Tetrahedron Letters 55 (2014) 6286–6291

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Amicoumacins from the marine-derived bacterium *Bacillus* sp. with the inhibition of NO production

Jian Bai [a], Dong Liu [a], Siwang Yu [a], Peter Proksch [b], Wenhan Lin [a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, PR China
[b] Institute für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb.26.23, 40225 Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 30 July 2014
Revised 18 September 2014
Accepted 22 September 2014
Available online 28 September 2014

Keywords:
Bacillus sp.
Isocoumarin derivatives
Bacillcoumacins
Inhibition of NO production

ABSTRACT

Chemical examination of the fermentation broth of the marine-derived bacterium *Bacillus* sp. resulted in the isolation of seven new amicoumacin-type isocoumarin derivatives, namely bacillcoumacins A–G (**1**–**7**), together with four known analogues. Their structures were elucidated on the basis of extensive spectroscopic analysis, while the absolute configurations of the new compounds were determined by CD, Mosher's method, and chemical conversion. Compounds **7** and **9** showed inhibitory effects against the NO production induced by lipopolysaccharide (LPS) in mouse macrophage RAW 264.7 cells.

Marine-derived microorganisms are the prolific sources of structurally diverse and biologically active secondary metabolites, which are considered as a promising source for the discovery of drug-related lead compounds.[1,2] Under marine stress environment (cold, lightless, and high pressure), microorganisms probably produce the structurally unique secondary metabolites to play the role of chemical defense for survival.[3–6] Among the metabolites derived from marine microorganisms, amicoumacin-based isocoumarins[7] are a small group of structurally unusual PKS-NRPS derived natural products characterized by an isopentyl unit bonded to a dihydroisocoumarin nucleus, while the structure variation was mainly attributed to an amide side chain.[7–11] These compounds have been reported to exhibit a range of bioactivities, including antibacterial,[11–16] cytotoxic,[17] antiulcer against stress-induced ulcers,[12,13] growth inhibition against barnyard millet,[10] as well as anti-inflammatory effects. Most amicoumacins were isolated from marine-derived bacterial genus *Bacillus*.[18] In the course of our investigation of bioactive and structurally diverse natural products from marine-derived microorganisms, a chemical assay guided selection from our microbial library informed that a *Bacillus* sp. strain from the Pacific sediments presented the HPLC–ESIMS fingerprints with rich metabolites. Chromatographic separation of the fermentation broth resulted in the isolation of eleven amicoumacin derivatives (**1**–**11**), including seven new analogues namely bacillcoumacins A–G (**1**–**7**). Four known analogues were identical to AI77-H (**8**),[19] AI-77-F (**9**),[20] AI-77-C (**10**),[20] and AI-77-D (**11**),[20] on the basis of the spectroscopic analyses and comparison of their spectroscopic data with those in literature.

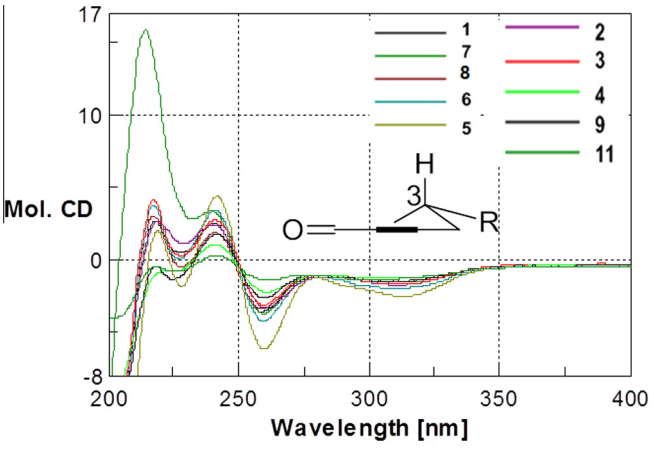
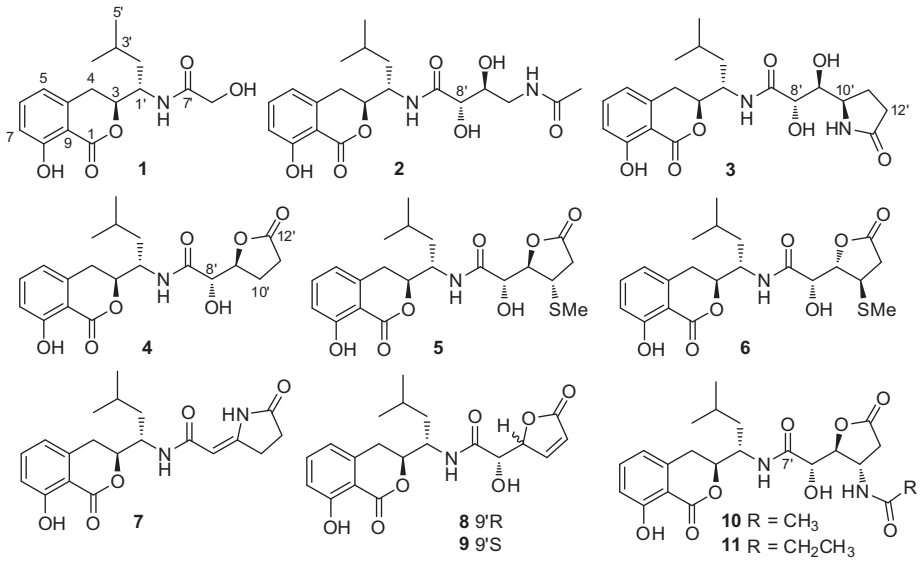


Figure 1. CD spectra of **1**–**9** and **11**.

* Corresponding author. Tel./fax: +86 10 82806188.
E-mail address: whlin@bjmu.edu.cn (W. Lin).

http://dx.doi.org/10.1016/j.tetlet.2014.09.100
0040-4039/



Bacillcoumacin A (**1**) was isolated as a colorless amorphous solid, and its molecular formula was determined as $C_{16}H_{21}NO_5$ on the basis of the HRESIMS (*m*/*z* 308.1497 $[M+H]^+$) and NMR data, requiring seven degrees of unsaturation. The IR absorptions at 3292, 1672, 1620, and 1462 cm^{-1} suggested the presence of hydroxy, carbonyl, and aromatic functionalities, while the UV absorption bands at 204, 246, and 313 nm were characteristic of amicoumacins.[7] The ^1H NMR and COSY spectra showed an aromatic ABC spin system at δ_H 6.84 (1H, d, *J* = 7.3 Hz, H-5), 7.50 (1H, dd, *J* = 7.3, 8.4 Hz, H-6), and 6.86 (1H, d, *J* = 8.4 Hz, H-7) for a 1,2,3-trisubstituted aromatic ring, as well as two methyl doublets and a number of alkyl protons. Analyses of the COSY, HMQC, and HMBC spectra revealed **1** possessing a dihydroisocoumarin nucleus, in which C-8 (δ_C 161.3) was positioned by a hydroxy group on the basis of a D_2O exchangeable proton at δ_H 10.83 (1H, s, 8-OH) correlated to C-7 (δ_C 115.8), C-8 and C-9 (δ_C 108.8) in the HMBC spectrum. The COSY couplings extended a spin system from H_3-4′/H_3-5′ to the amide NH (δ_H 7.47) and H_2-4 (δ_H 2.94), establishing an isopentyl unit linked to C-3. Thus, the partial structure of an amicoumacin was established. In addition, the COSY relationship between H_2-8′ (δ_H 3.86) and OH (δ_H 5.44) and the HMBC correlation between H_2-8′ and the carbonyl carbon C-7′ (δ_C 172.5) led to assign a hydroxyacetyl unit. This unit was positioned at C-1′ through an amide bond according to the COSY relationship between H-1′ (δ_H 4.21) and NH (δ_H 7.47, d, *J* = 9.5 Hz) in addition to the HMBC interactions from C-7′ to H-1′ and NH. Based on the helicity rule of the chiral benzoic ester chromophore,[21] the negative sign of Cotton effect of **1** at 259 nm for the n–π^* transition of the carbonyl group reflected the half chair conformation of the heterocyclic ring (Fig. 1), in which the side chain at C-3 is oriented equatorially.[22] Thus, C-3 was determined as *S* configuration. Acidic hydrolysis of **1** yielded a chromophoric moiety **1a** (Fig. 2), whose NMR data as well as the sign and the magnitude of specific rotation ($[\alpha]_D^{25}$ −52.0) were similar to those of the hydrolyzed product of AI-77-C (**10**) ($[\alpha]_D^{25}$ −43.0).[20] Thus, the absolute configuration of the stereogenic center C-1′ was the same as that of AI-77-C, indicating 1′*S* configuration.

The molecular formula of bacillcoumacin B (**2**) was established as $C_{20}H_{28}N_2O_7$ by the HRESIMS (*m*/*z* 409.1954 $[M+H]^+$) and NMR data. Comparison of the NMR spectroscopic data (Tables 1 and 2) revealed both **2** and **1** sharing the partial structure of dihydroisocoumarin nucleus. The difference was attributed to the amide substructure, of which six carbon resonances including two carbonyl carbons, a methyl carbon, two oxymethines, and a methylene were observed in the DEPT spectrum. The COSY correlations from H-9′ (δ_H 3.69) to H-8′ (δ_H 3.89, dd, *J* = 4.9, 5.8 Hz) and H_2-10′ (δ_H 3.08, 3.22) and between H-8′/OH-8′ (δ_H 5.58, d, *J* = 5.8 Hz), H-9′/OH-9′ (δ_H 4.95, d, *J* = 5.0 Hz), and H_2-10′/NH (δ_H 7.79, t, *J* = 5.5 Hz), in association with the HMBC interactions from H_2-10′ and NH to an acetyl carbon at δ_C 170.3 and from H-8′ and H-9′ to the second carbonyl carbon at δ_C 172.5 (C-7′), established an 10′-acetamido-8′,9′-dihydroxybutyryl moiety. In order to assign the relative configuration of H-8′ and H-9′, conversion of **2** to acetonide **2a** was achieved (Fig. 3). The NOE interactions between H-8′ (δ_H 4.61) and H-9′ (δ_H 4.33) and from the methyl protons of acetonide at δ_H 1.31 to both H-8′ and H-9′, without the correlation of H-8′ and H-9′ with the second methyl protons (δ_H 1.50), clarified an *erythro* 8′,9′-diol. Thus, the chiral configurations were depicted as 8′*R*/9′*R* or 8′*S*/9′*S*. Based on the in situ dimolybdenum CD method,[23,24] a metal complex of **2** with dimolybdenum tetraacteate $Mo_2(OAc)_4$ in DMSO was generated. The signs of the induced CD bands at 300 (band IV) reflected the O–C–C–O torsion angle, while the negative Cotton effect (CE) observed at 300 nm (Fig. 4) permitted the assignment of 8′*S* and 9′*S* configurations. Apart from *erythro* (1*R*,2*S*)-indane-1,2-diol,[24] this is an additional example to apply ICD data for the configurational determination of *erythro* diol.

Bacillcoumacin C (**3**) had a molecular formula of $C_{21}H_{28}N_2O_7$ as determined by the HRESIMS (*m*/*z* 443.17751 $[M+Na]^+$) data. Comparison of the NMR data (Tables 1 and 2) revealed the gross structure of **3** closely related to xenocoumacin.[15] The distinction was found at the terminal moiety, where a -lactam ring of **3** replaced the pyrrolidine of the known analogue. This finding was evident

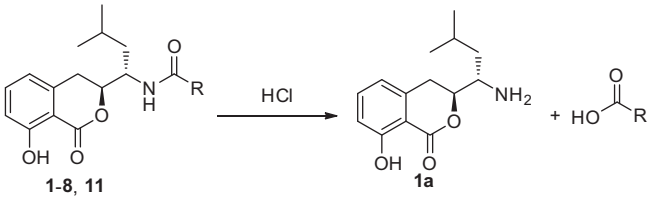


Figure 2. Chemical conversion of **1**–**8** and **11** to **1a**.

Table 1
^{1}H and ^{13}C NMR data for bacillcoumacins A–G (**1**–**7**)

	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]
3	4.70, br t (7.2)	4.71, br dd (2.5, 12.0)	4.71, br dd (2.5,12.6)	4.70, br t (7.6)	4.71, br dd (2.5,12.6)	4.71, br dd (2.5,12.6)	4.70, br dd (2.5,12.6)
4	2.94, d (7.2)	2.88, dd (2.5, 16.8) 3.02 dd (12.0, 16.8)	2.87, dd (2.5, 16.3) 3.04, dd (12.6, 16.3)	2.96, d (7.6)	2.95, dd (2.5, 16.0) 3.00, dd (12.6, 16.0)	2.95, dd (2.5, 16.0) 2.98, dd (12.6, 16.0)	2.90, dd (2.5, 16.0) 2.92, dd dd (12.6, 16.0)
5	6.84, d (7.3)	6.84, d (7.3)	6.83, d (7.4)	6.85, d (7.4)	6.84, d (7.7)	6.84, d (7.8)	6.84, d (7.2)
6	7.50, dd (7.3, 8.4)	7.50, dd (7.3, 8.3)	7.50, dd (7.4, 8.3)	7.50, dd (7.4, 8.3)	7.50, dd (7.7, 8.7)	7.50, dd (7.8, 8.8)	7.49, dd (7.2, 8.3)
7	6.86, d (8.4)	6.86, d (8.3)	6.86, d (8.3)	6.87, d (8.3)	6.87, d (8.7)	6.87, d (8.8)	6.86, d (8.3)
1′	4.21, ddd (9.5, 10.4, 12.8)	4.20, ddd (9.5, 10.4, 12.8)	4.20, ddd (9.5, 10.4, 12.8)	4.19, ddd (9.2, 10.4, 12.8)	4.21, ddd (9.5, 10.4, 12.8)	4.22, ddd (9.5, 10.4, 12.8)	4.21, ddd (9.2, 10.4, 12.8)
2′	1.37, dd (10.4, 12.4) 1.70, dd (12.4, 12.8)	1.35, dd (10.4, 12.4) 1.71, dd (12.4, 12.8)	1.34, dd (10.4, 12.4) 1.71, dd (12.4, 12.8)	1.31, dd (10.4, 12.4) 1.72, dd (12.4, 12.8)	1.32, dd (10.4, 12.4) 1.76, dd (12.4, 12.8)	1.32, dd (10.4, 12.4) 1.68, dd (12.4, 12.8)	1.36, dd (10.4, 12.4) 1.64, dd (12.4, 12.8)
3′	1.60, m	1.66, m	1.67, m	1.57, m	1.61, m	1.67, m	1.59, m
4′	0.91, d (6.4)	0.91, d (6.5)	0.91, d (6.5)	0.91, d (6.5)	0.92, d (6.5)	0.91, d (6.5)	0.91, d (6.5)
5′	0.86, d (6.4)	0.86, d (6.5)	0.87, d (6.5)	0.84, d (6.5)	0.86, d (6.5)	0.85, d (6.5)	0.86, d (6.5)
8′	3.86 s	3.89, dd (4.9, 5.8)	3.93, dd (4.8, 6.2)	4.31, dd (2.7, 5.5)	4.31, dd (2.7, 5.8)	4.22, m	5.11, s
9′		3.69, dddd (2.5, 4.9,5.0,11.0)	3.58, ddd (4.8,5.4,9.8)	4.80, m	4.64, dd (2.4, 2.7)	4.67, dd (2.4, 4.0)	
10′		3.08, ddd (5.5,11.0,12.0) 3.22, ddd (2.5, 5.5, 12.0)	3.68, m	2.06, m 2.07, m	3.50, ddd (2.4,2.7, 8.8)	3.60, m	2.77, t (7.6)
11′			1.92, m 2.02, m	2.45, m 2.46, m	2.42 dd (2.7, 18.4) 3.08 dd (8.8, 18.4)	2.53, dd (4.4, 16.0) 2.99, dd (9.0, 16.0)	2.38, t (7.6)
12′			2.01, m 2.09, m				
NH	7.47, d (9.5)	7.60, d (9.5)	7.66, d (9.5)	7.81, d (9.2)	7.91, d (9.5)	7.66, d (9.5)	7.91, d (9.2)
OH-8	10.83, s	10.84, s	10.84, s	10.82, s	10.82, s	10.82, s	10.84, s
OH-8′	5.44, br s	5.58, d (5.8)	5.53, d (6.2)	6.18, d (5.5)	6.39, d (5.8)	6.30, d (5.2)	
OH-9′		4.95, d (5.0)	5.08, d (5.4)				
NH		7.79 t (5.5)	7.35, s				10.42, s
Ac		1.81, s					
SMe					2.03, s	2.14, s	

[a] Recorded in DMSO-d_6, at 400 MHz
[b] Recorded in DMSO-d_6, at 500 MHz.

Table 2
^{13}C NMR data for bacillcoumacins A–G (**1**–**7**)[a]

Position	**1** [a]	**2** [b]	**3** [a]	**4** [a]	**5** [a]	**6** [a]	**7** [a]
1	169.4	169.5	169.6	169.3	169.4	169.4	169.6
3	81.5	81.6	81.6	81.4	81.4	81.5	81.2
4	29.7	29.6	29.6	29.6	29.6	29.7	29.8
5	119.0	119.0	119.0	119.0	119.0	119.0	119.0
6	136.8	136.8	136.7	136.8	136.8	136.8	136.8
7	115.8	115.7	115.7	115.8	115.8	115.8	115.8
8	161.3	161.3	161.4	162.4	161.3	161.3	161.3
9	108.8	108.8	108.8	109.4	108.8	108.8	108.8
10	140.8	141.1	141.2	140.7	140.8	140.8	140.8
1′	48.4	48.4	48.5	48.7	48.8	48.9	22.0
2′	39.8	39.7	39.7	40.2	39.4	39.6	23.7
3′	24.6	24.4	24.5	24.6	24.6	24.2	24.7
4′	23.7	23.8	23.8	23.8	23.9	23.9	39.8
5′	22.0	22.0	22.0	21.7	21.8	21.9	48.4
7′	172.5	172.5	173.0	170.8	170.3	171.0	168.2
8′	61.7	73.7	73.8	72.2	72.5	71.2	92.3
9′		71.9	73.0	80.8	85.8	85.2	154.5
10′		40.2	55.4	21.1	40.0	41.0	25.9
11′			21.4	28.5	36.2	35.6	27.8
12′			30.4	177.7	175.8	175.2	177.7
13′			177.4				
Ac		170.3 23.1					
SMe					13.3	13.3	

[a] Recorded in DMSO-d_6, at 100 MHz.
[b] Recorded in DMSO-d_6, at 125 MHz.

from the COSY correlation between H-10′ (δ_H 3.68, m) and NH (δ_H 7.35) in association with the HMBC interactions from the carbonyl carbon at δ_C 177.4 (C-13′) to H-10′, NH (δ_H 7.35), H_2-11′ (δ_H 1.92, 2.02), and H_2-12′ (δ_H 2.01, 2.09). The $J_{H\text{-}8'/H\text{-}9'}$ (4.8 Hz) value was in agreement with an *erythro* configuration of the diol,[25] the same as that of **2**. Based on the in situ dimolybdenum tetraacteate [Mo_2(OAc)$_4$] CD method,[23,24] the negative Cotton effect around 300 nm (band IV) (Fig. 4) was in agreement with 8′*S* and 9′*S* configurations. The $J_{H\text{-}9'/H\text{-}10'}$ (9.8 Hz) value indicated both H-9′ and H-10′ to be in an *anti* conformation. Thus, there are two possible relative conformations for the chiral center C-10′ (Fig. 5). Irradiation of H-8′ induced the NOE enhancement of the lactam NH (δ_H 7.35), indicating that **3** adopted **3a** confirmation. Thus, the absolute configuration of C-10′ was supposed to be 10′*R*.

The molecular formula of bacillcoumacin D (**4**) was established as $C_{20}H_{25}NO_7$ by the HRESIMS (*m*/*z* 390.15523 $[M-H]^-$) and NMR data. Apart from the NMR data for the dihydroisocoumarin which was identical to that of **3**, the remaining NMR resonances of **4** showed six carbon signals instead of seven of **3**. Interpretation of the COSY and HMBC correlations, such as the HMBC interaction of the oxymethine proton H-9′ (δ_H 4.80) with the carbonyl carbon at δ_C 177.7 (C-12′), disclosed the side chain to be terminated by a -lactone. This unit was bonded to the hydroxymethine C-8′ (δ_C 72.2) according to the HMBC interactions from OH-8′ (δ_H 6.18, d, *J* = 5.5 Hz) to C-7′ (δ_C 170.8), C-8′, and C-9′. According to the bulkiness rule,[26,27] a $Rh_2(OCOCF_3)_4$ complex of **4** was generated in $CHCl_3$. The positive sign of Cotton effect for E band at 346 nm in the CD spectrum (Fig 6) was in agreement with 8′*S* configuration. In addition, the $J_{H\text{-}8'/H\text{-}9'}$ (2.7 Hz) value was indicative of *cis* conformation of H-8′ and H-9′. Thus, four possible relative configurations (Fig. 7) were depicted. Irradiation of the amide NH (δ_H 7.81, d, *J* = 9.2 Hz) induced the NOE enhancement of H-9′ (δ_H 4.80, m) and H_2-10′ (δ_H 2.06, 2.07, m), while these results were in agreement with **4d** conformation.[28] Accordingly, the stereogenic center C-9′ was determined as 9′*S* configuration.

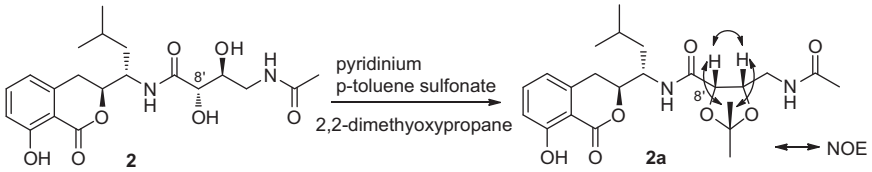


Figure 3. Chemical conversion of **2** to **2a** and the NOE interactions of **2a**.

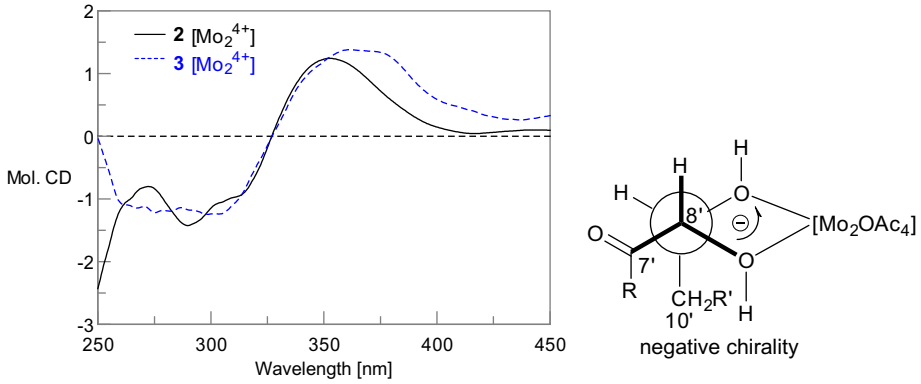


Figure 4. ICD curves of **2** and **3** induced by $Mo_2(OAc)_4$ in DMSO-d_6.

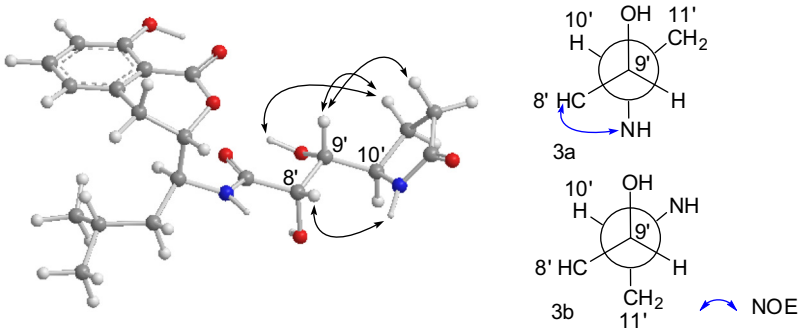


Figure 5. Key NOE correlations and the Newman projects of **3**.

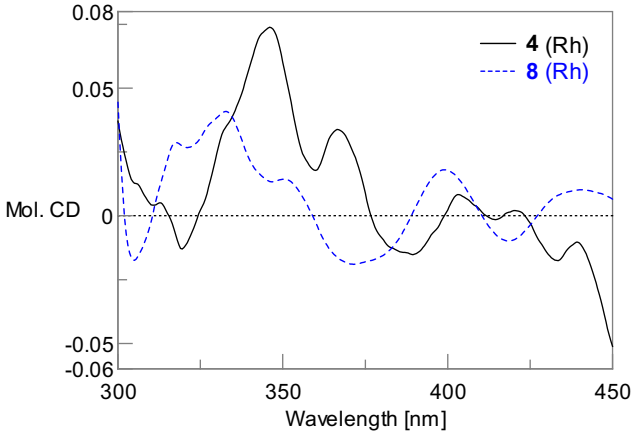


Figure 6. ICD curves of **4** and **8** induced by $Rh_2(OCOCF_3)_4$ in $CHCl_3$.

Bacillcoumacin E (**5**) has a molecular formula of $C_{21}H_{27}NO_7S$ as provided by the HRESIMS (*m*/*z* 897.29213 [2M+Na]$^+$) data, containing a sulfur atom. The close similarity of the NMR data between **5** and **4** with the exception of C-10′ (δ_C 40.0) of 5 to be a methine instead of a methylene conducted to assign C-10′ of **5** being substituted. The observation of a methyl singlet (δ_H 2.03, s) correlated to C-10′ and in turn H-10′ (δ_H 3.50, ddd, J = 2.4, 2.7, 8.8 Hz) to the methyl carbon (δ_C 13.3) in the HMBC spectrum deduced C-10′ to be bonded by a methylthio group. On the basis of Mosher's method,[20] the stereogenic center at C-8′ was determined as *S* configuration (Fig. 8). The NOE interactions observed from the methylthio protons (δ_H 2.03) to H-9′ (δ_H 4.64, dd, J = 2.4, 2.7 Hz) and H-11a′ (δ_H 2.42, dd, J = 2.7, 18.4 Hz) and between H-9′ and H-11a′ clarified the same orientation of H-9′ and the methylthio group. A *cis* relationship of H-8′ and H-9′ was recognized by the $J_{H\text{-}8'/H\text{-}9'}$ (2.7 Hz) value, which resulted in four possible relative conformations (**5a**–**5d**) (Fig. 7). The NOE interactions from amide NH (δ_H 7.91, d, J = 9.5 Hz) to H-9′ and H-10′ revealed **5a** to be a suitable confirmation. Accordingly, the stereogenic center C-9′ was determined as 9′*R* configuration, while C-10′ was assigned to *S* configuration.

Analysis of the NMR and MS data revealed the same gross structure of bacillcoumacin F (**6**) and **5**. Calculation of the $\Delta\delta(\delta_R-\delta_S)$ data of the MPA esters of **6**[20] (Fig. 6) assigned the same configuration of C-8′ as that of **5**, while the same orientation of methylthio group toward H-9′ (δ_H 4.67) was evident from the NOE interaction between H-9′ and SMe (δ_H 2.14, s). Similarly, the $J_{H\text{-}8'/H\text{-}9'}$ (2.4 Hz)

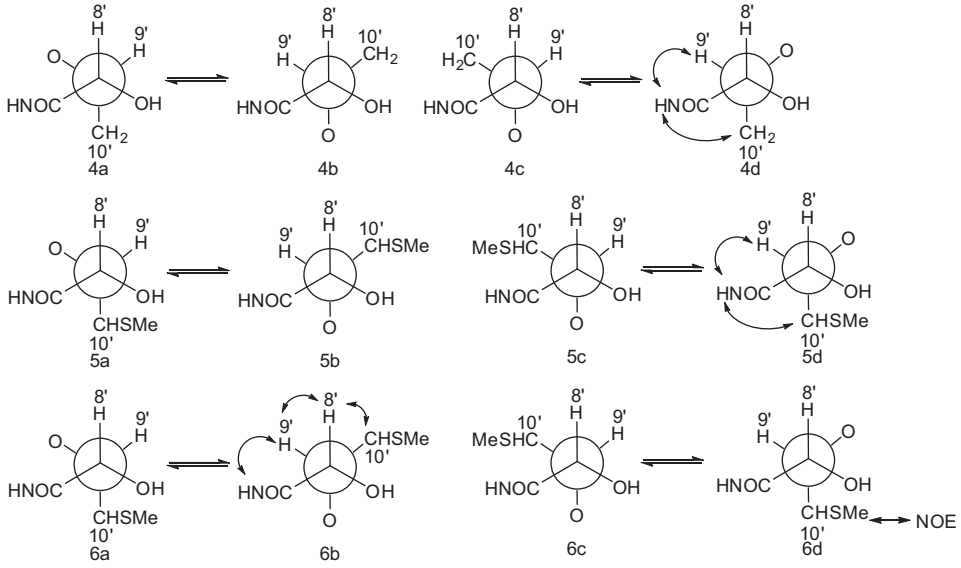


Figure 7. Newman projects for C-8′/C-9′ and key NOE interactions for **4**–**6**.

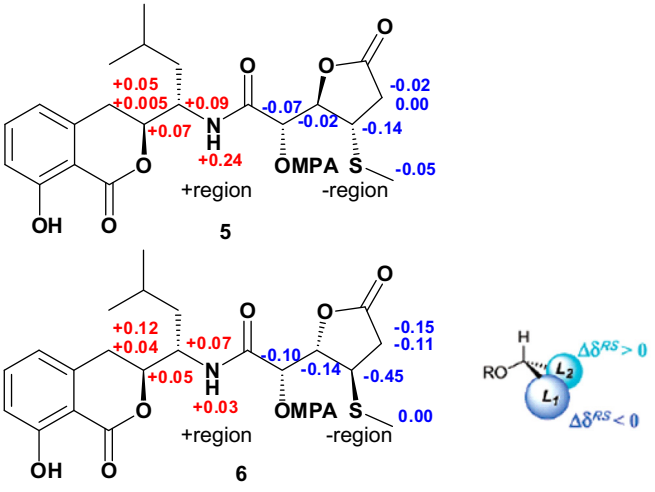


Figure 8. $\Delta\delta^{RS}(\delta_R-\delta_S)$ data for the MPA esters of **5** and **6**.

value for a *cis* relationship of H-8′ and H-9′ resulted in four possible relative conformations (**6a**–**6d**). However, the observation of the NOE interactions from H-8′ (δ_H 4.22, m) to H-9′ and H-10′ (δ_H 3.60, m) and the absence of NOE interaction between amide NH and H-10′ clarified a suitable conformation of **6** to be **6b**. Thus, the absolute configurations at the lactone were assigned to 9′*S* and 10′*R*.

Bacillcoumacin G (**7**) has a molecular formula of $C_{20}H_{24}N_2O_5$ as provided by the HRESIMS and NMR data. Its NMR spectroscopic data (Tables 1 and 2) closely resembled those of **4**, except for C-8′ (δ_C 92.3)/C-9′ (δ_C 154.5) to be resided by a double bond. This assignment was supported by the HMBC correlations from H-8′ (δ_H 5.11) to C-7′ (δ_C 168.2) and C-10′ (δ_C 25.9). The NOE correlation between H-8′ and H_2-10′ (δ_H 2.77) was indicative of 8′*Z* geometry.

Compounds **1**–**9** and the known analogue **11** were hydrolyzed with 4 M HCl, respectively, to derive the same chromophoric product (**1a**) (Fig. 2), indicating the new compounds sharing the same absolute configurations (3*S* and 1′*S*) in the nucleus part of **1**-**7**.

Compounds **1**–**11** were measured against five human tumor cell lines, including BGC-823, HCT-116, HepG2, NCI-H1650, and A2780 by the MTT method, showing weak inhibitory activities with the dose less than 10 μM. The inhibitory effects of these compounds against NO production induced by LPS in mouse macrophage RAW 264.7 cells were tested. As shown in Table 3, compounds **7** and **9** exhibited significantly inhibitory activities toward the production of NO with the IC_{50} values of 27.8 and 30.9 μM, respectively, comparable to the data for positive control curcumine (IC_{50} = 20.5 μM).[29] Compound **7** is the only one bearing a γ-lactam with a double bond resided at C-8′/C-9′, suggesting the olefin bond C-8′/C-9′ in the scaffolds contributed as a functional group. For the analogues with an unsaturated γ-lactone (**8** and **9**), the stereogenic center C-9′ directly affected the inhibitory effects, as evident from AI-77-F with 9′*S* (**9**) showing stronger inhibition than AI-77-H with 9′*R* (**8**). The NO is produced from the enzymatic oxidation of L-arginine by iNOS in numerous mammalian cells and tissues, and serves as a key signaling molecule in physiological processes, such as host defense, neuronal communication, and vascular regulation.[30,31] Amicoumacin A has been reported to exhibit potent anti-inflammatory activity to reduce carrageenan mice paw edema, but the high toxicity limited its application.[32,33] The potent inhibition of **7** and **9** against NO production with low cytotoxicity implied these analogues to be possible for the development as anti-inflammatory agents.

Biogenetically, amicoumacin-based isocoumarins are considered to be synthesized by the hybrid pathway involving non ribosomal peptide synthetases (NRPSs) and polyketide synthtases (PKSs). It is noted that all amicoumacins from nature exclusively possess a leucine unit to take part in the biosynthesis, implying the NRPS enzymes stringently selecting amino acid. Thus, detection of the specific NRPS synthesis gene may provide a

Table 3
Inhibitory activities of **7** and **9** on NO production induced by LPS in mouse macrophage RAW 264.7 cells[a,b,c]

Compound	IC_{50} (μM)
7	30.9 ± 0.8
9	27.8 ± 1.6
Curcumine[d]	20.5 ± 2.0

[a] No cytotoxicity was observed.
[b] Data are mean ± SD (n = 3).
[c] The other compounds were inactive (IC_{50} >50 μM).
[d] Positive control.

biomarker to find new microorganisms which are able to derive amicoumacin diversity. Previous investigation revealed that 14 gene clusters (*xcnA–N*) are required for the synthesis of amicoumacins.[34,35] These findings are helpful not only to find new source producing new amicoumacins but also to provide a molecular tool to regulate the yield of target compounds.

Acknowledgments

This work was supported by Grants from 973-Project (2015CB 755900), the National Hi-Tech 863-Projects (2011AA090701, 2013AA092902), COMRA (DY125-15-T-01), and Sino-German Project GZ816.

Supplementary data

NMR spectroscopic data for the new compounds (**1–7**) including ^{1}H, ^{13}C, and 2D NMR spectra, IR, and ESIMS/MS data, experimental section. This material is available free of charge via the Internet at http://www.sciencedirect.com.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.09.100. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Chen, G.; Wang, H. F.; Pei, Y. H. *J. Asian Nat. Prod. Res.* **2014**, *16*, 105–122.
2. Butler, M. S. *J. Nat. Prod.* **2004**, *67*, 2141–2153.
3. Zobell, C. E.; Morita, R. Y. *J. Bacteriol.* **1957**, *73*, 563–568.
4. Horikoshi, K. *Curr. Opin. Microbiol.* **1998**, *1*, 291–295.
5. Saleem, M.; Ali, M. S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y. S. *Nat. Prod. Rep.* **2007**, *24*, 1142–1152.
6. Debbab, A.; Aly, A. H.; Lin, W. H.; Proksch, P. *Microbiol. Biotechnol.* **2010**, *3*, 544–563.
7. Itoh, J.; Omoto, S.; Nishizawa, N.; Kodama, Y.; Inouye, S. *Agric. Biol. Chem.* **1982**, *46*, 2659–2665.
8. Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M.; Iitaka, Y. *Tetrahedron Lett.* **1982**, *23*, 5435–5438.
9. Huang, Y.; Li, L.; Tian, L.; Qiao, L.; Hua, H.; Pei, Y. *J. Antibiot.* **2006**, *59*, 355–357.
10. Azumi, M.; Ogawa, K.-I.; Fujita, T.; Takeshita, M.; Yoshida, R.; Furumai, T.; Igarashi, Y. *Tetrahedron* **2008**, *64*, 6420–6425.
11. Li, Y.; Xu, Y.; Liu, L.; Han, Z.; Lai, P. Y.; Guo, X.; Zhang, X.; Lin, W.; Qian, P.-Y. *Mar. Drugs* **2012**, *10*, 319–328.
12. Itoh, J.; Shomura, T.; Omoto, S.; Miyado, S.; Yuda, Y.; Shibata, U.; Inouye, S. *Agric. Biol. Chem.* **1982**, *46*, 1255–1259.
13. Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M. *Agric. Biol. Chem.* **1982**, *46*, 1823–1829.
14. Hashimoto, M.; Taguchi, T.; Nishida, S.; Ueno, K.; Koizumi, K.; Aburada, M.; Ichinose, K. *J. Antibiot.* **2007**, *60*, 752–756.
15. McInerney, B. V.; Taylor, W. C.; Lacey, M. J.; Akhurst, R. J.; Gregson, R. P. *J. Nat. Prod.* **1991**, *54*, 785–795.
16. Sato, T.; Nagai, K.; Suzuki, K.; Morioka, M.; Saito, T. *J. Antibiot.* **1992**, *45*, 1949–1952.
17. Canedo, L. M.; Puentes, J. L. F.; Baz, J. P.; Acebal, C.; De La Calle, F.; Gravalos, D. G.; De Quesada, T. G. *J. Antibiot.* **1997**, *50*, 175–176.
18. Han, X.; Liu, S.; Wang, F.; Liu, J.; Chen, C.; Hu, X.; Jiang, Z.; You, X.; Shang, G.; Zhang, Y.; Sun, C. *World Notes Antibiot.* **2013**, *34*, 106–115.
19. Shimojima, Y.; Hayashi, H.; Ooka, T.; Shibukawa, M.; Iitaka, Y. *Tetrahedron* **1984**, *40*, 2519–2527.
20. Liu, S.; Jin, J.; Chen, C.; Liu, J.; Li, J.; Wang, F.; Jiang, Z.; Hu, J.; Gao, Z.; Yao, F.; You, X.; Si, S.; Sun, C. *J. Antibiot.* **2013**, *66*, 281–284.
21. Shi, H.; Yu, S.; Liu, D.; Ofwegen, L.; Proksch, P.; Lin, W. *Mar. Drugs* **2012**, *10*, 1331–1344.
22. Krohn, K.; Bahramsari, R.; Flsrke, U.; Ludewig, K.; Klichespory, C.; Michel, A.; Aust, H. J.; Draeger, S.; Schulz, B.; Antus, S. *Phytochemistry* **1997**, *45*, 313–320.
23. Gorecki, M.; Jabłonska, E.; Kruszewska, A.; Suszczynska, A.; Urbanczyk-Lipkowska, Z.; Gerards, M.; Morzycki, J. W. *J. Org. Chem.* **2007**, *72*, 2906–2916.
24. Bari, L. D.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. *J. Org. Chem.* **2001**, *66*, 4819–4825.
25. Alam, M. S.; Sultana, D.; Suzuki, T.; Katayama, T. *Emir. J. Food Agric.* **2010**, *22*, 437–447.
26. Frelek, J.; Szczepek, W. J. *Tetrahedron: Asymmetry* **1999**, *10*, 1507–1520.
27. Bai, J.; Chen, H.; Fang, Z. F.; Yu, S. S.; Ma, S. G.; Li, Y.; Qu, J.; Xu, S.; Ren, J. H.; Lu, H. N.; Chen, X. *J. Asian Nat. Prod. Res.* **2012**, *14*, 940–949.
28. Chlipala, G. E.; Tri, P. H.; Hung, N. V.; Krunic, A.; Shim, S. H.; Soejarto, D. D.; Orjala, J. J. Nat. Prod. doi: 10.1021/np100002q.
29. Esatbeyoglu, T.; Huebbe, P.; Ernst, I. M.; Chin, D.; Wagner, A. E.; Rimbach, G. *Angew. Chem., Int. Ed.* **2012**, *51*, 5308–5332.
30. Mulligan, M. S.; Hevel, J. M.; Marletta, M. A.; Ward, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6338–6342.
31. Kuo, P. C.; Schroeder, R. A. *Ann. Surg.* **1995**, *221*, 220–235.
32. Gross, S. S.; Wolin, M. S. *Annu. Rev. Physiol.* **1995**, *57*, 737–769.
33. Itoh, J.; Omoto, S.; Shomura, T.; Nishizawa, N.; Miyado, S.; Yuda, Y.; Shibata, U.; Inouye, S. *J. Antibiot.* **1981**, *34*, 611–613.
34. Reimer, D.; Pos, K. M.; Thines, M.; Grün, P.; Bode, H. B. *Nat. Chem. Biol.* **2011**, *7*, 888–890.
35. Park, D.; Ciezki, K.; Hoeven, R.; Singh, S.; Reimer, D.; Bode, H. B.; Forst, S. *Mol. Microbiol.* **2009**, *73*, 938–949.