Gong, Y. H.; Yong, E. L. *Phytochemistry* **2006**, *67*, 728–734.
(21) Liu, L.; Ning, Z. Q.; Shan, S.; Zhang, K.; Deng, T.; Lu, X. P.; Cheng, Y. Y. *Planta Med.* **2005**, *71*, 808–813.
(22) Zhang, W.; Krohn, K.; Draeger, S.; Schulz, B. *J. Nat. Prod.* **2008**, *71*, 1078–1081.
(23) Aldridge, D. C.; Turner, W. B. *J. Chem. Soc., Sect. C* **1970**, *18*, 2598–2600.
(24) Mori, K.; Takaishi, H. *Tetrahedron* **1989**, *45*, 1639–1646.
(25) Grove, J. F.; Pople, M. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2048–2051.
(26) Claydon, N.; Grove, J. F.; Pople, M. *J. Invertebr. Pathol.* **1979**, *33*, 364–367.
(27) Takahashi, H.; Tsubuki, T.; Higashiyama, K. *Chem. Pharm. Bull.* **1991**, *39*, 3136–3139.
(28) Nakano, H.; Kumagai, N.; Matsuzaki, H.; Kabuto, C.; Hongo, H. *Tetrahedron: Asymmetry* **1997**, *8*, 1391–1401.
(29) Sappapan, R.; Sommit, D.; Ngamrojanavanich, N.; Pengpreecha, S.; Wiyakrutta, S.; Sriubolmas, N.; Pudhom, K. *J. Nat. Prod.* **2008**, *71*, 1657–1659.
(30) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jone, P. G.; Doring, D. *Mycol. Res.* **1995**, *99*, 1007–1015.
(31) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–942.
(32) Doyle, A.; Griffiths, J. B. *Mammalian Cell Culture: Essential Techniques*; John Wiley & Sons: Chichester, UK, 1997.
(33) Gerhauser, C.; Klimo, K.; Heiss, E.; Neumann, I.; Gamal-Eldeen, A.; Knauft, J.; Liu, G. Y.; Sitthimonchai, S.; Frank, N. *Mutat. Res.* **2003**, *523−524*, 163–172.
(34) Stresser, D. M.; Turner, S. D.; McNamara, J.; Stocker, P.; Miller, V. P.; Crespi, C. L.; Patten, C. J. *Anal. Biochem.* **2000**, *284*, 427–430.

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