Butyrolactone I Derivatives from Aspergillus terreus Carrying an Unusual Sulfate Moiety

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 2008

Impact Factor: 3.8 · DOI: 10.1021/np070341r · Source: PubMed

Notes

Butyrolactone I Derivatives from *Aspergillus terreus* Carrying an Unusual Sulfate Moiety

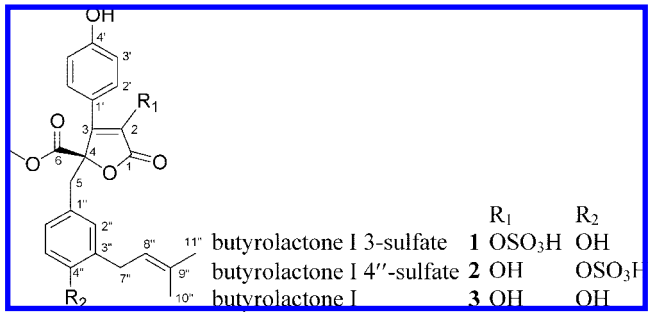
Xuemei Niu,[†] Hans-Martin Dahse,[†] Klaus-Dieter Menzel,[†] Olivier Lozach,[‡] Grit Walther,[§] Laurent Meijer,[‡] Susanne Grabley,[†] and Isabel Sattler*[,†]

Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstrasse 11a, D-07745 Jena, Germany, CNRS, Station Biologique, Place G. Teissier, B.P. 74, 29682 ROSCOFF Cedex, France, and CBS Fungal Biodiversity Centre, P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands

Received July 13, 2007

In our ongoing search for new bioactive metabolites from microbial resources, *Aspergillus terreus* (HKI0499) was examined by chemical metabolite profiling. Together with the known butyrolactone I (**3**), the unusual sulfate derivatives butyrolactone I 3-sulfate (**1**) and butyrolactone I 4″-sulfate (**2**) were discovered. The chemical structures were determined by NMR and MS data analyses. All compounds were tested on CDK1/cyclin B, CDK5/p25, DYRK1A, CK1, and GSK-3α/β kinases; compounds **2** and **3** were also evaluated for their cytotoxic and antiproliferative activities. Butyrolactone I 3-sulfate (**1**) exhibited specific inhibitory activity against CDK1/cyclin B and CDK5/p25, yet 15–30-fold less than butyrolactone I (**3**). Likewise, butyrolactone I 3-sulfate (**1**) exhibited moderate cytotoxicity solely against HeLa cells (CC_{50} = 80.7 μM).

In our ongoing search for new secondary metabolites from microbial resources by physicochemical analysis, we have directed particular attention to the hydrophilic portions of the metabolite pattern of the producing organisms.[1] Thus, chromatographic analysis (Si gel TLC, $CHCl_3$/MeOH = 2:1) of the culture filtrate extract of *Aspergillus terreus* (HKI0499) revealed compounds **1**–**3** as red spots under UV light (254 nm) and rose-red spots with anisaldehyde/H_2SO_4. From a 200 L cultivation of the strain, compounds **1** (R_f 0.45) and **2** (R_f 0.50) were purified from the H_2O-soluble portion of the culture filtrate, while compound **3** was obtained as major constituent from the EtOAc extract of the culture filtrate. We present results on the isolation and structure elucidation of the new compounds **1** and **2**, as well as *in vitro* inhibition assays on cyclin-dependent kinases related to Alzheimer's disease, as well as cytotoxicity and antiproliferative testing.



		R_1	R_2
butyrolactone I 3-sulfate	**1**	OSO_3H	OH
butyrolactone I 4″-sulfate	**2**	OH	OSO_3H
butyrolactone I	**3**	OH	OH

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of Compounds **1** and **2** (500 MHz, CD_3OD)

position	butyrolactone I 3-sulfate (**1**) δ_C, mult.	δ_H, mult. (*J* in Hz)	butyrolactone I 4″-sulfate (**2**) δ_C, mult.	δ_H, mult. (*J* in Hz)
1	169.3 s		170.5 s	
2	139.9 s		139.7 s	
3	146.0 s		131.1 s	
4	86.8 s		87.8 s	
5	40.1 t	3.47, d (14.7)	40.1 t	3.57, d (14.7)
		3.37, d (14.7)		3.43, d (14.7)
6	170.8 s		172.9 s	
1′	121.5 s		123.6 s	
2′, 6′	131.9 d	7.68, d (9.0)	130.0 d	7.66, d (8.8)
3′, 5′	116.6 d	6.86, d (9.0)	116.8 d	6.83, d (8.8)
4′	161.1 s		157.9 s	
1″	124.7 s		132.0 s	
2″	132.5 d	6.39, d (2.1)	133.2 d	6.54, d (2.1)
3″	128.7 s		135.0 s	
4″	156.4 s		151.2 s	
5″	115.3 d	6.49, d (8.1)	122.1 d	7.16, d (8.3)
6″	129.7 d	6.55, dd (2.1, 8.1)	130.0 d	6.71, dd (2.1, 8.1)
7″	28.8 t	3.07, d (7.2)	29.4 t	3.25, d (7.4)
8″	123.7 d	5.08, t (7.2)	123.6 d	5.05, t (7.4)
9″	132.9 s		133.9 s	
10″	25.9 q	1.67, s	26.3 q	1.65, s
11″	17.8 q	1.58, s	18.3 q	1.56, s
OCH_3	54.0	3.80, s	54.0	3.76, s

Compound **1** was isolated as a syrup and its molecular formula was determined as $C_{24}H_{23}O_{10}{}^{32}S_1$ by HRESIMS at *m/z* 503.0922 $[M - H]^-$ (calcd 503.1006 for $C_{24}H_{22}O_{10}{}^{32}S_1$). An ESIMS fragment peak at *m/z* 423 $[M - H - 80]^-$ indicated the loss of a $[HSO_3]^-$ unit from the quasi-molecular ion.[2] The presence of a sulfate moiety was confirmed by the appearance of a distinctive absorption at ν_{max} 1052 cm^{-1} in the IR spectrum of **1**, which was ascribed to the conjugated S−O bond stretching vibration.[3,4] The ^1H NMR spectrum revealed two methyl singlets at δ_H 1.67 and 1.58, one methoxy singlet at δ_H 3.80, one methylene doublet at δ_H 3.07 (2H, d, 7.2), another methylene at δ_H 3.47 (1H, d, 14.7) and 3.37 (1H, d, 14.7), an olefinic triplet at δ_H 5.08 (t, 7.2), three aromatic proton signals of a 1,2,4-trisubstituted phenol (δ_H 6.39, 6.49, 6.55), and two doublets of a 1,4-disubstituted phenolic moiety at δ_H 7.68 (2H, d, 9.0) and 6.86 (2H, d, 9.0) (Table 1). The ^1H NMR data of compound **1** closely resembled those of butyrolactone I (**3**), with the only difference being the chemical shift difference of the two

* Corresponding author. Tel: (03641)65 6920. Fax: (03641)65 6679. E-mail: isabel.sattler@hki-jena.de.
[†] Leibniz-Institute for Natural Product Research and Infection Biology.
[‡] CNRS.
[§] CBS Fungal Biodiversity Centre.

10.1021/np070341r CCC: $40.75
Published on Web 02/14/2008

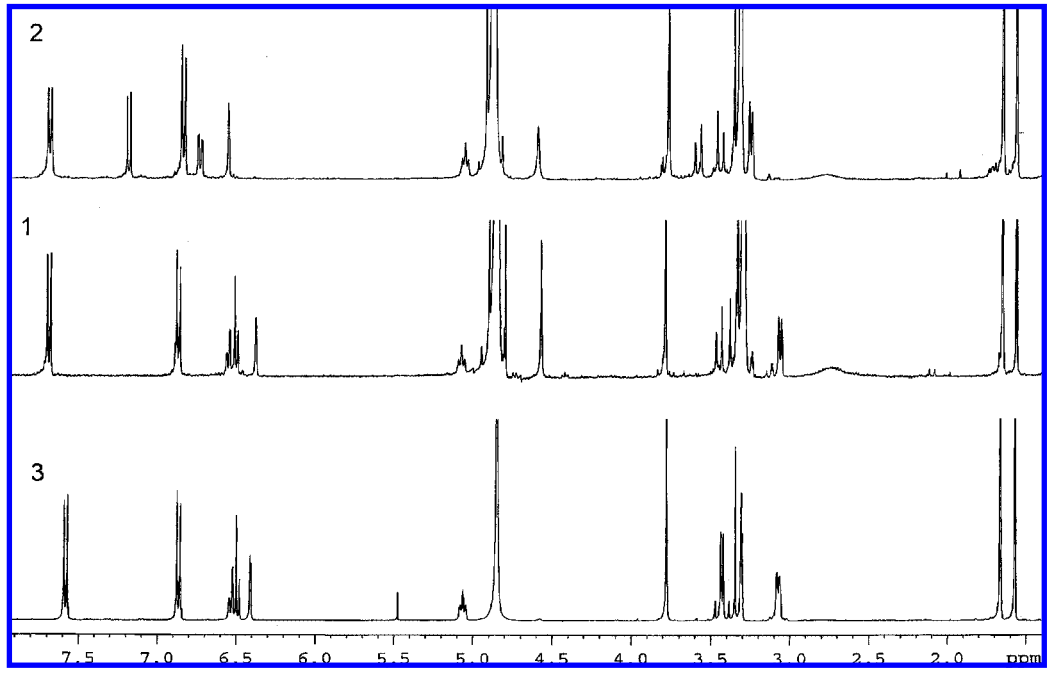


Figure 1. ^{1}H NMR spectra (CD_3OD) of compounds **1**–**3**.

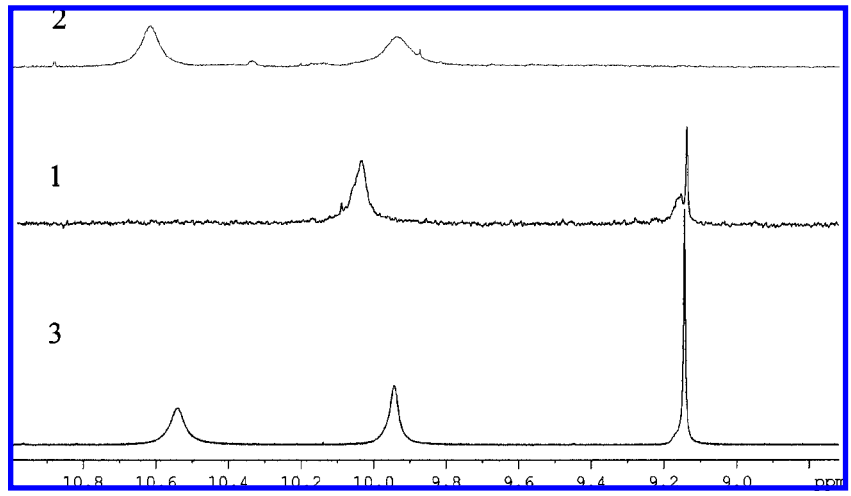


Figure 2. Partial ^{1}H NMR spectra (DMSO-d_6) of compounds **1**–**3**.

doublets of the AA′ system at C-5, which is 0.05 ppm larger than that of compound **3** (Figure 1).[5,6] In addition, the aromatic H-2′ and H-6′ resonances were shifted downfield by 0.1 ppm as compared to **3**. Comparison of the ^{13}C NMR and DEPT spectra of **1** with those of **3** showed that C-3 was shifted downfield (δ_C 129.27 (s) in **3**, 146.0 (s) in **1**). The above data suggested that compound **1** contained a sulfate group attached to C-3, instead of the hydroxy group of compound **3**. This was further confirmed by the disappearance of the 3-OH resonance of compound **3** (δ_H 10.57 ppm in DMSO-d_6) in the ^{1}H NMR spectrum of **1** (Figure 2).[5] Thus, compound **1** was determined to be butyrolactone I 3-sulfate. As the cometabolite butyrolactone I was determined to be 4*R*-configured by comparison of specific rotation data ($[\alpha]^{23.5}_D$ = +68.30 vs +84.0 in ref 6), we suggest butyrolactone I 3-sulfate, on the basis of biosynthetic considerations and similarity of specific rotation ($[\alpha]^{23.5}_D$ = +25.46), to also have the 4*R*-configuration.[6]

For compound **2**, HRESIMS at *m*/*z* 503.1009 $[M - H]^-$ revealed the identical molecular formula of $C_{24}H_{23}O_{10}{}^{32}S_1$ as for **1** (calcd for $C_{24}H_{23}O_{10}{}^{32}S_1$, 503.1006). In addition, the IR spectrum of **2** was almost identical to that of **1**, indicating a very similar chemical structure including the presence of a sulfate group. Concerning the ^{13}C NMR data, differences were found for **2** in an upfield shift of C-4″ by 5.2 ppm and downfield shifts of C-1″, C-3″, and C-5″ by about 7 ppm. Comparison of the ^{1}H NMR data of compound **2** with those of **1** revealed downfield shifts of H-2″, H-5″, and H-6″ and the attached H-7″ by 0.15–0.57 ppm (Table 1 and Figure 1). Together with the absence of the 4″-OH signal in DMSO-d_6 (δ_H 9.14 ppm in **1**) the sulfate group was assigned to C-4″, and thus, compound **2** was determined to be butyrolactone I 4″-sulfate (Figure 2). Although more than 1000 plant secondary metabolites with sulfate moieties are known, similar metabolites of microbial origin are very rare.[7] About 100 sulfated bacterial metabolites can be found in the literature and only about 20 of fungal origin, with none of them being produced by *Aspergillus*. Thus, butyrolactone I 3-sulfate and butyrolactone I 4″-sulfate are the first examples of sulfate-bearing secondary metabolite isolated from *Aspergillus*.

Butyrolactone I, a metabolite of *Aspergillus terreus* var. *africans* IFO 8355 discovered in 1977, has antiproliferative activity against colon and pancreatic carcinoma, human lung cancer, and prostatic cancer cell lines.[8–10] It selectively inhibits cyclin-dependent kinases (CDKs), which play important roles in cell cycle progression, neuronal functions, apoptosis, and transcription in mammalian

Table 2. Kinase Inhibitory Effects (IC_{50} in μM) of Compounds **1–3**

compound	CDK1	CDK5	GSK-3α/β	DYRK1A	CK1
butyrolactone I 3-sulfate (**1**)	6.0	2.4	>100	>100	>10
butyrolactone I 4″-sulfate (**2**)	>10	>100	>100	>100	>10
butyrolactone I (**3**)	0.43	0.077	>100	>100	>10
R-roscovtine[a]	0.45	0.16	>100	18.0	20.0

[a] Control.

cells.[11] CDKs are involved in numerous diseases, among which are cancer, Alzheimer's disease, Parkinson's disease, stroke, diabetes, polycystic kidney disease, glomerulonephritis, inflammation, and AIDS.[12–15] In the present study, compounds **1**−**3** were evaluated for inhibition of CDK1/cyclin B and CDK5/p25 (cyclin-dependent kinases 1 and 5), as well as for inhibition of DYRK1A (dual-serine/threonine-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A), CK1 (casein kinase 1), and GSK-3α/β (glycogen synthase kinase 3) (Table 2).[16] All these kinases are involved in Alzheimer's disease. Whereas DYRK1A, CK1, and GSK-3α were insensitive to the three butyrolactones, butyrolactone I 3-sulfate (**1**) exhibited specific inhibitory activity against CDK1/cyclin B and CDK5/p25, yet 15–30-fold less than butyrolactone I (**3**). Butyrolactone I 4″-sulfate (**2**) was inactive in all assays. These results indicate that the substitution of the hydroxy group at the alkenyl-substituted phenolic ring of butyrolactone I with a sulfate group renders it inactive against CDK5 and CDK1, while the presence of a sulfate group at the lactone moiety did not prohibit CDK1 and CDK5 inhibition of butyrolactone I. These results provide experimental proof for molecular modeling studies on the binding mode of butyrolactone I to the closely related CDK2 that are pointing to a strong electrostatic interaction of the 4″-OH group with an Asp residue.[17] This interaction is most likely disturbed in butyrolactone I 4″-sulfate (**2**) due to electronic and spatial requirements of the sulfate moiety.

Butyrolactone I (**3**) displayed antiproliferative activities toward L-929 (mouse fibroblasts) and K562 (human chronic myeloid leukemia) cell lines (GI_{50} 32.3 and 20.2 μM, respectively) and cytotoxicity toward HeLa (human cervix carcinoma) cells (CC_{50} value of 66.3 μM) similar to literature data, whereas butyrolactone I 3-sulfate (**1**) exhibited cytotoxicity only against HeLa cells (CC_{50} = 80.7 μM); butyrolactone I 4″-sulfate (**2**) was not tested.[17] These results confirm previous reports on SAR studies that the electronic nature of substituents of the aromatic rings does not significantly affect cytotoxic activity.[17]

Thus, our studies provide structurally unusual sulfate derivatives of fungal origin and new insights into the inhibitory activity of butyrolactones on cyclin-dependent kinases. Earlier reports on synthetic analogues of butyrolactone I for SAR did not include sulfates or any other related derivatives.[5,17,18]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Propol digital automatic polarimeter. IR spectra for KBr disks were recorded on a Bruker IFS55 spectrometer. UV spectra were obtained using a Varian UV–visible Cary spectrophotometer. 1D and 2D NMR were recorded on Bruker DPX-300 and DRX-500 instruments, respectively, using residual solvents as internal standards. The chemical shift values (δ) are given in parts per million (ppm), and coupling constants in Hz. ESI-MS were measured with Thermo Electron LCQ and Thermo Electron MAT95XL.

Strain and Cultivation. *Aspergillus terreus* (HKI0499), isolated from a soil sample, was cultivated in Petri dishes on malt extract agar (3.0%) at 24 °C and on Czapek yeast agar at 24 and 37 °C in the dark.[19] After 7 days macroscopic and microscopic characteristics of the strain were ascribed and colony diameters were measured. The strain was characterized by columnar yellow-brown conidial heads, densely packed metulae, and small smooth-walled conidia reaching 2.0–2.5 μm in diameter. The submerged hyphae formed numerous lateral hyaline ellipsoidal to subspherical cells (aleurioconidia). After 7 days the colonies reached the following diameter on the respective media and at the respective temperatures: malt extract agar (24 °C) 40 mm; Czapek yeast agar (24 °C) 46 mm; Czapek yeast agar (37 °C) >85 mm. By the morphological features of the conidiophores and the submerged hyphae and rates of growth strain HKI 0499 was identified as *Aspergillus terreus* THOM. The isolate was deposited as HKI0499 in the strain collection of the Leibniz Institute for Natural Products Research and Infection Biology. Cultivation was performed in a 300 L fermentor (Braun Biostat K) in 200 L of malt medium (malt extract 20.0 g/L, yeast extract 2.0 g/L, glucose 10.0 g/L, $(NH_4)_2HPO_4$ 0.5 g/L, pH 6.0) at 28 °C, while stirring at 200 rpm for 4 days.

Extraction and Isolation. The culture filtrate was separated from the mycelium by filtration and subjected to XAD-16 resin, which was washed with deionized H_2O and eluted with MeOH. The eluate was concentrated *in vacuo* and then lyophilized. The residue (3.55 g) was dissolved in H_2O and partitioned between EtOAc and H_2O (1 L:1 L, three times). The organic fraction was concentrated to dryness to give 2.02 g of residue, which was successively chromatographed on Sephadex LH-20 (MeOH) and on silica gel ($CHCl_3$ and $CHCl_3$/MeOH (9:1, 4:1, 2:1)) to yield 100.9 mg of crude compound **3**. The water fraction was subjected to an MCI gel column and, after washing with H_2O, eluted with 50% MeOH/H_2O and MeOH. The 50% MeOH/H_2O subfraction was repeatedly chromatographed on Sephadex LH-20 (MeOH) and on a RP-18 column (20% MeOH/H_2O) to give compounds **1** (2.0 mg) and **2** (12.5 mg).

Butyrolactone I 3-sulfate (1): colorless oil; $[\alpha]^{23.5}_D$ +68.30 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 311 nm (4.4); IR (KBr) ν_{max} 3367, 2925, 1743, 1608, 1517, 1438, 1351, 1252, 1182, 1127, 1052, 959, 842, 737, 722 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 1; ^{13}C NMR (125 MHz, CD_3OD) see Table 1; HRESIMS *m/z* 503.0922 [M − H]$^-$, calcd 503.1006 for $C_{24}H_{23}O_{10}{}^{32}S_1$.

Butyrolactone I 4″-sulfate (2): colorless oil; $[\alpha]^{23.5}_D$ +28.22 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 312 nm (4.4); IR (KBr) ν_{max} 3354, 2924, 1739, 1705, 1608, 1517, 1493, 1437, 1368, 1228, 1180, 1044, 840, 707 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 1; ^{13}C NMR (125 MHz, CD_3OD) see Table 1; HRESIMS *m/z* 503.1009 [M − H]$^-$, calcd 503.1006 for $C_{24}H_{23}O_{10}{}^{32}S_1$.

Butyrolactone I (3): yellow syrup, $[\alpha]^{23.5}_D$ +25.46 (*c* 0.1, MeOH); ^1H NMR and ^{13}C NMR data were in agreement with the literature;[5] UV (MeOH) λ_{max} (log ϵ) 317 nm (4.4); IR (KBr) ν_{max} 3271, 2927, 1741, 1703, 1609, 1516, 1436, 1361, 1222, 1180, 840 cm^{-1}; ESIMS *m/z* 422.94 ([M − H]$^-$, 100%).

Biological Testing. Compounds **1–3** were evaluated for their inhibitory activities on CDK1 (cyclin-dependent kinase 1/cyclin B; native, starfish oocytes), CDK5 (cyclin-dependent kinase 5/p25; recombinant, human), DYRK1A (dual-serine/threonine-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A; human, recombinant), CK1 (casein kinase 1; pig brain, native), and GSK-3α/β (glycogen synthase kinase 3; pig brain, native) as described.[16]

Compounds **2** and **3** were assayed against L-929 (mouse fibroblasts, DSM ACC 2) and K562 (human chronic myeloid leukemia cells, DSM ACC 10) for their antiproliferative effects (GI_{50}) and against HeLa (human cervix carcinoma, DSM ACC 57) cells for their cytotoxic (CC_{50}) effects as previously described.[20] Inhibitory concentrations are provided as 50% inhibition of cell growth (GI_{50}; the concentration needed to reduce the growth of treated cells to half that of untreated cells) or 50% cytotoxic concentration (CC_{50}; the concentration that kills 50% of treated cells).

Acknowledgment. This work was supported by a DAAD-Leibniz postdoctoral research fellowship (X.N.) and by grants from the EEC (FP6-2002-Life Sciences & Health, PRO-KINASE Research Project) and a "Cancéropole Grand-Ouest" grant (L.M.).

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

References and Notes

(1) Niu, X. M.; Li, S. H.; Görls, H.; Schollmeyer, D.; Hilliger, M.; Grabley, S.; Sattler, I. *Org. Lett.* **2007**, *9*, 2437–40.
(2) Chou, C. J.; Lin, L. C.; Tsai, W. J.; Hsu, S. Y.; Ho, L. K. *J. Nat. Prod.* **1997**, *60*, 375–377.

(3) Kitajima, J.; Shindo, M.; Tanaka, Y. *Chem. Pharm. Bull.* **1990**, *38*, 714–716.
(4) Sun, T. V.; Adam, G. *Phytochemistry* **1991**, *30*, 2717–2720.
(5) Morishima, H.; Fujita, K.; Nakano, M.; Atsumi, S.; Okubo, M.; Kitagawa, M.; Matsumoto, H.; Okuyama, A.; Okabe, T. (Banyu Pharma Co. Ltd.) Jpn. Kokai Tokkyo Koho 06 100, 445, 1994.
(6) Rao, K. V.; Sadhukhan, A. K.; Veerender, M.; Ravikumar, V.; Mohan, E. V. S.; Dhanvantri, S. D.; Sitaramkumar, M.; Babu, J. M.; Vyas, K.; Reddy, G. O. *Chem. Pharm. Bull.* **2000**, *48*, 559–562.
(7) *Dictionary of Natural Products*; Chapman & Hall/CRC: Boca Raton, FL, Hampden Data Services, Ltd., 2005; Version 13:1.
(8) Kiriyama, N.; Nitta, K.; Sakaguchi, Y.; Taguchi, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1977**, *25*, 2593–2601.
(9) Nishio, K.; Ishida, T.; Arioka, H.; Kurokawa, H.; Fukuoka, K.; Nomoto, T.; Fukumoto, H.; Yokote, H.; Saijo, N. *Anticancer Res.* **1996**, *16*, 3387–3396.
(10) Suzuki, M.; Hosaka, Y.; Matsushima, H.; Goto, T.; Kitamura, T.; Kawabe, K. *Cancer Lett.* **1999**, *138*, 121–130.
(11) Kitagawa, M.; Higashi, H.; Takahashi, I. S.; Okabe, T.; Ogino, H.; Taya, Y.; Hishimura, S.; Okuyama, A. *Oncogene* **1994**, *9*, 2549–2557.
(12) Malumbres, M.; Barbacid, M. *Trends Biochem. Sci.* **2005**, *30*, 630–41.
(13) Knockaert, M.; Greengard, P.; Meijer, L. *Trends Pharmacol. Sci.* **2002**, *23*, 417–25.
(14) Shapiro, G. I. J. *Clin. Oncol.* **2006**, *24*, 1770–83.
(15) Smith, P. J., Yue, E., Eds. *CDK Inhibitors of Cyclin-Dependent Kinases as Anti-tumor Agents. "Monographs on Enzyme Inhibitors"*; CRC Press: Boca Raton, 2006; Vol. 2, 448 pp.
(16) (a) Ribas, J.; Bettayeb, K.; Ferandin, Y.; Garrofé-Ochoa, X.; Knockaert, M.; Totzke, F.; Schächtele, C.; Mester, J.; Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A. L.; Boix, J.; Meijer, L. *Oncogene* **2006**, *25*, 6304–6318. (b) Bach, S.; Knockaert, M.; Lozach, O.; Reinhardt, J.; Baratte, B.; Schmitt, S.; Coburn, S. P.; Tang, L.; Jiang, T.; Liang, D. C.; Galons, H.; Dierick, J. F.; Totzke, F.; Schächtele, C.; Lerman, A. S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. J. *Biol. Chem.* **2005**, *280*, 31208–19.
(17) Brana, M. F.; Garcia, M. L.; Lopez, B.; De Pascual-Teresa, B.; Rramos, A.; Pozuelo, J. M.; Dominguey, M. T. *Org. Biomol. Chem.* **2004**, *2*, 1984–1871.
(18) Nitta, K.; Fujita, N.; Yoshimura, T.; Arai, K.; Yamamoto, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1528–1533.
(19) Pitt, J. I. *The Genus Penicillium and its Teleomorphic States: Eupenicillium and Talaromyces*; Academic Press: London, 1979.
(20) Dahse, H. M.; Schlegel, B.; Gräfe, U. *Pharmazie* **2001**, *56*, 489–491.

NP070341R