Gan, M. et al. Polyketides with New Delhi metallo-β-lactamase 1 inhibitory activity from Penicillium sp. J. Nat. Prod. 76, 1535-1540

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2013

Impact Factor: 3.8 · DOI: 10.1021/np4000944 · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Polyketides with New Delhi Metallo-β-lactamase 1 Inhibitory Activity from *Penicillium* sp.

Maoluo Gan,[†,‡,⊥] Yufeng Liu,[‡,§,⊥] Yinlei Bai,[†] Yan Guan,[†] Li Li,[‡] Rongmei Gao,[†] Weiying He,[†] Xuefu You,[†] Yuhuan Li,[†] Liyan Yu,[†] and Chunling Xiao*[,†]

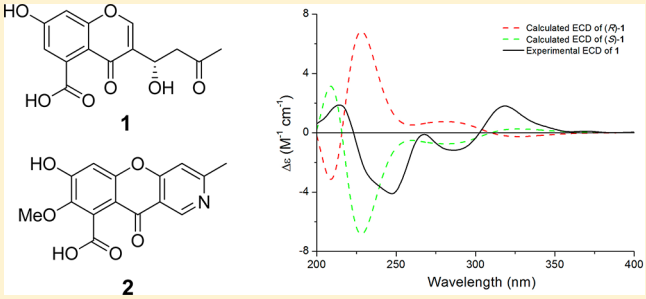
[†]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

[‡]State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

[§]Jining Medical College, Jining, Shandong 272067, People's Republic of China

Supporting Information

ABSTRACT: Three new polyketide compounds (**1**–**3**), a new quinolone alkaloid (**4**), and seven known polyketide derivatives were identified from the cultures of *Penicillium* sp. I09F 484, a strain isolated from the rhizosphere soil of the plant *Picea asperata* from Kanas Lake, Xinjiang, China. Their structures were elucidated by extensive spectroscopic data analysis. The absolute configurations of **1** and **4** were established by quantum chemical time-dependent density functional theory electronic circular dichroism calculation and Marfey's method, respectively. Compounds **1** and **2** displayed inhibitory activity against New Delhi metallo-β-lactamase 1 with IC_{50} values of 94.9 and 87.9 μM, respectively.



Infections with carbapenem-resistant Enterobacteriaceae, particularly *Klebsiella pneumoniae*, are emerging as an important challenge in healthcare settings worldwide.[1] The resistance of Enterobacteriaceae to carbapenems is increasing because of the production of various carbapenemases, including metallo-β-lactamases (MBLs).[2] In 2008, a novel MBL, called New Delhi metallo-β-lactamase 1 (NDM-1), was identified from a *K. pneumoniae* clinical isolate and has attracted great attention worldwide since then.[3] NDM-1 inactivates all β-lactams except aztreonam, conferring upon enteric bacteria nearly complete resistance to all β-lactam antibiotics in clinical use, including the late-generation carbapenems meropenem and imipenem.[3a,4] NDM-1 is encoded on a readily transferable plasmid, which facilitates its transmission.[4] Of particular concern is the fact that NDM-1 producers are already becoming highly prevalent, and NDM-1 has been observed in clinical isolates of the opportunistic bacterium *Acinetobacter baumannii* and in strains of *Escherichia coli* and other Enterobacteriaceae.[3b] NDM-1 is not inhibited by current β-lactamase inhibitors such as clavulanic acid and tazobactam.[3] Although some MBL inhibitors have been reported, none of them are clinically approved, and to date, there are few reports of compounds with inhibitory activity against the newly emerging NDM-1.[5]

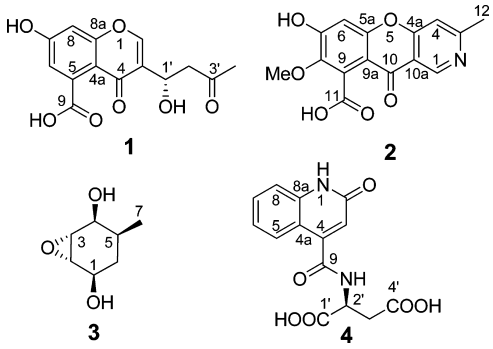
Penicillium species, known for their ability to produce bioactive compounds, are a rich source of polyketide metabolites.[6] Some important pharmaceutical agents from *Penicillium* sp., such as griseofulvin[7] and compactin (mevastatin),[8] are derived from the polyketide biosynthesis pathway.[9] In our ongoing search for bioactive compounds from microbial metabolites,[10] a fungal strain, *Penicillium* sp. I09F 484, was isolated from the rhizosphere soil of the plant *Picea asperata*, collected from Kanas Lake, Xinjiang, China. The crude extract of this strain showed considerable antibacterial activity against a multidrug-resistant enteric bacterium, *Morganella morganii* 07-09, in preliminary screening. Bioassay-guided isolation of the extract resulted in the isolation and characterization of three new polyketide compounds (**1**–**3**), a new quinolone alkaloid (**4**), and seven known polyketides, patulin,[11] gentisyl alcohol,[11] 2-methylhydroquinone,[12] epoformin,[13] epiepoformin,[13] (4*S*,5*S*)-4,5-dihydroxy-2-methylcyclohex-2-enone,[14] and 3-hydroxybenzyl alcohol.[15] Compound **2** was a polyketide alkaloid with an unusual chromeno[3,2-*c*]pyridine moiety. Compared with other reported MBL inhibitors,[5a] **1** and **2** showed moderate inhibitory activity against NDM-1, and the antibacterial activity of the crude extracts was mainly due to the presence of the known patulin. Herein we report the isolation, structure elucidation, and biological activities of these compounds.

RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous powder. Its molecular formula was established as $C_{14}H_{12}O_7$ by analysis of

Received: January 31, 2013
Published: August 23, 2013

dx.doi.org/10.1021/np4000944 | *J. Nat. Prod.* 2013, 76, 1535–1540



nuclear magnetic resonance (NMR) and HRAPCIMS data. The infrared (IR) bands at 1503, 1578, 1602, and 1700 cm^{-1} suggested the presence of an aromatic ring and conjugated carbonyl groups, which was supported by the UV absorptions at 216 and 295 nm. The ^1H NMR spectrum exhibited signals that could be attributed to a conjugated double bond proton at δ 8.06 (s) and a pair of *meta*-coupled aromatic protons (δ 6.74, brs; δ 6.84, brs), indicative of a 1,3,4,5-tetrasubstituted benzene ring. Other signals in the ^1H NMR spectrum included one singlet methyl (δ 2.13), two geminal-coupled methylene protons (δ 2.80, dd, J = 15.6, 3.0 Hz; δ 2.57, dd, J = 15.6, 9.0 Hz), and one oxymethine (δ 5.03, dd). The ^{13}C NMR and DEPT data revealed one methyl (δ 30.4), one methylene (δ 50.0), one oxygenated sp^3-hybridized methine (δ 62.2), three carboxyl/carbonyl carbons (δ 169.8, 174.1, and 206.6), and eight aromatic/olefinic carbons, three of which were methine carbons. The spectroscopic data were similar to those of chromone derivatives from *Penicillium* species,[16] suggesting that **1** was also a chromone analogue with an aliphatic side chain moiety. This supposition was corroborated by two-dimensional (2D) NMR data analysis (Figures S8 and S9 of the Supporting Information). Cross-peaks in the HMBC spectrum of **1** from H-8 to C-4a, C-6, C-7, and C-8a; from H-6 to C-4a, C-8, and the carboxyl group (C-9); and from H-2 to C-3, C-4, and C-8a indicated that **1** had a 5-carboxyl-7-hydroxychromone nucleus. Additionally, HMBC correlations from H-1′ to C-2′ and C-3′; from H-2′ to C-1′ and C-3′; and from H-4′ to C-2′ and C-3′ suggested the presence of the 1′-hydroxy-3′-butanoyl side chain. The linkage of C-1′ of the side chain to C-3 of the chromone moiety was established by HMBC correlations from H-1′ to C-2, C-3, and C-4 and from H-2′ to C-3. Accordingly, the planar structure of **1** was determined as shown.

To determine the absolute configuration of **1**, attempts were made to prepare α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester derivatives by application of the modified Mosher's method.[17] Unfortunately, the approaches employed did not yield the desired product. More extensive efforts toward this end were hampered by sample limitations. Finally, we decided to use circular dichroism (CD) analysis because of the presence of an α,β-unsaturated carbonyl function adjacent to the asymmetric center in **1**. The electronic CD spectrum (ECD) was experimentally recorded, and the theoretical ECD was calculated using the time-dependent density functional theory (TDDFT) method.[18] The experimental CD spectrum of **1** exhibited a positive Cotton effect (CE) at 215 nm, two negative CEs near 249 and 286 nm, and a positive CE at 319 nm and was in excellent agreement with the calculated ECD spectrum of **1** with the *S* configuration at C-1′ (Figure 1). Therefore, the structure of **1** was established as (1′*S*)-7-hydroxy-3-(1′-hydroxy-3′-butanoyl)chromone-5-carboxylic

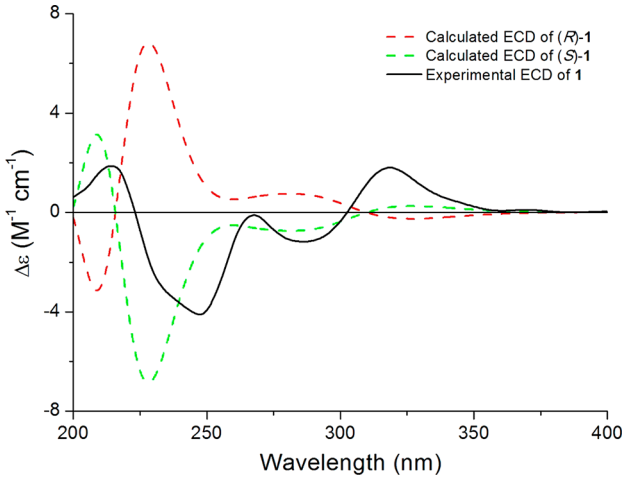


Figure 1. Experimental and TDDFT-calculated ECD spectra of **1**.

acid. Chromone derivatives with a similar aliphatic side chain have been reported,[16,19] but the configuration of the chiral carbon in the side chain had not been determined. On the basis of the configuration assignment of **1** described above, PI-3, previously isolated from *Penicillium italicum*[16] with the same negative optical rotation as **1**, should also possess the 1′*S* configuration.

Compound **2** was isolated as a brown powder with a molecular formula of $C_{15}H_{11}O_6N$ by HRESIMS (m/z 300.0514 $[M - H]^-$) and NMR data. This formula required 11 degrees of unsaturation, two more than compound **1**. The NMR data for **2** were similar to those for **1** (Table 1). The main differences were the absence of signals for the 1′-hydroxy-3′-butanoyl side chain and the presence of signals for an allylic methyl (δ_H 2.61) and a methoxyl group (δ_H 3.78) in the ^1H NMR spectrum of **2**. Additionally, the downfield shifts of three aromatic protons [H-1 (δ_H 9.09), H-4 (δ_H 7.46), and H-6 (δ_H 7.03)] were observed. In total, 15 carbon resonances were observed in the ^{13}C NMR and DEPT spectra of **2**, which were ascribed to one allylic methyl (δ_C 24.9), one methoxyl (δ_C 61.6), three sp^2 methines [one nitrogen-bearing (δ_C 149.1)], and 10 sp^2 quaternary carbons (including one carboxyl at δ_C 167.4 and one conjugated carbonyl at δ_C 173.6). On the basis of the carbon chemical shifts of these resonances, **2** was composed of a carboxyl, a ketone, five C=C groups, and a C=N bond that accounted for 8 of the 11 degrees of unsaturation. This suggested that **2** possessed a fused chromeno-pyridine tricyclic ring structure.[20] This suggestion was reflected in the UV chromophore of **2**, which showed a significant bathochromic shift (absorption maximum at 330 nm) as compared to **1**, and was further confirmed by 2D NMR data analysis (Figures S15−S17 of the Supporting Information). In the HMBC spectrum of **2**, H-6 displayed intense $^2J_{CH}$ or $^3J_{CH}$ correlations to C-5a, C-7, C-8, and C-9a and weak $^4J_{CH}$ correlations to C-9 and the carbonyl (C-10). Furthermore, the methoxy protons were correlated to C-8 (δ 142.7). These correlations, combined with the chemical shifts of these carbons and protons, unequivocally established a 7-hydroxy-8-methoxylchromone-9-carboxylic acid moiety for **2**. The lack of ROESY correlations between the methoxyl and H-6 confirmed that the methoxyl group was substituted at C-8, instead of C-7. In addition, HMBC correlations of H-1/C-3, C-4a, C-10, and C-10a; H-4/C-4a, C-10 and C-10a; and H_3-12/C-3 and C-4 demonstrated that the chromone moiety was fused with a 3-

dx.doi.org/10.1021/np4000944 | J. Nat. Prod. 2013, 76, 1535−1540

Table 1. NMR Spectroscopic Data for Compounds 1−4 in DMSO-d_6[a]

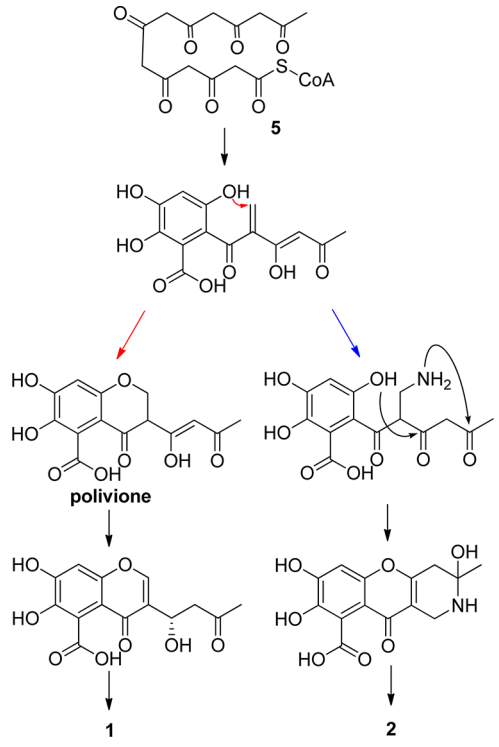
	1		2		3		4	
no.	δ_C	δ_H mult. [J (Hz)]	δ_C	δ_H mult. [J (Hz)]	δ_C	δ_H mult. [J (Hz)]	δ_C	δ_H mult. [J (Hz)]
1			149.1	9.09 s	64.6	3.64 ddd (10.2, 6.0, 4.8)		
2	152.5	8.06 s			56.8	2.94 brd (4.8)	161.2	
3	126.1		164.2		55.7	3.07 dd (2.4, 2.4)	119.7	6.45 s
4	174.1		111.1	7.46 s	66.3	3.76 ddd (5.4, 2.4, 2.4)	146.1	
4a	112.0		160.9				116.1	
5	137.4				26.3	1.38 m	126.0	7.77 d (7.8)
5a			158.4					
6	113.1	6.74 brs	104.7	7.03 s	32.2	1.28 ddd (12.6, 6.0, 2.4)	122.0	7.18 t (7.8)
						1.23 ddd (12.6, 12.6, 10.2)		
7	162.2		153.6		17.0	0.82 d (6.6)	130.9	7.53 t (7.8)
8	102.3	6.84 brs	142.7				115.6	7.35 d (7.8)
8a	157.7						139.2	
9	169.8		129.5				165.6	
9a			111.2					
10			173.6					
10a			115.2					
11			167.4					
12			24.9	2.61 s				
OMe			61.6	3.78 s				
1′	62.2	5.03 dd (9.0, 3.0)					172.4[b]	
2′	50.0	2.80 dd (15.6, 3.0)					49.2	4.72 dd (7.8, 6.0)
		2.57 dd (15.6, 9.0)						
3′	206.6						36.2	2.83 dd (16.8, 6.0)
								2.66 dd (16.8, 7.8)
4′	30.4	2.13 s					171.9[b]	

[a]The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b]Exchangeable. Abbreviation: mult., multiple.

methylpyridine ring via C-4a and C-10a to form a chromeno-[3,2-*c*]pyridine ring. Consequently, **2** was established as 7-hydroxy-8-methoxy-3-methyl-10-oxo-10*H*-chromeno[3,2-*c*]-pyridine-9-carboxylic acid. Compound **2**, and SB236049 isolated from the fungus *Chaetomium funicola*,[20] represent the only examples of alkaloids possessing the unique chromeno-[3,2-*c*]pyridine nucleus. Compounds **1** and **2** share the same carbon skeleton with the well-known polyketide metabolites fulvic acid,[21] polivione,[22] and lapidosin[23] isolated from *Penicillium* species. The close resemblance between these compounds, in the carbon skeleton and oxygenation pattern, indicated that they are probably generated by a common biosynthesis strategy from a heptaketide precursor (**5**) as suggested by Staunton et al (Scheme 1).[24]

Compound **3** had the molecular formula $C_7H_{12}O_3$, as indicated by HRESIMS. The NMR data (Table 1 and Experimental Section) in DMSO-d_6 and a pyridine-d_5/D_2O mixture (5:1) were very similar to those of ampelomin B,[25] suggesting that **3** is a stereoisomer of the reported compound with the same planar structure. This was confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC data analyses (Figures S24−S26 of the Supporting Information). However, detailed ^{1}H NMR coupling constant analysis and the NOESY experiment revealed an equatorial hydroxyl group at C-1 in **3** rather than the axial hydroxyl found in ampelomin B. The large coupling constant between H-1 and H-6ax ($^3J_{H\text{-}1/H\text{-}6ax}$ = 10.2 and 10.8 Hz in DMSO-d_6 and a 5:1 pyridine-d_5/D_2O mixture, respectively) and between H-5 and H-6ax ($^3J_{H\text{-}5/H\text{-}6ax}$ = 12.6 and 11.4 Hz in DMSO-d_6 and a 5:1 pyridine-d_5/D_2O mixture, respectively) revealed their axial relationships, thus also indicating an equatorial orientation for OH-1. This was further verified by the obvious correlation of H-1 and H-5 and the lack of correlation between H-5 and OH-1 in the NOESY spectrum of **3** (Figure S27 of the Supporting Information). Thus, compound **3** was determined to be an epimer of ampelomin B with the opposite configuration at C-1, and its relative

Scheme 1. Plausible Biosynthetic Pathways for 1 and 2



dx.doi.org/10.1021/np4000944 | J. Nat. Prod. 2013, 76, 1535−1540

structure was established as (1*R**,2*S**,3*R**,4*S**,5*S**)-2,3-epoxy-5-methylcyclohexane-1,4-diol. Polyoxygenated methyl cyclohexanoids of fungal origin, such as theobroxide,[26] terremutin, epoformin, and gentisyl alcohol are biosynthetically derived from a polyketide pathway via 6-methylsalicylic acid (6-MSA).[27] Besides ampelomin B, the same *trans* relative configuration between OH-4 and the 2,3-epoxy group in **3** is also seen in isoepiepoformin.[28] Therefore, on the basis of the biosynthesis consideration, the absolute configuration of **3** was tentatively assigned as shown.

The molecular formula of compound **4** was established as $C_{14}H_{12}O_6N_2$ with 10 degrees of unsaturation by HRAPCIMS. Compound **4** showed UV absorptions at 229 and 334 nm and IR absorptions for OH/NH (3277 cm^{-1}) and the carbonyl (1652 cm^{-1}) functionalities. The NMR data indicated the presence of an *ortho*-substituted benzene ring, a trisubstituted double bond, an sp^3 methine, a methylene, and four carbonyl groups. In addition, resonances for two amide NH protons (δ_H 9.03, NH-2′; δ 11.93, NH-8a) and two additional exchangeable protons (δ_H 12.85) were observed in the ^1H NMR spectrum. These data, considering the unsaturation of **4**, indicated that this compound had an additional ring. The presence of characteristic signals for an α-amino methine (δ_C 49.2 and δ_H 4.72) and carbonyl carbons (δ_C 161.2−172.4) was indicative of the incorporation of an amino acid residue in **4**. Analysis of the $^1H-^1H$ COSY data (Figure S33 of the Supporting Information) led to the identification of the -NHCHCH$_2$- unit of an amino residue and confirmed the *ortho*-substituted aromatic ring. HMBC correlations of H-3/C-2, C-4, and C-4a established the connection between C-4 and C-4a. Although cross-peaks of NH-8a/C-2, C-3, C-4a, and C-8a were not observed in the HMBC spectrum, the connection of C-2 and C-8a through an amide bond was suggested by the chemical shift of C-8a (δ_C 139.2) and the long-range *W* coupling between H-3 and NH-8a observed in the $^1H-^1H$ COSY spectrum. HMBC correlations of H-3/C-9 (δ_C 165.6) and H-5/C-4 indicated that an amide carbon (C-9) was attached at C-4 and established the basic skeleton of 4-carboxyl-2(1*H*)-quinolinone for **4**. HMBC correlations of H-2′/C-1′, C-3′, and C-4′ revealed the presence of an aspartic acid (Asp) residue. Finally, HMBC correlations of H-2′/C-9 and NH-2′/C-9 suggested that C-2′ of the aspartic acid unit was connected to C-9 of the 2(1*H*)-quinolinone ring moiety through an amide bond. The linkage between these units was also confirmed by the negative-mode ESIMS/MS fragment ion at *m/z* 259 $[M - CO_2 - H]^-$ and *m/z* 187 $[M - C_4H_4O_4 - H]^-$. The absolute configuration of the Asp residue was determined after hydrolysis of **4** and derivatization HPLC analysis by using Marfey's method.[29] The 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) derivative of the hydrolysate of **4** gave the same HPLC retention time as that of authentic L-Asp. Therefore, the Asp residue in **4** was assigned the L configuration. On the basis of the foregoing evidence, the structure of **4** was established as (*S*)-2-(2-oxo-1,2-dihydroquinoline-4-carboxamido)succinic acid.

Chromone derivatives isolated from *Chaetomium funicola* were reported to display potent broad-spectrum inhibitory activities against metallo-β-lactamases (K_i values ranging from 3.4 to 88 μM) and good antibacterial potentiation with meropenem.[20] The structures of **1** and **2** possess the same chromone-5-carboxylic acid skeleton as the reported compounds; therefore, we assessed their inhibitory activity against NDM-1. In the evaluation, **1** and **2** exhibited inhibitory activity against NDM-1 with 50% inhibitory concentration (IC_{50}) values of 94.9 and 87.9 μM, respectively (the positive controls L-captopril and EDTA gave IC_{50} values of 107.6 and <0.1 μM, respectively). To further evaluate the antibacterial synergy of **1** and **2** with meropenem, the MICs of meropenem, alone and in combination with **1** or **2**, against the NDM-1-producing strain *K. pneumoniae* ATCC BAA-2146 were measured. **1** and **2** had no inherent antibacterial activity at 256 μg/mL, and the MIC of meropenem (128 μg/mL) for this strain was not reduced even in combination with **1** or **2** at a concentration of 128 μg/mL. A variety of structurally disparate compounds have been examined as MBL inhibitors; however, there are few compounds with efficacy at the submicromolar level.[5a] Among these compounds, L-captopril, clinically used as an antihypertensive agent, and its D diastereomer are the most promising candidates, which displayed potent and broad-spectrum MBLs, including NDM-1 inhibitory activity (the reported IC_{50} value against NDM-1 was 202 μM for L-captopril and 8 μM for D-captopril).[5c−e] The data indicated the potency of **1** and **2** was close to that of L-captopril.

In addition, compounds **1**−**4** were also assessed for *in vitro* antimicrobial, antiviral, and cytotoxic activity but found to be inactive at a concentration of 10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 343 polarimeter. UV spectra were obtained on an Agilent 8453 UV−vis spectrometer. CD spectra were recorded on a JASCO-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission). One-dimensional (1D) and 2D NMR spectra were obtained at 500 or 600 MHz for ^1H and 125 or 150 MHz for ^{13}C, respectively, on Varian INOVA 500 or 600 MHz spectrometers in DMSO-d_6 or a 5:1 pyridine-d_5/D_2O mixture with solvent peaks used as a reference. ESIMS data were measured with a Thermo LTQ XL linear ion trap mass spectrometer. HRESIMS and HRAPCIMS data were measured using a Thermo LTQ Orbitrap XL mass spectrometer. Column chromatography (CC) was performed with Diaion HP-20 macroporous resin (Mitsubishi Chemical Inc.) and Toyopearl HW-40F (Tosoh Co.). Flash chromatography was performed on an Ez Purifier (Suzhou Lisure Science Co., Ltd.). HPLC separation was performed on a Shimadzu HPLC apparatus consisting of an LC-20AD pump and an SPD-M20A diode array detector with a Capcell-Pak C_{18} AQ column (10 mm × 250 mm, 5 μm, Shiseido Co.). TLC was conducted with glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% aqueous EtOH followed by heating.

Microorganism and Fermentation. Strain I09F 484 was isolated from the rhizosphere soil of the plant *Picea asperata*, collected from Kanas Lake, Buerjin County, Altay Prefecture, Xinjiang Uygur Autonomous Region, China. Using its morphological characteristics, the fungus could be identified as a member of the genus *Penicillium*. The 18S rRNA sequence (GenBank entry JX134614) was strongly homologous to *Penicillium freii* IBT 3464 (GenBank entry AJ005446, 1686 bp of 1691, 99%), while its internal transcribed spacer regions 1 and 2 and 5.8S rRNA genes (GenBank entry JX134613) were identical to those of *Penicillium griseofulvum* (GenBank entry DQ339549). The strain was deposited in the China Medicinal Microbiological Culture Collection Center (Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, entry I09F00484).

Strain I09F 484 was cultivated on a rotary shaker (180 rpm) for 96 h at 28 °C in 500 mL × 20 Erlenmeyer flasks each containing 150 mL of sterile medium consisting of 1% glycerol, 2.0% glucose, 1% sucrose, 0.2% soybean meal, 1% peptone, 0.25% polyethylene glycol 6000, 0.03% KH_2PO_4, 0.3% $NaNO_3$, and 0.3% $(NH_4)_2SO_4$ in deionized water (pH 6.0 before sterilization).

Isolation. The harvested culture broth (3 L) was filtered, and the resulting filtrate was subjected to a Diaion HP-20 macroporous adsorbent resin column (0.7 L). A successive elution of the column with H_2O and 30, 60, 80, and 100% MeOH yielded five corresponding fractions (F_1−F_5, respectively) after the solvents had been removed. The fraction eluted by 30% MeOH (F_2, 6.6 g) was further separated into eight subfractions ($F_{2\text{-}1}$−$F_{2\text{-}8}$) by reversed-phase (RP) C_{18} flash chromatography using step-gradient elution with an increasing MeOH concentration (5 to 60%) in H_2O. $F_{2\text{-}4}$ (0.7 g) was subjected to a Toyopearl HW-40F CC column (3 cm × 100 cm), eluting with 15% MeOH, to give five mixtures ($F_{2\text{-}4\text{-}1}$−$F_{2\text{-}4\text{-}5}$). $F_{2\text{-}4\text{-}5}$ was purified by semipreparative RP C_{18} HPLC using a mobile phase of 30% MeOH in a 20 mM formic acid aqueous solution to yield **1** (3.2 mg) and **4** (5.9 mg). $F_{2\text{-}5}$ (0.35 g) was separated by a Toyopearl HW-40F CC column (3 cm × 100 cm) using 20% MeOH as an eluent, and the resulting subfraction was further purified by RP C_{18} HPLC with 13% MeOH in H_2O to afford **3** (3.5 mg). Compound **2** (19.4 mg) was precipitated from the MeOH solution of fraction $F_{2\text{-}8}$ and also purified by RP C_{18} HPLC (30% MeOH in a 0.1% trifluoroacetic acid aqueous solution).

*(1′S)-7-Hydroxy-3-(1′-hydroxy-3′-butanoyl)chromone-5-carboxylic acid (**1**):* amorphous solid; $[\alpha]^{20}_{D}$ −54.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.03), 295 nm (3.74); CD (MeOH) 215 ($\Delta\varepsilon$ +1.40), 249 ($\Delta\varepsilon$ −3.08), 268 ($\Delta\varepsilon$ −0.08), 286 ($\Delta\varepsilon$ −0.88), 319 nm ($\Delta\varepsilon$ +1.35); IR ν_{max} 3348, 3112, 2924, 1700, 1602, 1578, 1503, 1432, 1278, 1171, 1088, 1056, 1011, 872, 768, 614 cm^{-1}; for ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; HRAPCIMS *m*/*z* 293.0688 $[M + H]^+$ (calcd for $C_{14}H_{13}O_7$, 293.0656).

*7-Hydroxy-8-methoxy-3-methyl-10-oxo-10H-chromeno[3,2-c]-pyridine-9-carboxylic acid (**2**):* brown amorphous powder; UV (MeOH) λ_{max} (log ε) 201 (4.38), 224 (4.36), 241 (4.37), 299 (3.97), 330 nm (3.85); IR ν_{max} 3056, 2932, 1667, 1641, 1605, 1569, 1543, 1492, 1401, 1211, 1192, 1042, 864 cm^{-1}; for ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; ESIMS *m*/*z* 300 $[M - H]^-$ and 302 $[M + H]^+$; HRESIMS *m*/*z* 302.0661 $[M + H]^+$ (calcd for $C_{15}H_{12}O_6N$, 302.0659) and 300.0514 $[M - H]^-$ (calcd for $C_{15}H_{10}O_6N$, 300.0503).

*(1R,2S,3R,4S,5S)-2,3-Epoxy-5-methylcyclohexane-1,4-diol (**3**):* colorless oil; $[\alpha]^{20}_{D}$ +28.3 (*c* 0.11, $CHCl_3$); IR ν_{max} 3360, 2924, 1794, 1722, 1676, 1459, 1263, 1041, 989, 862, 820, 726 cm^{-1}; for ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; ^1H NMR (5:1 C_5D_5N/D_2O, 600 MHz) δ 4.22 (1H, dd, *J* = 10.8, 6.6 Hz, H-1), 3.53 (1H, brd, *J* = 3.6 Hz, H-2), 3.54 (1H, dd, *J* = 3.6, 2.4 Hz, H-3), 4.19 (1H, dd, *J* = 2.4, 2.4 Hz, H-4), 1.62 (1H, m, H-5), 1.67 (1H, ddd, *J* = 13.2, 6.6, 1.2 Hz, H-6eq), 1.91 (1H, ddd, *J* = 13.2, 11.4, 10.8 Hz, H-6ax), 0.99 (3H, d, *J* = 7.2 Hz, H_3-7); HRESIMS *m*/*z* 145.0855 $[M + H]^+$ (calcd for $C_7H_{13}O_3$, 145.0859) and 167.0675 $[M + Na]^+$ (calcd for $C_7H_{12}O_3Na$, 167.0679).

*(S)-2-(2-Oxo-1,2-dihydroquinoline-4-carboxamido)succinic acid (**4**):* amorphous powder; $[\alpha]^{20}_{D}$ +84.1 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.88), 334 nm (3.10); CD (MeOH) 210 ($\Delta\varepsilon$ +0.33), 221 ($\Delta\varepsilon$ −0.26), 248 nm ($\Delta\varepsilon$ +1.22); IR ν_{max} 3277, 2927, 1652, 1429, 1203, 1139, 879, 758 cm^{-1}; for ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; ESIMS *m*/*z* 303 $[M - H]^-$; HRAPCIMS *m*/*z* 305.0797 $[M + H]^+$ (calcd for $C_{14}H_{13}O_6N_2$, 305.0768) and 303.0607 $[M - H]^-$ (calcd for $C_{14}H_{11}O_6N_2$, 303.0612).

ECD Calculation of 1. Conformational analysis of the *R* isomer of **1** [(*R*)-**1**] was conducted via Monte Carlo searching with the MMFF94 molecular mechanics force field using MOE.[30] Three lowest-energy conformers for (*R*)-**1** (Figure S2 of the Supporting Information), whose relative energies were within 4 kcal/mol of each other, were considered for further DFT calculations. Subsequently, the conformers were reoptimized using DFT at the B3LYP/6-31G(d) level in the gas phase with GAUSSIAN 03.[31] The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability and to provide their relative thermal free energy (ΔG), which are used to assess their equilibrium populations. The energies, oscillator strengths, and rotational strengths of the 20 weakest electronic excitations of the conformers were calculated using the TDDFT methodology at the B3LYP/6-31G(d) level in the gas phase, and the ECD spectra were then simulated by using a Gaussian function with a bandwidth σ of 0.30 eV. To obtain the final spectra of (*R*)-**1**, the simulated spectra of the corresponding lowest-energy conformations were averaged according to the Boltzmann weighting of each conformer. The corresponding theoretical ECD spectrum of (*S*)-**1** was depicted by inverting that of (*R*)-**1**.

Acid Hydrolysis and Marfey's Analysis of 4. Approximately 1 mg of **4** was hydrolyzed with 1 mL of 6 M HCl at 110 °C for 16 h, and then the hydrolysate was evaporated to dryness and redissolved in H_2O (120 μL). To one portion (60 μL) were added 100 μL of a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone and 20 μL of 1 M $NaHCO_3$. The reaction mixture was heated at 40 °C for 1 h, cooled to room temperature, neutralized with 2 M HCl (10 μL), and diluted with MeCN (100 μL). Similarly, the standard D- and L-Asp were derivatized separately. Marfey's derivatives of the hydrolysate and standards were analyzed by HPLC using the following conditions: column, Cosmosil MG II C_{18} column (5 μm, 4.6 mm × 150 mm); flow rate, 1.0 mL/min; solvent A, 0.1 M ammonium acetate in an aqueous solution adjusted to pH 3.0 by addition of trifluoroacetic acid; solvent B, MeCN; linear gradient elution from 15 to 45% B in A over 45 min; UV detection at 340 nm; column temperature, 40 °C. The retention times for the FDAA derivatives of the hydrolysate of **4** and standard D- and L-Asp were 11.5, 13.9, and 11.4 min, respectively (Figure S36 of the Supporting Information).

NDM-1 Inhibitory Assay. The NDM-1 enzyme was expressed in *E. coli* BL21 and purified by nickel-NTA chromatography as described previously.[5d] The assay was performed at 30 °C in 10 mM HEPES buffer (pH 7.5).[3a] Compounds was prepared as 40 mM stocks in DMSO and diluted with the buffer mentioned above. The compound dilution and 0.0025 μM enzyme were incubated at their final concentration for 15 min at 30 °C before the reaction was initiated by addition of 100 μM meropenem substrate. The change in absorption at 300 nm caused by the opening of the meropenem β-lactam ring was followed at 30 °C on a microplate reader (Enspire 2300, Perkin-Elmer) with 96-well plates. The first 5 min of the reaction was used to measure initial rates. EDTA and L-captopril were used as the positive controls, while tazobactam was used as the negative control. The percent inhibition of each compound concentration was calculated on the basis of the control enzyme activity. All tests were run in triplicate and averaged. The calculation of the IC_{50} values was performed using GraphPad Prism version 5.

Antimicrobial Assay and Antibacterial Synergy Assay. Antimicrobial assays were performed as described previously.[10a] In the antibacterial synergy assay, the minimal inhibitory concentration (MIC) of meropenem against *K. pneumoniae* ATCC BAA-2146 was determined in combination with 128, 64, and 32 μg/mL **1**, **2**, or EDTA.

Antiviral Assay. Antiviral assays against influenza virus A strains A/FM/1/47 (H1N1) and A/Hanfang/359/95 (H3N2) and coxsackie virus B3 were performed as described previously.[32]

Cytotoxic Assays. See ref 10a.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS, IR, 1D, and 2D NMR spectra of compounds **1**−**4** and ECD calculation details of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 86-10-63165277. Fax: 86-10-63020226. E-mail: xiaocl318@163.com or ganml@hotmail.com.

Author Contributions

⊥M. Gan and Y. Liu contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the High Performance Computing Center of Peking Union Medical College for computational facilities. Financial support from the Natural Sciences Foundation of China (NSFC, Grants 81273414 and 81001386), the Beijing Natural Science Foundation (Grant 5122032), the National Science and Technology Project of China (Grant 2014ZX09507009-008), and the National Infrastructure of Microbial Resources (Grant NIMR-2011-3) is gratefully acknowledged. Y. Liu was supported by the Visiting Scholarship Fund from Shandong Provincial Education Department.

■ REFERENCES

(1) Van Duin, D.; Kaye, K. S.; Neuner, E. A.; Bonomo, R. A. *Diagn. Microbiol. Infect. Dis.* **2013**, *75*, 115−120.
(2) (a) Livermore, D. M.; Woodford, N. *Trends Microbiol.* **2006**, *14*, 413−420. (b) Hsueh, P.-R. *J. Formosan Med. Assoc.* **2010**, *109*, 685−687.
(3) (a) Yong, D.; Toleman, M. A.; Giske, C. G.; Cho, H. S.; Sundman, K.; Lee, K.; Walsh, T. R. *Antimicrob. Agents Chemother.* **2009**, *53*, 5046−5054. (b) Nordmann, P.; Poirel, L.; Walsh, T. R.; Livermore, D. M. *Trends Microbiol.* **2011**, *19*, 588−595.
(4) Kumarasamy, K. K.; Toleman, M. A.; Walsh, T. R.; Bagaria, J.; Butt, F.; Balakrishnan, R.; Chaudhary, U.; Doumith, M.; Giske, C. G.; Irfan, S.; Krishnan, P.; Kumar, A. V.; Maharjan, S.; Mushtaq, S.; Noorie, T.; Paterson, D. L.; Pearson, A.; Perry, C.; Pike, R.; Rao, B.; Ray, U.; Sarma, J. B.; Sharma, M.; Sheridan, E.; Thirunarayan, M. A.; Turton, J.; Upadhyay, S.; Warner, M.; Welfare, W.; Livermore, D. M.; Woodford, N. *Lancet Infect. Dis.* **2010**, *10*, 597−602.
(5) (a) Walsh, T. R.; Toleman, M. A.; Poirel, L.; Nordmann, P. *Clin. Microbiol. Rev.* **2005**, *18*, 306−325. (b) Bebrone, C. *Biochem. Pharmacol.* **2007**, *74*, 1686−1701. (c) Guo, Y.; Wang, J.; Niu, G.; Shui, W.; Sun, Y.; Zhou, H.; Zhang, Y.; Yang, C.; Lou, Z.; Rao, Z. *Protein Cell* **2011**, *2*, 384−394. (d) Thomas, P. W.; Zheng, M.; Wu, S.; Guo, H.; Liu, D.; Xu, D.; Fast, W. *Biochemistry* **2011**, *50*, 10102−10113. (e) King, D. T.; Worrall, L. J.; Gruninger, R.; Strynadka, N. C. J. *J. Am. Chem. Soc.* **2012**, *134*, 11362−11365.
(6) (a) Bérdy, J. *J. Antibiot.* **2005**, *58*, 1−26. (b) Turner, W. B. *Fungal Metabolites*; Academic Press: London, 1971; p 74.
(7) Harris, C. M.; Roberson, J. S.; Harris, T. M. *J. Am. Chem. Soc.* **1976**, *98*, 5380−5386.
(8) Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkamp, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165−1170.
(9) Cox, R. J.; Simpson, T. J. In *Comprehensive Natural Products II*; Lew, M., Liu, H.-W., Eds.; Elsevier: Oxford, U.K., 2010; pp 347−383.
(10) (a) Gan, M.; Zheng, X.; Gan, L.; Guan, Y.; Hao, X.; Liu, Y.; Si, S.; Zhang, Y.; Yu, L.; Xiao, C. *J. Nat. Prod.* **2011**, *74*, 1142−1147. (b) Gan, M.; Zheng, X.; Liu, Y.; Guan, Y.; Xiao, C. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6151−6154. (c) Gan, M.; Guan, Y.; Zheng, X.; Yang, Y.; Hao, X.; Liu, Y.; Yu, L.; Xiao, C. *J. Antibiot.* **2012**, *65*, 513−516.
(11) Smetanina, O. F.; Kalinovskii, A. I.; Khudyakov, Y. V.; Moiseenko, O. P.; Pivkin, M. V.; Menzorova, N. I.; Sibirtsev, Y. T.; Kuznetsova, T. A. *Chem. Nat. Compd.* **2005**, *41*, 243−244.
(12) Pouchert, C. J. *The Aldrich library of ^{13}C and ^{1}H FT NMR spectra*; Aldrich: Milwaukee, WI, 1993; Vol. 2, p 305A.
(13) (a) Nagasawa, H.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1978**, *42*, 1303−1304. (b) Toribio, G.; Marjanet, G.; Alibés, R.; De March, P.; Font, J.; Bayón, P.; Figueredo, M. *Eur. J. Org. Chem.* **2011**, *2011*, 1534−1543.
(14) Takei, R.; Takahashi, K.; Matsuura, H.; Nabeta, K. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 2069−2073.
(15) Alfaro, C.; Urios, A.; González, M. C.; Moya, P.; Blanco, M. *Mutat. Res.* **2003**, *539*, 187−194.
(16) Arai, K.; Miyajima, H.; Mushiroda, T.; Yamamoto, Y. *Chem. Pharm. Bull.* **1989**, *37*, 3229−3235.
(17) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451−2458.
(18) (a) Diedrich, C.; Grimme, S. *J. Phys. Chem. A* **2003**, *107*, 2524−2539. (b) Crawford, T. D.; Tam, M. C.; Abrams, M. L. *J. Phys. Chem. A* **2007**, *111*, 12057−12068.
(19) (a) Elson, S. W.; Perez Rodrigo, P.; Diez Monedero, E.; Hueso Rodriguez, J. A.; Donoso Carrero, R.; Valmaseda Jadu, M. Spainish Patent ES 2143916, 2000. (b) Conrad, J.; Förster-Fromme, B.; Constantin, M.-A.; Ondrus, V.; Mika, S.; Mert-Balci, F.; Klaiber, I.; Pfannstiel, J.; Möller, W.; Rösner, H.; Förster-Fromme, K.; Beifuss, U. *J. Nat. Prod.* **2009**, *72*, 835−840.
(20) Payne, D. J.; Hueso-Rodríguez, J. A.; Boyd, H.; Concha, N. O.; Janson, C. A.; Gilpin, M.; Bateson, J. H.; Cheever, C.; Niconovich, N. L.; Pearson, S.; Rittenhouse, S.; Tew, D.; Díez, E.; Pérez, P.; De la Fuente, J.; Rees, M.; Rivera-Sagredo, A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1880−1886.
(21) Dean, F. M.; Eade, R. A.; Moubasher, R. A.; Robertson, A. *Nature* **1957**, *179*, 366−366.
(22) Demetriadou, A. K.; Laue, E. D.; Leeper, F. J.; Staunton, J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 763−768.
(23) Turner, W. B. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1621−1621.
(24) (a) Demetriadou, A. K.; Laue, E. D.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1985**, 764−766. (b) Demetriadou, A. K.; Laue, E. D.; Staunton, J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 769−772.
(25) Zhang, H.; Xue, J.; Wu, P.; Xu, L.; Xie, H.; Wei, X. *J. Nat. Prod.* **2009**, *72*, 265−269.
(26) Li, P.; Takei, R.; Takahashi, K.; Nabeta, K. *Phytochemistry* **2007**, *68*, 819−823.
(27) (a) Kiriyama, N.; Higuchi, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1977**, *25*, 1265−1272. (b) Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 380−416.
(28) Jarvis, B. B.; Yatawara, C. S. *J. Org. Chem.* **1986**, *51*, 2906−2910.
(29) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(30) *Molecular Operating Environment (MOE 2009.10)*; Chemical Computing Group Inc.: Montreal, 2009.
(31) *Gaussian 03*, revision A.1; Gaussian, Inc.: Wallingford, CT, 2003. A full list of authors can be found in the Supporting Information.
(32) (a) Hao, L.-H.; Li, Y.-P.; He, W.-Y.; Wang, H.-Q.; Shan, G.-Z.; Jiang, J.-D.; Li, Y.-H.; Li, Z.-R. *Eur. J. Med. Chem.* **2012**, *55*, 117−124. (b) Wang, Y.-X.; Li, Y.-H.; Li, Y.-H.; Gao, R.-M.; Wang, H.-Q.; Liu, Y.-X.; Gao, L.-M.; Lu, Q.-N.; Jiang, J.-D.; Song, D.-Q. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5787−5790.