

Citrinin Derivatives from the Soil Filamentous Fungus *Penicillium* sp. H9318Guangmin Yao,^{a,d} Fred Musoke Sebisubi,^{b,e} Lok Yung Christopher Voo,^c Coy Choke Ho,^c
Ghee Teng Tan^b and Leng Chee Chang^{*,a,b}^aDepartment of Chemistry and Biochemistry, College of Science and Engineering,
University of Minnesota Duluth, 1039 University Drive, Duluth, MN 55812, USA^bDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Hawaii Hilo,
34 Rainbow Drive, Hilo, HI 96720, USA^cBiotechnology Program, School of Science and Technology, University Malaysia Sabah,
88999 Kota Kinabalu, Sabah, Malaysia^dHubei Key Laboratory of Natural Medicinal Chemistry and Resources Evaluation, School of
Pharmacy, Tongji Medical College, Huazhong University of Science and Technology,
13 Hangkong Road, Wuhan 430030, P. R. China^eDivision of Pharmaceutical Services, Ministry of Health,
PO Box 7272 Kampala, Uganda

A investigação do extrato orgânico produzido na fermentação microbiológica de *Penicillium* sp. H9318 conduziu ao isolamento de um novo alcaloide isoquinolínico, o composto (5*S*)-3,4,5,7-tetrametil-5,8-di-hidróxi-6(5*H*)-isoquinolinona (**1**), juntamente com quatro outros conhecidos compostos derivados da citrinina (**2-5**). Uma atividade inibitória significativa no ensaio da inibição da formação dos halos (HFI), foi exibida pela citrinina (**2**), semelhante àquela observada pelo *Streptomyces* 85E, enquanto os compostos **1**, **3**, **4** e **5** não mostraram atividade inibitória com respeito ao ensaio HFI quando testados com 20 µg/disco. Em relação ao ensaio de citotoxicidade, a citrinina (**2**) demonstrou uma atividade inibitória mais fraca quando comparada as linhagens de células cancerosas MCF-7 (IC₅₀ 71,93 µmol L⁻¹), LNCaP (IC₅₀ 77,92 µmol L⁻¹), LU-1 (147,85 µmol L⁻¹) e KB (IC₅₀ 65,93 µmol L⁻¹).

Investigation of a microbial fermentation organic extract of *Penicillium* sp. H9318 led to the isolation of a new isoquinolinone alkaloid, (5*S*)-3,4,5,7-tetramethyl-5,8-dihydroxyl-6(5*H*)-isoquinolinone (**1**), along with four known citrinin derivatives (**2-5**). Citrinin (**2**) exhibited significant inhibitory activity against *Streptomyces* 85E in the hyphae formation inhibition (HFI) assay, while compounds **1**, **3-5** were not active when tested at 20 µg/disk in the HFI assay. Citrinin (**2**) further demonstrated a weak inhibitory activity against MCF-7 (IC₅₀ 71.93 µmol L⁻¹), LNCaP (IC₅₀ 77.92 µmol L⁻¹), LU-1 (147.85 µmol L⁻¹) and KB (IC₅₀ 65.93 µmol L⁻¹) cell lines, respectively, in the cytotoxicity assay.

Keywords: *Streptomyces* 85E, isoquinolinone alkaloid, citrinin, *Penicillium* sp. H9318, kinase inhibitor

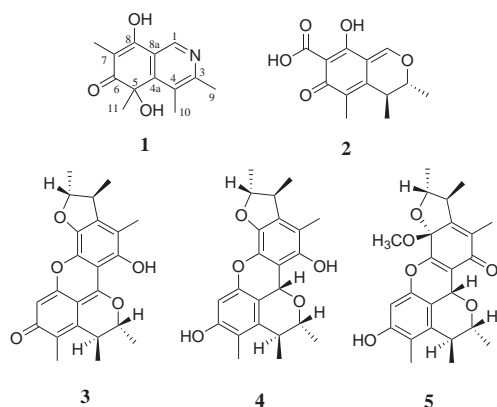
Introduction

The phosphorylation of proteins on serine/threonine and tyrosine residues by protein kinases is one of the major regulatory mechanisms in biological processes including apoptosis, cell proliferation, cell differentiation,

and metabolism. Deregulated phosphorylation associated with these pathways can result from genetic alterations acquired early in tumorigenesis, and are often the cause of cancer. In this regard, protein kinases have emerged as promising inhibitory targets in cancer treatment.^{1,2} Aerial hyphae formation of *Streptomyces* sp. requires protein kinase activity. It has been shown that a variety of kinase inhibitors block this process.^{3,4}

*e-mail: lengchee@hawaii.edu

During the course of our search for novel protein kinase inhibitors from natural resources,⁵⁻¹⁰ an acetone-soluble extract of the fungus *Penicillium* sp. H9318 showed inhibitory activity towards both mammalian protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) in an *in vitro* phosphatase assay.⁵ The *n*-BuOH-soluble extract exhibited inhibitory activity in the hyphae formation inhibition (HFI) assay with *Streptomyces* 85E giving a 20 mm clear zone of inhibition at 80 µg/disk. These preliminary results encouraged us to investigate the fungus *Penicillium* sp. H9318 for the discovery of novel protein kinase inhibitors. A bioassay-directed fractionation of the organic extract has resulted in the isolation of a novel isoquinolinone alkaloid 3,4,5,7-tetramethyl-5,8-dihydroxyl-6(5*H*)-isoquinolinone, namely, isoquinocitrinin A (**1**), and four known compounds, citrinin (**2**), penicitrone A (**3**), penicitrinols A (**4**) and B (**5**). Herein, we report the isolation, structure elucidation, and biological activities of compounds **1-5**.



Results and Discussion

The fungus *Penicillium* sp. H9318 was isolated from a soil sample collected at a heath forest in Maliau Basin, Sabah, Malaysia. The CHCl₃-soluble partition (26.6 g) of the *n*-BuOH extract of fermented *Penicillium* sp. H9318 significantly inhibited the growth of hyphae formation in *Streptomyces* 85E at a concentration of 80 µg/disk. A bioassay-guided isolation of the active fractions by repeated Sephadex LH-20 and silica gel MPLC afforded compounds **1-5**. The known compounds **2-5** were identified as citrinin (**2**),¹¹⁻¹³ penicitrone A (**3**) (also known as dicitrinin A),¹⁴ penicitrinol A (**4**)¹⁵ and penicitrinol B (**5**),¹⁶ respectively, by spectroscopic analysis and comparison with literature data.

Compound **1** was obtained as a colorless oil, with the molecular formula C₁₃H₁₅NO₃ determined from the ¹H and ¹³C NMR data (Table 1) and HRESIMS (*m/z* 234.11248

[M+H]⁺, calc. 234.11247), indicating seven degrees of unsaturation. The UV absorption bands at λ_{max} 249, 269, 293, 307, 330 nm indicated the presence of an extended conjugated chromophore. The ¹H NMR spectrum of **1** (Table 1) displayed signals of an aromatic proton at δ_H 8.85 (1H, s, H-1), three aromatic methyls at δ_H 2.66 (3H, s, H-10), 2.57 (3H, s, H-9), 1.89 (3H, s, H-12); and a tertiary methyl at δ_H 1.62 (3H, s, H-11). The ¹³C NMR and DEPT spectrum exhibited 13 carbon signals, including one carbonyl (δ_C 188.5), one sp² methine (δ_C 143.9), six sp² quaternary carbons (δ_C 178.8, 161.1, 152.4, 131.9, 126.0 and 108.5), one oxygen-bearing sp³ quaternary carbon (δ_C 73.6), and four methyls (δ_C 29.3, 22.9, 16.8 and 8.3). One carbonyl and four double bonds from the ¹³C NMR spectra accounted for five degrees of unsaturation, thus the remaining two degrees of unsaturation requires the presence of two rings in **1**.

Table 1. NMR spectroscopic data for compounds **1** in CD₃OD (500 MHz)

Position	δ _H , multiplicity	δ _C	HMBC (H→C)
1	8.85, s	143.9	C3, C4a, C8, C8a
3		161.1	
4		131.9	
4 ^a		152.4	
5		73.6	
6		188.5 ^a	
7		108.5	
8		178.8 ^a	
8 ^a		126.0	
9	2.57, s	22.9	C3, C4
10	2.66, s	16.8	C3, C4, C4a
11	1.62, s	29.3	C4a, C5, C6
12	1.89, s	8.3	C6, C7, C8

^a Weakly observed in ¹³C NMR spectrum, assigned by HMBC.

Comparison of its ¹H and ¹³C NMR data (Table 1) with those of citrinin (**2**) showed that these compounds differ in the presence of C-3 and C-4 sp² quaternary carbons (δ_C 161.1 and 131.9) in **1** rather than the C-3 and C-4 sp³ methines (δ_C 82.0 and 33.4) in **2**. The ¹H and ¹³C assignments of **1** were confirmed by analysis of the HMBC spectrum (Figure 1). Long-range correlations were observed between the signal of H-1 (δ_H 8.85) and C-3 (δ_C 161.1); between CH₃-9 (δ_H 2.57) and C-3 (δ_C 161.1), C-4 (δ_C 131.9); as well as between CH₃-10 (δ_H 2.66) with C-3 (δ_C 161.1), C-4 (δ_C 131.9) and C-4a (δ_C 152.4). Another major difference in the ¹³C NMR spectrum of compounds **1** and **2** was that C-12 was changed from a carbonyl group (δ_C 174.0) in **2** into a methyl group (δ_C 8.3) in **1**. Also, HMBC correlations of **1** from CH₃-12 (δ_H 1.89, s)

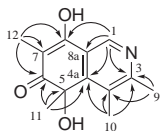
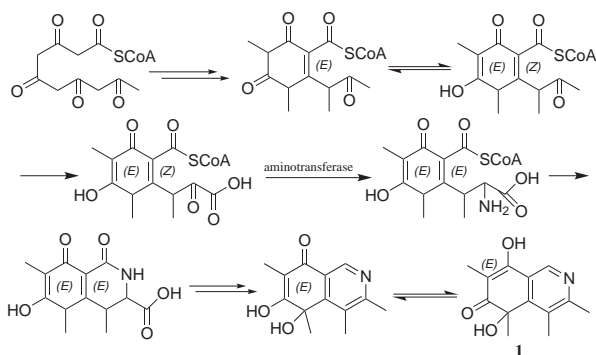


Figure 1. Key HMBC correlations (H→C) for compound **1**.

to C-6 (δ_c 188.5), C-7 (δ_c 108.5), and C-8 (δ_c 178.8) revealed that the methyl C-12 was located at C-7. Furthermore, the cross peaks of the methyl CH₃-11 (δ_H 1.62, s) with C-4a (δ_c 152.4), C-5 (δ_c 73.6) and C-6 (δ_c 188.5), along with a NOESY correlation observed between CH₃-11 and CH₃-10 confirmed that the methyl C-11 was connected to an oxygen bearing sp³ quaternary carbon at C-5. In consideration of the molecular formula and the ¹³C NMR chemical shift, C-5 (δ_c 73.6) and C-8 (δ_c 178.8) were substituted by hydroxyl groups, and a N atom must be assigned to position 2. In spite of our utmost efforts, the absolute configuration at C-5 of compound **1** has yet to be ascertained. Therefore, compound **1** was elucidated as 3,4,5,7-tetramethyl-5,8-dihydroxyl-6(5*H*)-isoquinolinone and named isoquinocitrinin A.

Several similar novel isoquinoline alkaloids have been reported from the genus of *Penicillium*,¹⁷ *Aspergillus*,¹⁸ *Streptomyces*^{19,20} and *Chaetomium*.²¹ Most isoquinoline alkaloids discovered from plants exhibit complicated structures, which were mainly synthesized from tyrosine, whereas most isoquinolines from lichens, fungi, and sponges show simple skeletons and are probably biosynthesized via mixed pathways.²¹

Compound **1** possesses a carbon skeleton similar to citrinin (**2**), and it is possibly biosynthesized via mixed routes. The proposed biosynthesis of compound **1** proceeds via an enzyme-bonded poly- β -ketone chain²² and the amino acid formation is accomplished by an aminotransferase (Scheme 1).



Scheme 1. Proposed biogenesis pathway of compound **1**.

Citrinin (**2**) was first isolated from *P. citrinum* in 1931 and it has been isolated from ten or more species of *Penicillium* and *Aspergillus*. Compounds **3-5** are known citrinin dimer derivatives. Until now, more than ten citrinin dimers have

been discovered from natural resources.^{14,16} This is the first report of an isoquinolinone alkaloid from the genus *Penicillium* possessing a carbon skeleton similar to that of citrinin.

Compounds **1-5** were evaluated for their inhibitory activities against *Streptomyces* 85E in the hyphae formation inhibition assay, according to an established protocol.⁴ Compound **2** exhibited significant inhibitory activity and gave a 21 mm clear zone of inhibition (ZOI) at 80 $\mu\text{g}/\text{disk}$, 17 mm clear ZOI at 40 $\mu\text{g}/\text{disk}$, 12 mm ZOI at 20 $\mu\text{g}/\text{disk}$, and a 10 mm bald ZOI at 2.5 $\mu\text{g}/\text{disk}$. All other isolates were inactive. It is hypothesized that compounds which inhibit hyphae formation in *Streptomyces* 85E may block the proliferation of cancer. In the human breast cancer MCF-7 cells, proliferation can be blocked in numerous ways, including serum deprivation, pharmacological inhibition of specific kinase and steroid hormone pathways.^{23,24} Therefore, compound **2** was further evaluated for its cytotoxicity activity using several cancer cell lines. Compound **2** demonstrated a weak activity with the MCF-7 (IC₅₀ 71.93 $\mu\text{mol L}^{-1}$ or 18 $\mu\text{g mL}^{-1}$), LNCaP (IC₅₀ 77.92 $\mu\text{mol L}^{-1}$ or 19.5 $\mu\text{g mL}^{-1}$), and LU-1 (147.85 $\mu\text{mol L}^{-1}$ or 37.0 $\mu\text{g mL}^{-1}$), KB cells (IC₅₀ 65.93 $\mu\text{mol L}^{-1}$ or 16.5 $\mu\text{g mL}^{-1}$), respectively.

Citrinin (**2**), a well-known mycotoxin, is both nephrotoxic¹³ and carcinogenic.²⁵ It was reported to show moderate cytotoxicity against the mouse NS-1 cell line with LD₉₉ values of 25 $\mu\text{g mL}^{-1}$,¹⁵ and antifungal activities against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *A. niger* at a concentration of 100 $\mu\text{g}/\text{disk}$.¹⁴ It was documented too that penicitrone A (also known as dicitrinin A) (**3**) showed moderate cytotoxicity against the mouse NS-1 cell line with LD₉₉ values of 6.3 $\mu\text{g mL}^{-1}$,¹⁵ and displayed scavenging activity with IC₅₀ values of 55.3 mmol L^{-1} ,¹⁶ while penicitrinol B (**5**) did not show cytotoxicity against P388, A-549, BEL-7402, and HL-60 cells (IC₅₀ > 50 $\mu\text{mol L}^{-1}$).¹⁶

Experimental

General procedures

Optical rotations were measured using a JASCO P-1010 automatic polarimeter. UV spectra were measured on a HP 8453 UV-Visible spectrophotometer. FT-IR spectra were acquired on a Perkin-Elmer Spectrum BX spectrometer using AgCl film. Mass spectra and high-resolution mass spectra were taken with a BioTOF II ESI mass spectrometer. 1D and 2D NMR spectra were recorded in MeOH-*d*₄ and DMSO-*d*₆ on a Varian INOVA Unity (500 MHz) spectrometer. The chemical shift

(δ) values are given in ppm with the residual CD₃OD signals (3.31 ppm for ¹H and 49.15 ppm for ¹³C) as internal standard, and the coupling constants (J) are in Hz. Chromatographic fractions and pure compounds were monitored by TLC, detected by absorption of UV light at 254 nm and a color reaction by spraying with a solution of 10%, v/v, sulfuric acid/EtOH followed by 5 min heating at 120 °C. MPLC (medium pressure column chromatography) was carried out on a Büchi® pump system and a Büchi column packed with Merck silica gel 60 and/or reversed-phase C₁₈.

Fungal material

The fungal strain H9318 was isolated from the soil sample (MB 75) collected under an unidentified tree in a “Kerangas” or a heath forest at about 3000 feet (900 meters) above sea level located within the rim perimeter (south side) of Maliau Basin, Sabah,²⁶ Malaysia in May 2001. The morphological characteristics of the *Penicillium* structure of H9318 as observed through light microscopic are circular conidia, the *Ampulliform philiades* are shorter than the metula, 3 to 4 metulas were observed to branch from a single smooth stipe (of which some metulas were non-uniform in length). These characteristics points most likely to the subgenus *Furcatum*.²⁷ The strain is maintained in the Laboratory Collection at the University of Malaysia Sabah. The isolation medium used was dichloran rose bengal chloramphenicol (DRBC) medium with NaCl (2.5%) at pH 5.6. Fungi were purified, and the producing strain was prepared on potato dextrose agar slants containing NaCl 10%, m/v, and stored at 4 °C. Conidia of single colonies of microfungi were kept in anhydrous silica gel particles at 4 °C. It was then ready to inoculate to a seed medium.

Fermentation

The composition of the seed and production medium (in g L⁻¹) was as follows: yeast extract (10), peptone (10), sucrose (10), KH₂PO₄ (1), and MgSO₄·7H₂O (0.3). The seed medium was prepared with distilled water, and the pH was adjusted to 5.5 prior to sterilization. The medium was dispensed at 50 mL per 250 mL in Belco baffled shaker flasks. A single colony of *Penicillium* from the agar plate was used as inoculum into each flask of seed media, cultured at 30 °C at 250 rpm for two days. An aliquot (1.5%) strain H9318 from the seed medium was inoculated into the production medium (similar composition) and were incubated at 30 °C at 220 rpm for seven days and were then harvested.

Extraction and isolation

The microfungi fermentation culture (90 L) was centrifuged, and the supernatant was filtered and partitioned with *n*-BuOH. Both the *n*-BuOH-soluble and aqueous layers were tested against the hyphae formation inhibition (HFI) assay. The organic extracts were concentrated, suspended in H₂O (1:1, v/v) and subsequently partitioned with hexane, CHCl₃, and EtOAc (3 × 500 mL each). The CHCl₃-soluble partition (26.6 g) of *n*-BuOH extract of fermented *Penicillium* sp. H9318 significantly inhibited the growth of hyphae formation in *Streptomyces* 85E at a concentration of 80 µg/disk, and was subjected to bioassay-guided fractionation. This extract was chromatographed using silica gel CC, eluting with a gradient of CH₂Cl₂-MeOH, to afford ten fractions (H1-H10). Fraction H2 (5.3 g) was further separated on Sephadex LH-20 eluting with CHCl₃-MeOH (1:1, v/v) to give one major fraction, which was crystallized from CH₂Cl₂ to afford the yellow crystalline compound **2** (2 g). Fraction H4 (100 mg, CH₂Cl₂-MeOH (50:1, v/v)) was chromatographed on silica gel and then Sephadex LH-20 (CHCl₃-MeOH (1:1, v/v)), to afford compounds **3** (10.5 mg), **4** (5.6 mg), and **5** (7.0 mg). Fraction H6 (80 mg, CH₂Cl₂-MeOH (30:1, v/v)) was separated further on Sephadex LH-20 (CHCl₃-MeOH (1:1, v/v)) and then purified by reversed-phase C₁₈ MPLC, eluting with 50% MeOH to give compound **1** (8.5 mg).

Isoquinocitrinin A (I): colorless oil; [α]_D²³ -5.2 (c 0.42, MeOH); λ_{max} /nm (MeOH) (log ϵ): 207 (4.15), 229 (sh, 3.78), 249 (sh, 3.66), 269 (3.54), 293 (3.58), 307 (3.59), 330 (sh, 3.53); IR (AgCl, film) ν_{max} /cm⁻¹ 3350, 2928, 1600, 1508, 1383, 1237, 1124, 1071, 910; ¹H and ¹³C NMR see Table 1; LRESIMS m/z 234 (M⁺); LRESIMS m/z 232 (M⁻); HRESIMS [M+H]⁺ m/z Found: 234.11248. Calc. for C₁₃H₁₆NO₃: 234.11247.

Hyphae formation inhibition assay

The inhibition assay observed with hyphae formation in *Streptomyces* 85E was performed on purified isolates as described previously.⁴ The mycelia fragments of *Streptomyces* were spread on minimal medium ISP 4 agar plates for the generation of a bacteria lawn. Compounds of known concentration dissolved in MeOH were dispensed onto disks in 20 µL aliquots. The impregnated paper disks (with a concentration of 80 µg/disk) were applied directly on the surface of the agar plates seeded with *Streptomyces* 85E. After 30 h of growth (during which the development of hyphae in *Streptomyces* species takes place), the results are identified by a clear zone of inhibition or bald phenotype around the disk. Surfactin, a sporulation inhibitor, and

MeOH were employed as positive and negative controls, respectively. An inhibition zone of greater than 9 mm is considered active. Subfractions were tested at 80 µg/disk on 7 mm filter disks. Active compounds were tested at lower concentrations (20, 10, 5, 2.5 µg/disk). The assays were performed in duplicate.

Cytotoxicity assay

The standard protocol for the assessment of cellular toxicity measures the ability of cultured cells to proliferate in the presence of test samples, and subsequently quantitates total protein content with sulforhodamine B dye as a measure of the percentage of surviving cells.²⁸ The cytotoxic potential of citrinin was determined against the KB (human oral epidermoid carcinoma), LNCaP (androgen-sensitive human prostate adenocarcinoma), MCF-7 (human breast adenocarcinoma), and LU-1 (human lung carcinoma) cell lines. Cells were seeded in 96-well plates (2.5×10^4 cells mL⁻¹), and six two-fold serial dilutions of samples in 10% DMSO (10 µL) were added to each well. The plates were incubated for 72 h at 37 °C, after which cell viability was determined with sulforhodamine B staining. IC₅₀ values were determined as the concentration of sample required to inhibit cell growth by 50% relative to a control treated with 0.5% DMSO, and represent the average of triplicate values obtained from two independent experiments.²⁸

Supplementary Information

HRESIMS, ¹H and ¹³C NMR spectra, HMQC, HMBC and NOESY spectra for compound **1** are available free of charge at <http://jbc.sbj.org.br> as a PDF file.

Acknowledgments

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References

- Persidis, A.; *Nat. Biotechnol.* **1998**, *16*, 1082.
- Cohen, P.; *Nat. Rev. Drug Discovery* **2002**, *1*, 309.
- Hong, S. K.; Matsumoto, A.; Horinouchi, S.; Beppu, T.; *Mol. Genet. Genomics* **1993**, *236*, 347.

- Waters, B. D.; Saxena, G.; Wanggui, Y.; Kau, D.; Wrigley, S.; Stokes, R.; Davies, J.; *J. Antibiot.* **2002**, *55*, 407.
- Ong, S. M.; Voo, L. Y. C.; Lai, N. S.; Stark, M. J. R.; Ho, C. C.; *J. Appl. Microbiol.* **2007**, *102*, 680.
- Xiang, W.; Chang, L. C.; *Planta Med.* **2006**, *72*, 735.
- Shao, N.; Yao, G. M.; Chang, L. C.; *J. Nat. Prod.* **2007**, *70*, 869.
- Yao, G. M.; Vidor, N. B.; Foss, A. P.; Chang, L. C.; *J. Nat. Prod.* **2007**, *70*, 901.
- Yao, G. M.; Chang, L. C.; *Org. Lett.* **2007**, *9*, 3037.
- Yao, G. M.; Kondratyuk, T. P.; Tan, G. T.; Pezzuto, J. M.; Chang, L. C.; *J. Nat. Prod.* **2009**, *72*, 319.
- Marinho, A. M. R.; Rodrigues-Filho, E.; Moitinho, M. L.; Santos, L. S.; *J. Braz. Chem. Soc.* **2005**, *16*, 280.
- Barber, J.; Cornford, J. L.; Howard, T. D.; Sharples, D.; *J. Chem. Soc., Perkin Trans. I* **1987**, *1*, 2743.
- Krogh, P.; Hasselager, E.; Friis, P.; *Acta Pathol. Microbiol. Scand., Sect. B: Microbiol. Immunol.* **1970**, *78*, 401.
- Clark, B. R.; Capon, R. J.; Lacey, E.; Tennant, S.; Gill, J. H.; *Org. Biomol. Chem.* **2006**, *4*, 1520.
- Wakana, D.; Hosoe, T.; Itabashi, T.; Okada, K.; Campos Takaki, G. M.; Yaguchi, T.; Fukushima, K.; Kawai, K.; *J. Nat. Med.* **2006**, *60*, 279.
- Lu, Z.-Y.; Lin, Z.-J.; Wang, W.-L.; Du, L.; Zhu, T.-J.; Fang, Y.-C.; Gu, Q.-Q.; Zhu, W.-M.; *J. Nat. Prod.* **2008**, *71*, 543.
- Trisuwan, K.; Rukachaisirikul, V.; Sukpondma, Y.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J.; *Tetrahedron* **2010**, *66*, 4484.
- Kohno, J.; Hiramatsu, H.; Nishio, M.; Sakurai, M.; Okuda, T.; Komatsubara, S.; *Tetrahedron* **1999**, *55*, 11247.
- Hawas, U. W.; Shaaban, M.; Shaaban, K. A.; Speitling, M.; Maier, A.; Kelter, G.; Fiebig, H. H.; Meiners, M.; Helmke, E.; Laatsch, H.; *J. Nat. Prod.* **2009**, *72*, 2120.
- Solecka, J.; Sitkowski, J.; Bocian, W.; Bednarek, E.; Kaweck, R.; Kozerski, L.; *J. Antibiot.* **2009**, *62*, 581.
- Li, G.-Y.; Li, B.-G.; Yang, T.; Liu, G.-Y.; Zhang, G.-L.; *Org. Lett.* **2006**, *8*, 3613.
- Rodrig, O. R.; Ellis, L. C.; Glover, I.T.; *Biochemistry* **1966**, *5*, 2458.
- Osborne, C. K.; Hobbs, K.; Clark, G. M.; *Cancer Res.* **1985**, *45*, 584.
- Lobenhofer, E. K.; Huper, G.; Iglehart, J. D.; Marks, J. R.; *Cell Growth Differ.* **2000**, *11*, 99.
- Ueno, Y.; Kubota, K.; *Cancer Res.* **1976**, *36*, 445.
- Voo, C. L.; Lai, N. S.; Kibat, C.; Puah, S. H.; Hew, C. S.; Lee, K.-H.; Ho, C. C.; *J. Trop. Biol. Conserv.* **2007**, *3*, 11.
- Pitt, J. I.; *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*, Academic Press: London, 1979.
- Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

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