ChemPubSoc Europe

DOI: 10.1002/open.201500198

Open Access ChemistryOPEN Full Papers

Novel Natural Oximes and Oxime Esters with a Vibralactone Backbone from the Basidiomycete *Boreostereum vibrans*

He-Ping Chen,[a, c] Zhen-Zhu Zhao,[a, c] Zheng-Hui Li,[a, b] Ze-Jun Dong,[a] Kun Wei,[a] Xue Bai,[a] Ling Zhang,[a] Chun-Nan Wen,[a, c] Tao Feng,*[a, b] and Ji-Kai Liu*[a, b]

A variety of novel natural products with significant bioactivities are produced by the basidiomycete *Boreostereum vibrans*. In the present study, we describe 16 novel natural oximes and oxime esters with a vibralactone backbone, vibralactoximes, which were isolated from the scale-up fermentation broth of *B. vibrans*. Their structures were determined through extensive spectroscopic analyses. These compounds represent the first oxime esters from nature. The hypothetical biosynthetic pathway of these compounds was also proposed. Seven compounds exhibited significant pancreatic lipase inhibitory activity, while ten compounds exhibited cytotoxicities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480), with IC_{50} values comparable with those of cisplatin.

Introduction

Obesity and overweight, caused by multifaceted physical and environmental factors, have become global issues because of their association with a high risk of cardiovascular diseases,[1] diabetes,[2] musculoskeletal disorders,[3] hypertension,[4] and several types of cancers.[5] According to the fact sheets of the World Health Organization, nearly 13% of the world's adult population were obese and 39% were overweight in 2014.[6] Fighting obesity is one of the top priorities of health departments. Although pharmacotherapy is subordinate to individual lifestyle adjustments, it is an indispensable treatment in combating obesity. Many therapeutic agents used to treat obesity have been found to be associated with serious adverse reactions.[7] Fenfluramine, an antiobesity medicine once approved by the US FDA in 1973, was withdrawn in 1997 due to the risk of valvular heart disease.[8] Although orlistat is now available, it is currently involved in a debate regarding the likelihood of causing liver damage.[9] Therefore, searching for new cost-effective antiobesity medicines with fewer side effects has been one of the hot research topics over the past decades.[10]

Higher fungi belonging to the genus *Stereum* produce diverse bioactive secondary metabolites. Several sesquiterpenoid skeletons were first isolated from this genus, including hirsutane-type,[11] sterpurane-type,[12] and stereumane-type[13] sesquiterpenes. Moreover, some homo- and heterodimeric sesquiterpenes with various biological activities were recently obtained from this genus.[14] We have investigated the basidiomycete *Boreostereum vibrans* (synonym *Stereum vibrans*), which is known to produce a significant pancreatic lipase inhibitor with an unusual fused β-lactone, named vibralactone (IC_{50} of 0.4 $\mu g\,mL^{-1}$).[15] Some important progress has been made in both elucidating the biosynthetic pathway and modifying the structure of this molecule.[16]

To explore more interesting compounds from this productive strain, a scale-up fermentation (1200 L) was conducted, which resulted in the isolation of sixteen oxime and oxime esters with a vibralactone backbone. Natural products possessing an oxime group are rare, and they have been found to originate from sponges, bacteria, fungi, and plants.[17] These types of compounds exhibit various activities, including strong antibiotic activity,[16a,17j–l] cytotoxicity,[17g] inhibition of insect juvenile hormone production,[17d] dexamethasone glucocorticoid receptor binding,[17f] and phosphorylated cholinesterase regeneration.[17d]

Interestingly, the monomers vibralactoxime A (**1**) and vibralactoxime B (**2**) can play the role of building blocks to form polymers via the formation of an ester bond between the carbonyl and oxime hydroxy groups. All of the polymers can be divided into four categories depending on the starting and terminating scaffolds: a) starting with **1** and ending with the 2,3-

[a] H.-P. Chen, Z.-Z. Zhao, Z.-H. Li, Z.-J. Dong, Dr. K. Wei, X. Bai, Dr. L. Zhang, C.-N. Wen, Dr. T. Feng, Prof. Dr. J.-K. Liu
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650201 (P. R. China)
E-mail: jkliu@mail.kib.ac.cn
fengtao@mail.kib.ac.cn

[b] Z.-H. Li, Dr. T. Feng, Prof. Dr. J.-K. Liu
School of Pharmaceutical Sciences
South-Central University for Nationalities
Wuhan 430074 (P. R. China)

[c] H.-P. Chen, Z.-Z. Zhao, C.-N. Wen
University of Chinese Academy of Sciences, Beijing 100049 (P.R. China)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/open.201500198.

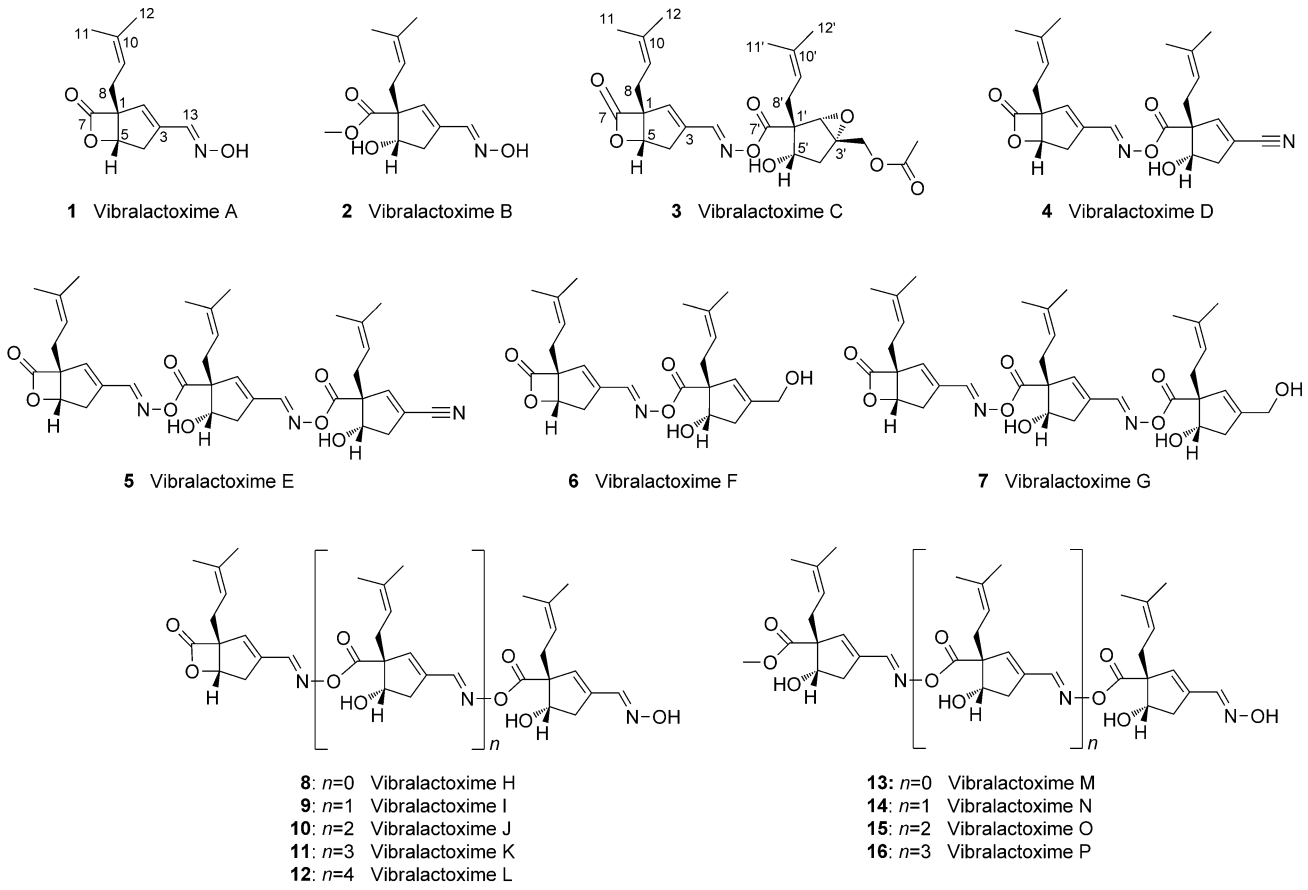


Figure 1. Structures of vibralactoxims A–P (**1**–**16**).

epoxy vibralactone derivative(**3**), b) starting with **1** and ending with the cyano group-containing derivative of **1** (**4**, **5**); c) starting with **1** and ending with vibralactone (**6**, **7**), and d) starting with **1** or **2** and ending with **1** (**8**–**16**). Polyoxime ester bonds were found for the first time in natural products.

Herein, we describe the isolation, structural elucidation, biosynthetic supposition, cytotoxicity, and pancreatic lipase inhibitory activity of vibralactoxims **1**–**16** (Figure 1).

Results and Discussion

The filtrate of a scale-up fermentation broth of *B. vibrans* (1200 L) was concentrated under reduced pressure and then partitioned between ethyl acetate and water four times to afford an ethyl acetate layer (856 g). Subsequently, the mycelia were soaked in choroform/methanol (1:1), and the extract was evaporated and then dissolved in water, which was extracted with ethyl acetate three times to afford an ethyl acetate layer (362 g). The total organic extract (1218 g) was purified using repeated silica gel, Sephadex LH-20, medium-pressure liquid chromatographic approaches and preparative high-performance liquid chrimatography (HPLC) to afford sixteen oximes and oxime esters (Figure 1).

Vibralactoxime A (**1**) was isolated as a pale yellow oil. Its molecular formula was established to be $C_{12}H_{15}NO_3$ based on the high-resolution electron ionization mass spectrometry (HR EI-MS) analysis (*m/z* 221.1049, calcd 221.1052), requiring six degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data in combination with the heteronuclear single quantum coherence (HSQC) spectrum revealed the presence of a hydroxy (δ_H = 10.53 ppm), two methyls (δ_H = 1.70, s 3H; 1.66, s, 3H; δ_C = 25.9, 18.0 ppm), two methylenes, one oxygenated methine (δ_C = 78.9 ppm), one quaternary carbon (δ_C = 76.5 ppm), five olefinic carbons [δ_C = 118.5 (CH), 131.3 (CH), 146.5 (CH), 136.4, 141.1 ppm], and one carbonyl (δ_C = 172.4 ppm) (Table 1). All of these data, as well as the partial heteronuclear multiple bond correlations (HMBCs), were highly similar to those of vibralactone, except that the hydroxymethyl at δ_C = 61.3 ppm of vibralactone was changed to an sp^2-hybridized methane at δ_C = 146.5 ppm (C-13), which was supported by the HMBC correlations from δ_H = 7.99 ppm (1H, s, H-13) to δ_C = 131.3 (d, C-2), 141.1 (s, C-3), and 36.7 ppm (t, C-4).The above results indicated that the main difference between them was the substituent at C-3. According to the molecular formula, the group substituted at C-3 in **1** must have the composition CH_2NO. In addition, the unique ^{3}J HMBC correlation from the hydroxyl proton at δ_H = 10.53 ppm (1H, s) to C-13 indicated that the carbon atom and hydroxyl group were separated by a nitrogen atom (Supporting Information, Figure 5S). Therefore, considering aforementioned evidences and the chemical shift feature of C-13 (δ_C = 146.5 ppm), the remaining unassigned atoms of a nitrogen, an oxygen, and two protons were determined to be an unusual

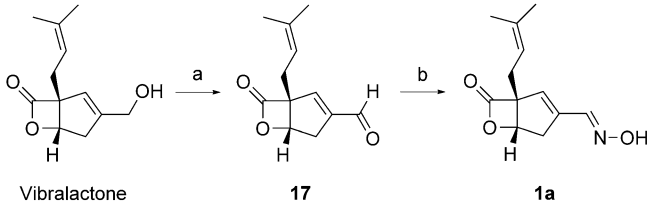
Table 1. NMR data of **1** and **2** in [D_6]acetone.

	Chemical shifts [ppm] and coupling constants (*J*) [Hz]			
	1		**2**	
No.	δ_C, type	δ_H, multi.	δ_C, type	δ_H, multi.
1	76.5, s		66.7, s	
2	131.3, d	6.04, s	136.4, d	6.06, s
3	141.1, s		138.5, s	
4	36.7, t	2.88, d (19.2)	39.9, t	2.54, dd (17.0, 1.8)
		2.94, dd (19.2, 5.4)		2.84, overlapped
5	78.9, d	4.97, d (5.4)	78.5, d	4.27, ddd (6.0, 5.9, 1.8)
7	172.4, s		173.5, s	
8	28.0, t	2.52, dd (15.0, 7.4)	35.7, t	2.20, dd (13.9, 7.6)
		2.67, dd (15.0, 7.4)		2.62, dd (13.9, 7.6)
9	118.5, d	5.19, t (7.4)	120.4, d	5.06, t (7.6)
10	136.4, s		134.9, s	
11	18.0, q	1.66, s, 3H	17.9, q	1.57, s, 3H
12	25.9, q	1.70, s, 3H	26.0, q	1.66, s, 3H
13	146.5, d	7.99, s	147.3, d	7.93, s
7-OCH_3			51.6, s	3.63, s, 3H
N-OH		10.53, s,		10.20, s
5-OH				4.18, d (5.9)

oxime moiety. The key rotating-frame Overhauser effect spectroscopy (ROESY) cross peaks between protons at $\delta_H = 6.04$ (1H, s, H-2) and 7.99 ppm, and between $\delta_H = 7.99$ and 10.53 ppm, suggested the conjugated diene (C2=C3–C13=N) was a *trans* configuration, while the oxime group was an *E* configuration. Other ROESY signals revealed that the relative configurations of remaining asymmetric centers in **1** to be 1*R*′, 5*S*′ (Figure 3).

In order to determine the absolute configurations of **1**, a semisynthesis of this molecule was achieved from vibralactone by two steps (Scheme 1). The semisynthesis of **1** commenced with vibralactone, a major compound in this crude extract, which was oxidized with pyridinium chlorochromate (PCC) to give **17** as a colorless oil in 78% yield.[15b,16a] Aldehyde **17** was subjected to react with hydroxylamine hydrochloride to afford **1a** as a pale yellow oil in high yield (90%).[18] The ^{1}H NMR data of **1a**, as well as circular dichroism (CD) and UV/Vis spectra matched those of the natural isolate **1** fabulously (Figure 2; Figures A, D, and E in the Supporting Information). Moreover, the presence of oxime group in the conjugated diene in **1** led to the red shift of its CD spectrum compared with that of vibralactone, as shown in Figure 2. All pieces of evidences above indicated that the absolute configurations of **1** were 1*R*, 5*S*, which were the same as vibralactone.

Vibralactoxime B (**2**) was obtained as a light yellow oil and determined to have a molecular formula of $C_{13}H_{19}NO_4$ based



Scheme 1. Semisynthesis of **1** from vibralactone. *Reagents and conditions*: a) PCC, CH_2Cl_2, 0 °C, 30 min, 78%; b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH/H_2O (1:1), 0 °C, 20 min, 90%.

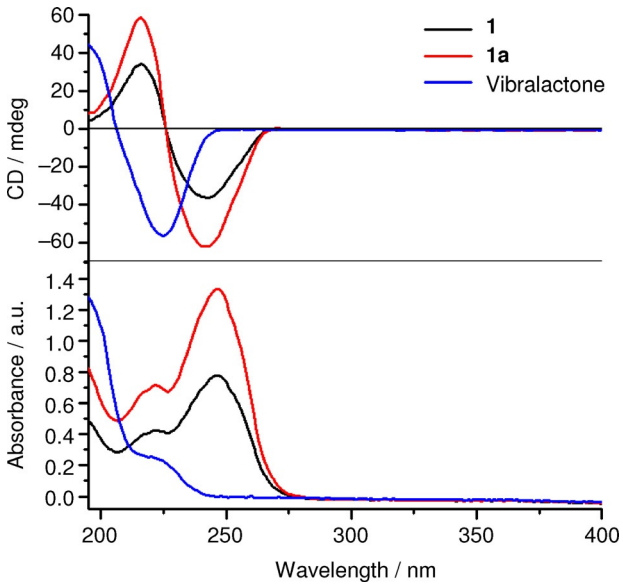


Figure 2. CD and UV/Vis spectra of compound **1**, **1a**, and vibralactone (CH_3CN).

on the ion peak of HR-EI-MS at *m/z* 253.1314 (calcd 253.1312). Many of the structural characteristics of **2**, including the oxime group and isopentenyl moiety, were found to be the same as those of vibralactoxime A (**1**) based on preliminary analyses of the 1D and 2D NMR spectra (Table 1). However, the protons at $\delta_H = 3.63$ ppm (3H, s) were correlated to the carbonyl at $\delta_C =$ 173.5 ppm (s, C-7) in the HMBC spectrum, whereas the cross peak between the hydroxyl proton at $\delta_H = 4.18$ ppm (1H, d, $J = 5.9$ Hz) and proton at $\delta_H = 4.27$ ppm (1H, ddd, $J = 6.0$, 5.9, 1.8 Hz, H-5) in the ^{1}H-^{1}H COSY spectrum was also observed (Figure 12S in the Supporting Information). All of the aforementioned spectral data suggested that **2** was a derivative of **1**, in which the β-lactone group was opened and the carboxyl was methyl esterified. The key ROESY correlation between H-5 and H-8 (2.62, dd, $J = 13.9$, 7.6 Hz; 2.20, dd, $J = 13.9$, 7.6 Hz) demonstrated the same configurations between **1** and **2**.

The molecular formula of vibralactoxime C (**3**) was determined to be $C_{26}H_{33}NO_8$ from the HR electrospray ionization (ESI)-MS data, *m/z* 510.2102 $[M+Na]^+$ (calcd for $C_{26}H_{33}NO_8Na$, 510.2098). The ^{13}C NMR spectroscopic data revealed the presence of 26 carbon signals, which were classified by distortionless enhancement by polarization transfer (DEPT) and HSQC spectra into the categories of five methyls, five methylenes (with one oxygenated), seven methines (with three oxygenated), and nine nonprotoned carbons (three carbonyls, three olefinic carbons, and one oxygen bearing carbon) (Table 2). Detailed analyses of the 1D and 2D NMR spectra indicated the existence of two isolated vibralactone skeletons. One of the moieties (fragment A, Figure 3) presented similar characteristic signals as vibralactoxime A (**1**). In fragment B, key HMBC correlations from $\delta_H = 4.49$ (1H, d, $J = 12.5$ Hz, H-13a), 4.20 (1H, d, $J = 12.5$ Hz, H-13b), and 2.07 ppm (s, 3H) to $\delta_C = 170.8$ ppm (C=O), as well as the mutual ^{1}H–^{1}H COSY correlation between $\delta_H = 2.98$ (5′-OH) and 3.95 ppm (H-5′) suggested that the hydroxyl of C-13′ was acetylated, and that the β-lactone group

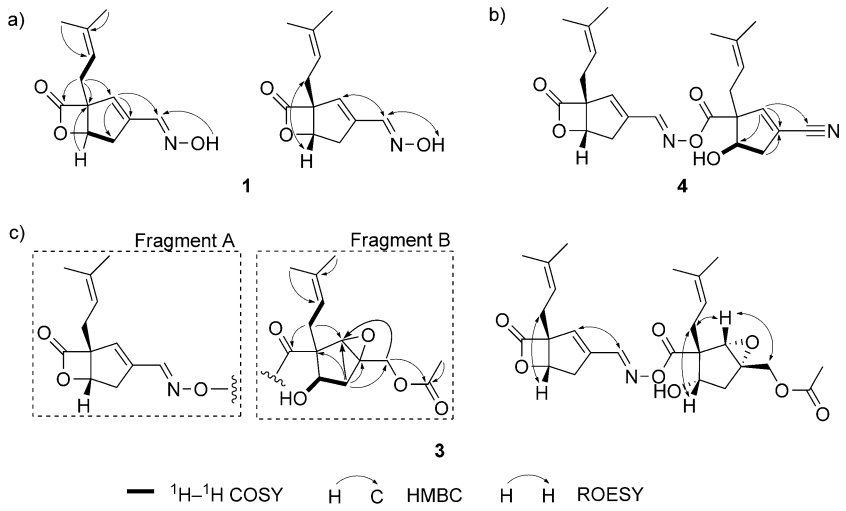


Figure 3. a) Key 2D NMR correlations of **1**; b) key ^{1}H–^{1}H COSY and HMBC correlations of **4**; c) key 2D NMR correlations of **3**.

Table 2. NMR data of **3** and **4** in [D$_6$]acetone.

	Chemical shifts [ppm] and coupling constants (*J*) [Hz]			
	3		**4**	
No.	δ_C, type	δ_H, multi.	δ_C, type	δ_H, multi.
1	77.1, s		77.0, s	
2	138.9, d	6.48, s	138.9, d	6.48, s
3	139.3, s		139.2, s	
4	36.6, t	3.08, dd (18.4, 5.8) 2.99, d (18.4)	36.5, t	3.08, dd (19.0, 5.7) 2.97, d (19.0)
5	79.1, d	5.08, d (5.8)	79.0, d	5.07, d (5.6)
7	171.7, s		171.6, s	
8	28.0, t	2.74, dd (14.8, 7.5) 2.60, overlapped	27.9, t	2.73, overlapped 2.59, overlapped
9	118.4, d	5.20, br.t (7.5)	118.3, d	5.20, br. t (7.4)
10	136.8, s		136.7, s	
11	18.1, q	1.67, s, 3H	18.0, q	1.67, s, 3H
12	26.1, q	1.71, s, 3H	25.9, q	1.71, s, 3H
13	154.2, d	8.44, s	154.3, d	8.45, s
1′	61.4, s		67.5, s	
2′	64.9, d	3.77, s	149.4, d	6.82, s
3′	65.3, s		115.0, s	
4′	37.2, t	2.44, dd (15.2, 6.1) 2.10, d (15.2)	43.3, t	3.11, dd (17.6, 5.8) 2.61, dd (17.6, 1.7)
5′	76.8, d	3.95, dd (7.5, 6.1)	77.8, d	4.44, ddd (5.8, 5.7, 1.7)
7′	168.7, s		168.2, s	
8′	32.8, t	2.60, overlapped 2.23, dd (14.3, 7.5)	34.8, t	2.74, overlapped 2.33, dd (14.2, 7.8)
9′	118.8, d	5.16, br. t (7.5)	119.0, d	5.11, br. t (7.8)
10′	136.4, s		136.3, s	
11′	18.1, q	1.63, s, 3H	18.0, q	1.60, s, 3H
12′	26.0, q	1.69, s, 3H	26.0, q	1.68, s, 3H
13′	64.0, t	4.49, d, (12.5) 4.20, d (12.5)	116.8, s	
CH_3CO-	20.6, q	2.07, overlapped[a]		
CH_3CO-	170.8, s			
7-OCH_3				
5′-OH		2.98, d (7.5)		4.83, d (5.7)

[a] Signal overlapped in solvent residual peaks.

was opened. Moreover, obvious HMBC correlations were observed from H-8′ (δ_H = 2.60, overlapped; 2.23 ppm, dd, *J* = 14.3, 7.5 Hz) to three quaternary carbons at δ_C = 168.7 (C-7′), 61.4 ppm (C-1′), and 64.9 ppm (C-2′), as well as H-4′ (δ_H = 2.44, dd, *J* = 15.2, 6.1 Hz; 2.10 ppm, d, *J* = 15.2 Hz) to C-1′, C-2′, C-3′ (δ_C = 65.3), and C-13′ (δ_C = 64.0) (Figure 3). The above results in conjunction with the elemental composition revealed that the double bond of C-2′ and C-3′ was oxygenated into an epoxy ring. This enabled the completion of the planar structure of fragment B. Further analyses of the ROESY data enabled the relative configuration of **3**, as described in Figure 3, simply as correlations between H-2′ (δ_H = 3.77 ppm, s) and H-8′, H-2′ and H-13′, as well as H-2′ and H-5′ (Figure 24S in the Supporting Information). Finally, to satisfy the requirement of the molecular weight, fragments A and B were connected by an ester linkage between the hydroxyl group of the oxime substituent in fragment A and the carbonyl (C-7′) in fragment B, which was also supported by the disappearance of the oxime hydroxyl of fragment A and the upfield shift of C-7′ (δ_C = 168.7 ppm).The additional observed HMBC and ^{1}H–^{1}H COSY correlations were completely consistent with the assignments.

Vibralactoxime D (**4**) was obtained as a yellow oil with the elemental composition $C_{24}H_{28}N_2O_5$ (twelve degrees of unsaturation), which was determined by HR EI-MS (ion peak at *m/z*: 424.1998, calcd 424.1987). In the ^{13}C NMR and DEPT spectra, the signals were present in pairs, implying that **4** might be a dimer. Detailed analyses of the 1D and 2D NMR spectroscopic data showed that a moiety of **4** was the same as fragment A of **3**, while the other part possessed similar signal characteristics to the β-lactone-opened vibralactone, except for the substituent at C-3′ (Table 2). In this moiety, key HMBC correlations from the proton at δ_H = 6.82 ppm (s, H-2′) to two quaternary carbons at δ_C = 115.0 (s, C-3′) and 116.8 ppm (s, C-13′) (Figure 32S in the Supporting Information) suggested that the substituent at C-3′ was a cyano group (Figure 3), which was biologically produced through the elimination of a water molecule of an oxime group. The infrared (IR) absorption bands at 2225 cm^{-1} confirmed the presence of the cyano group (Figure 35S in the Supporting Information).[19] Considering the overall formula of **4**, an ester bond was determined to be between the oxime hydroxyl and C-7′, as described in **3**.

Vibralactoxime E (**5**) was obtained as a yellow oil. Its molecular formula was established to be $C_{36}H_{43}N_3O_8$ according to the pseudomolecular ion peak at *m/z* 668.2938 $[M+Na]^+$ (calcd for $C_{36}H_{43}N_3O_8Na$, 668.2942) by HR ESI-MS. The trimeric structural features of **5** were based on the comparison between the 1D and 2D NMR data of **4** and **5**, as well as the requirement of molecular weight and IR spectroscopy (Figure 44S in the Supporting Information), which revealed that two moieties of this trimer were the same as those of **4**. Another moiety that was characterized by an opened β-lactone group and invisible oxime hydroxyl signal was constructed from the remaining signals. These data led to the connectivity of the aforementioned three moieties of **5**, as shown in Figure 1, for which we proposed the name vibralactoxime E.

Vibralactoxime F (**6**) presented a pseudomolecular ion peak at *m/z* 452.2046 $[M+Na]^+$ in the HR ESI-MS spectrum, indicating a molecular formula of $C_{24}H_{31}NO_6$ (calcd for $C_{24}H_{31}NO_6Na$, 452.2044). Preliminary analyses of its 1D and HSQC data suggested the presence of four methyl singlets (δ_H=1.58, 1.66, 1.67, 1.71 ppm; δ_C=18.1, 18.0, 26.1, 25.9 ppm), five methylenes (one was oxygenated), seven methines, and eight quaternary carbons (two were sp^3 hybridized and six were sp^2 hybridized). Detailed analyses of the HMBC and ^1H–^1H COSY spectra revealed the presence of two isolated vibralactone moieties which corresponded to **1** and β-lactone-opened vibralactone, respectively. Finally, an ester linkage was assigned between the oxime hydroxyl of the first unit and the carbonyl of the second unit. The relative configuration in the second unit was elucidated by ROESY correlations from H-5′ (δ_H=4.29 ppm, br. t, J=6.5 Hz) to H-8′ (δ_H=2.65, overlapped; 2.21 ppm, dd, J=14.3, 8.0 Hz).

The molecular formula of vibralactoxime G (**7**) was established as $C_{36}H_{46}N_2O_9$ by HR ESI-MS at *m/z* 673.3104 $[M+Na]^+$, which was 221 mass units greater than that of vibralactoxime F (**6**), indicating that **7** was a trimer. This assumption was confirmed by 1D and 2D NMR spectral analyses. The ^1H–^1H COSY and HMBC spectra displayed three isolated moieties, which belonged to two vibralactoxime A (**1**) scaffolds and a vibralactone scaffold. The upfield shift of two carbonyls (δ_C=170.6 and 169.1 ppm) and the disappearance of two oxime hydroxyls, as well as the active hydrogen at δ_H=3.91 ppm (t, J=5.5 Hz), which correlated to a methylene at δ_H=4.12 (dd, J=14.0, 5.5, H-13″a) and 4.13 ppm (dd, J=14.0, 5.5, H-13″b) in ^1H–^1H COSY spectrum, suggested that the β-lactone groups of two units were opened and had been esterified, and that the vibralactone component is the tail unit. Therefore, the sequence of the aforementioned three moieties and the connected positions of **7** are described in Figure 1.

Vibralactoxime H–L (**8**–**12**) were isolated as yellow gums and identified as a group of polyether derivatives ranging from dimer to hexamer. The molecular formulas of compounds **8** to **12** were assigned as $C_{24}H_{30}N_2O_6$, $C_{36}H_{45}N_3O_9$, $C_{48}H_{60}N_4O_{12}$, $C_{60}H_{75}N_5O_{15}$, and $C_{72}H_{90}N_6O_{18}$, respectively (Figures 68S, 75S, 86S, 89S, and 92S in the Supporting Information). These molecular weights generated a class of arithmetic sequence with a common difference of 221 mass units, which equaled $C_{12}H_{15}NO_3$, just as a vibralactoxime A (**1**) moiety. It was possible to identify this group of compounds by ^1H NMR spectra and HR MS analyses. The downfield of the ^1H NMR spectra of these compounds could be classified into three characteristic signal areas: a) peaks at approximately 10.2 ppm, the oxime hydroxyl signal was roughly displayed as a singlet in this area; b) peaks distributed in 7.9–8.5 ppm, the methines of the oxime double bond (H-13) exhibited in this area. The rules for this area were that H-13 of the first part of these polymers was mainly present as singlets at δ_H=8.42 ppm, while H-13 of the last part was presented as singlets at δ_H=7.95 ppm, and the others appeared as overlapped signals at approximately δ_H=8.36 ppm; c) the singlets that appeared at 6.10–6.60 ppm were associated with H-2. In vibralactoxime A (**1**), H-2 was a singlet at δ_H=6.04 ppm. When it polymerized, the H-2 of the first moiety shifted downfield to 6.46 ppm, the last moiety shifted downfield to 6.11–6.13 ppm, and the other moieties shifted downfield to 6.54 ppm as a cluster of signals (Figure 4). The number of polymer units could be easily deduced from the number of the integration of hydrogen atoms in the (b) and (c) areas, as well as the mass spectra. Although there is a lack of ^{13}C NMR spectra due to the scarcity of compounds **11** and **12**, all these data could enable completion of the structures of **8** to **12**, for which we proposed the name vibralactoxime H–L (Figure 1).

The chemical formula of vibralactoxime M–P (**13**–**16**), a group of polymers ranging from dimer to pentamer, were assigned by HR ESI-MS/HR EI-MS as $C_{25}H_{34}N_2O_7$, $C_{37}H_{49}N_3O_{10}$, $C_{49}H_{64}N_4O_{13}$, and $C_{61}H_{79}N_5O_{16}$, respectively (Figure 100S, 108S, 111S, and 114S in the Supporting Information). Compounds **15** and **16** were presented in limited quantities for their ^{13}C NMR experiments. The differences between these polymers and vibralactoxime H–L (**8**–**12**) were that the β-lactone group of the first scaffold was opened, and the carboxyl had been methyl esterified. The other parts of these polymers were the same as their counterparts, in which the β-lactone group remained unopened.

The biogenetic pathway of vibralactone has previously been illustrated.[16b] Here, we present a proposed biogenetic pathway of all the compounds by referring to the biosynthetic pathway of vibralactone (Scheme 2). It is hypothesized that *p*-hydroxybenzoyl methylamine was prenylated and oxidized by a series of oxidation reactions to produce vibralactamine (an assumed intermediate).[20] According to the literature, the oxime group is oxidized from an amino group, which was catalyzed by an open reading frame, NcoL.[21] Therefore, the amino group of vibralactamine was oxygenated followed by a dehydration process to produce vibralactoxime A (**1**). Vibralactoxime A could serve as a precursor to produce the other compounds. Because we have not yet isolated any C-13 cyano vibralactone monomers, it was hypothesized that the dehydration procedure from oxime to cyano group was performed after the polymerization.

Because natural products containing an oxime group are rarely encountered, their bioactivities greatly aroused our curiosity. Vibralactone exerted pancreatic lipase inhibitory activity through nucleophilic attack on its β-lactone group by the serine residue of pancreatic lipase to form a stoichiometric acyl-enzyme complex.[22] Some of the vibralactoxime still pos-

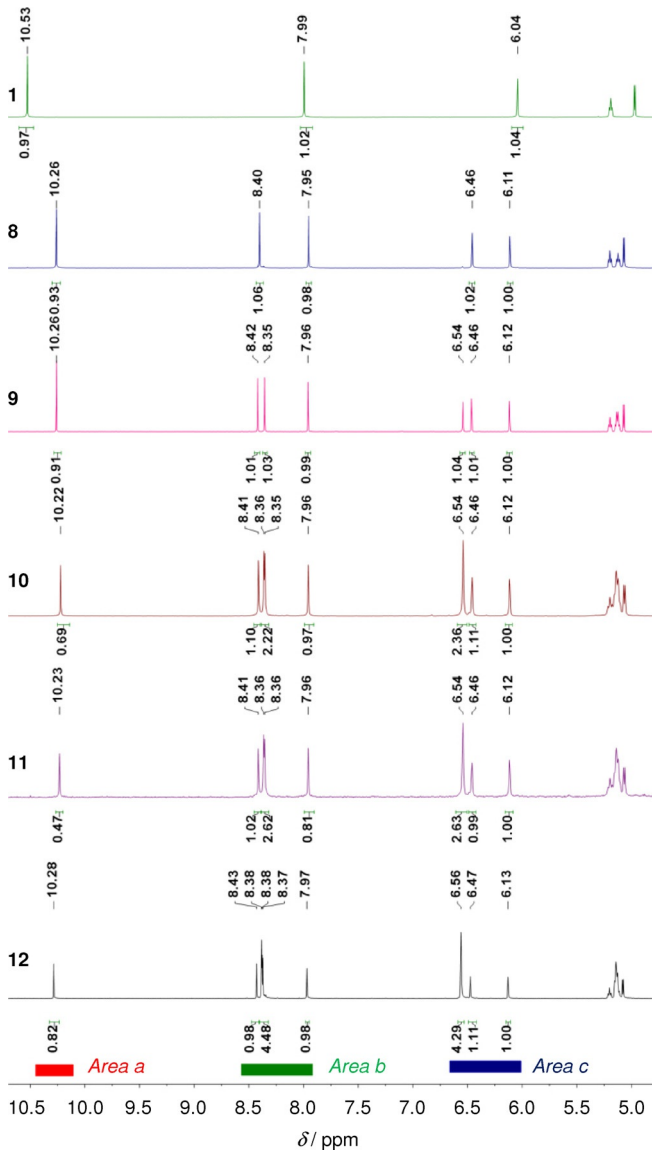


Figure 4. Chemical shifts ($\delta = 10.7–4.8$ ppm) of ^{1}H NMR spectra of compounds **1**, **8**–**12**.

sessed the β-lactone function, which is the reason why we tested their pancreatic lipase inhibitory activities. Interestingly, compounds **1**, **4**, **5**, **7**, **9**, **10**, and **11** exhibited stronger pancreatic lipase inhibitory activities than vibralactone, which was used as one of the positive controls (the other positive control was orlistat); compounds **6** and **12** exhibited low activities, and the other opened β-lactone group compounds were inactives (Table 3 and Page 7 in the Supporting Information). From the results we could postulate that a) the cleavage of the β-lactone function led to inactivity on pancreatic lipase, b) the existence of the oxime group could enhance the pancreatic lipase inhibitory activities, and c) it was more likely that the molecular lengths were related to the activities to a large extent because the pentamer vibralactoxime K (**11**) ranked first in terms of pancreatic inhibitory activity.

Moreover, all these compounds were evaluated for their cytotoxicities against five human cancer cell lines (promyelocytic leukemia HL-60, hepatoma SMMC-7721, lung adenocarcinoma A-549, breast adenocarcinoma MCF-7, and colon adenocarcinoma SW480 cells) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay, as previously reported.[23] Notably, compounds **7**, **9**, and **10** exhibited significant cytotoxicities against HL-60, MCF-7, and SW480 with IC_{50} values comparable with those of cisplatin. Compounds **4**–**6**, **8**, **11**, **12**, **14**, and **15** exhibited moderate cytotoxic activities (Table 4).

Table 3. Pancreatic lipase inhibitory activities (IC_{50}) of compounds **1**, **4**, **5**, **7**, **9**–**11**.

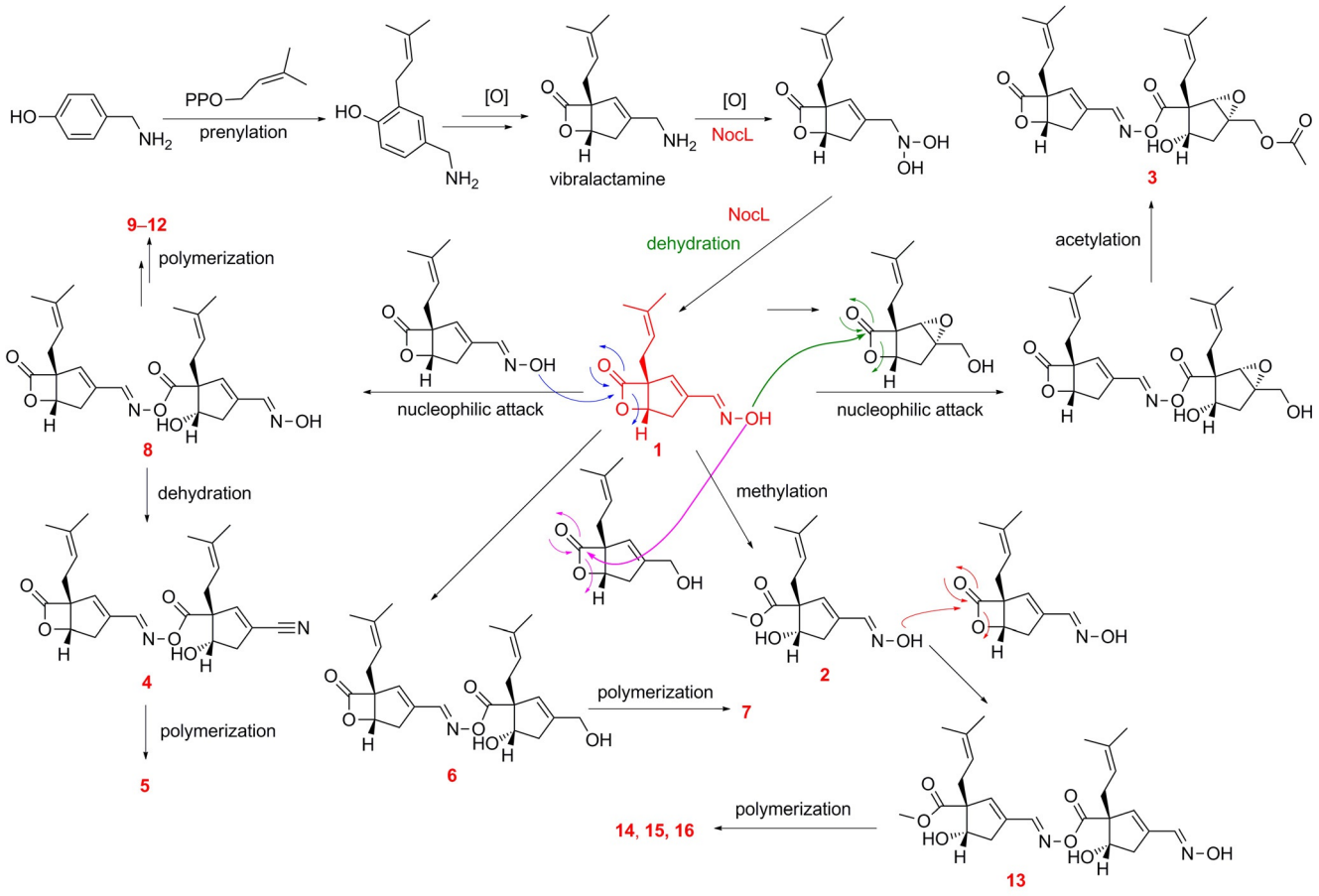
Compound	IC_{50} [μmol]	Compound	IC_{50} [μmol]
1	23.1	**10**	19.8
4	20.6	**11**	11.1
5	28.6	Vibralactone	48.7
7	16.8	Orlistat	0.0018
9	23.2		

Table 4. Cytotoxicities of compounds **4**–**10**, **12**, **14**, **15**.

	IC_{50} [μmol]				
Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
4	14.5	29.5	>40	29.7	>40
5	7.6	14.6	15.7	15.6	16.1
6	12.1	18.5	24.6	21.3	17.4
7	2.9	12.9	12.2	15.3	7.5
8	10.9	13.0	18.6	17.7	17.4
9	3.1	13.2	14.2	9.4	8.1
10	3.4	14.3	15.0	14.4	8.0
11	14.4	21.6	27.8	20.5	16.0
12	34.2	>40	33.5	>40	>40
14	15.7	21.4	23.9	30.2	>40
15	16.3	20.1	33.7	24.5	>40
Cisplatin	1.2	4.5	6.2	15.2	12.0
Taxol	<0.008	<0.008	<0.008	<0.008	<0.008

Conclusion

A further chemical investigation of the culture broth of *B. vibrans* led to the discovery of sixteen structurally and biogenetically novel oxime-group-containing vibralactone derivatives. Notably, this work exemplified oxime-group-bearing natural products discovered from higher fungi for the first time. Vibralactoxime A (**1**) was a building block assembling various polymers with diverse activities, including pancreatic lipase inhibitory activity and cytotoxicity. A postulated biosynthetic pathway for these compounds was provided in Scheme 1. Their diverse bioactivities have broadened our horizons in structural modifications of vibralactone for developing stronger bioactive molecules. Moreover, further biosynthetic effort should shed light on the proposed biosynthetic pathway for these compounds.



Scheme 2. Plausible biosynthetic pathway for compounds **1**–**16**.

Experimental Section

General

Optical rotations (OR) were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV/Vis spectra were obtained using a Shimadzu UV2401PC spectrometer (Shimadzu, Kyoto, Japan). CD spectra were tested on an Applied Photophysics Chirascan Circular Dichroism Spectrometer (Applied Photophysics Limited, Leatherhead, Surrey, UK). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA) with KBr pellets. HR EI-MS were recorded on a Waters AutoSpec Premier P776 instrument (Waters, Milford, MA, USA). HR ESI-MS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were measured on a Bruker Avance III 600 MHz spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, P. R. China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Medium-pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipped with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). Preparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with two type of Zorbax SB-C18 columns (9.4 mm×150 mm and 21.2 mm×150 mm, particle size 5 μm).

Five human cancer cell lines were used to evaluate the cytotoxicities of these isolated compounds: the HL-60 (ATCC CCL-240) human myeloid leukemia cell line, the SMMC-7721 human hepatocellular carcinoma cell line, the A549 (ATCC CCL-185) lung cancer cell line, the MCF-7 (ATCC HTB-22) breast cancer cell line, and the SW-480 (ATCC CCL-228) human colon cancer cell line. The cell line SMMC-7721 was bought from China Infrastructure of Cell Line Resources (Beijing, China), and others were bought from American Type Culture Collection (ATCC, Manassas, VA, USA)

Fungus material and cultivation conditions

The fungus *B. vibrans* was collected in Kunming Botanical Garden. A voucher specimen was deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (No. 20120920B). The culture medium consisted of potato (200.0 g), glucose (20.0 g), KH_2PO_4 (3.0 g), $MgSO_4$ (1.5 g), vitamin B_1 (10 mg), peptone from porcine meat (1.0 g), citric acid (0.1 g) in deionized water (1.0 L). The pH was adjusted to 6.0–6.5 before autoclaving 3400 bottles of 500 mL Erlenmeyer flasks, each containing 350 mL of above-mentioned culture medium, which were then inoculated with *B. vibrans* strains. Fermentation was carried out on a shaker at 150 rpm for 25 d in darkness seven times.

Extraction and isolation

The culture broth of *B. vibrans* (1200 L) was filtered to separate the culture fluid and mycelia. The filtrate was concentrated in vacuo then extracted with EtOAc four times (total 200 L). Meanwhile, the mycelia were soaked with $CHCl_3$/MeOH (1:1) (total 90 L, 3×30 L, 3 d), and the extraction was evaporated under reduced pressure then partitioned between EtOAc and water to give an EtOAc layer

(90 L total, 5×18 L). The combination of total EtOAc solution (290 L) was concentrated under reduced pressure to give a crude extract of 1218 g. Then this residue was subjected to column chromatography over silica gel (200–300 mesh) eluting with a gradient of petroleum ether/acetone (10:1 → 0:1) to give five fractions (A–H). Fraction B (6.4 g) was separated by Sephadex LH-20 CC (MeOH) followed by preparative HPLC (CH_3CN/H_2O, 40:60 → 70:30, 40 min, 20 mL min^{-1}) to give four subfractions (B1–B4). Subfraction B1 (160 mg) was purified by preparative HPLC (CH_3CN/H_2O, 35:65 → 55:45, 35 min, 10 mL min^{-1}) to yield **10** (12 mg, 9.85×10^{-6} % *w/w*),[24] **11** (26 mg, 2.13×10^{-5} % *w/w*), **14** (3 mg, 2.46×10^{-6} % *w/w*), **15** (3 mg, 2.46×10^{-6} % *w/w*). Subfraction B2 (30 mg) was separated using preparative HPLC (CH_3CN/H_2O, 45:55 → 65:35, 30 min, 10 mL min^{-1}) to give **12** (0.9 mg, 7.39×10^{-7} % *w/w*), **16** (0.6 mg, 4.93×10^{-7} % *w/w*). Fraction C (58.5 g) was separated by MPLC eluting with MeOH/H_2O (20:80 → 100:0) to afford four subfractions (C1–C8). Subfraction C2 (16.8 g) was purified by Sephadex LH-20 (acetone) to yield vibralactone (15.1 g). Subfraction C3 (4.1 g) was separated by preparative HPLC (CH_3CN/H_2O, 30:70 → 60:40, 30 min, 20 mL min^{-1}) to obtain **1** (25 mg, 2.05×10^{-5} % *w/w*), **2** (13 mg, 1.07×10^{-5} % *w/w*), **3** (0.5 mg, 4.11×10^{-7} % *w/w*), and **6** (10 mg, 8.21×10^{-6} *w/w*). Subfraction C6 (1.5 g) was separated using preparative HPLC (CH_3CN/H_2O, 36:64 to 56:44, 30 min, 20 mL min^{-1}) to afford **4** (9 mg, 7.39×10^{-6} *w/w*), **5** (1 mg, 8.21×10^{-7} % *w/w*), **7** (4 mg, 3.28×10^{-6} % *w/w*), **8** (4 mg, 3.28×10^{-6} % *w/w*), **9** (6 mg, 4.93×10^{-6} % *w/w*), **13** (11 mg, 9.03×10^{-6} % *w/w*).

Acknowledgements

This work was financially supported by the National Key Technology Support Program, China (2013BAI11B02) and National Natural Science Foundation of China (81561148013 and 81373289).

Keywords: cytotoxicity · natural products · oximes · oxime esters · pancreatic lipase · structure elucidation

[1] a) M. Raipuria, G. O. Hardy, H. Bahari, M. J. Morris, *Nutr. Metab. Cardiovas.* **2015**, *25*, 881–888; b) G. Twig, H. C. Gerstein, D. B.-A. Shor, E. Derazne, D. Tzur, A. Afek, A. Tirosh, *Eur. J. Endocrinol.* **2015**, *173*, 305–312.

[2] O. Saidi, M. O'Flaherty, N. B. Mansour, W. Aissi, O. Lassoued, S. Capewell, J. A. Critchley, D. Malouche, H. B. Romdhane, *BMC Public Health* **2015**, *15*, 104.

[3] P. J. Mork, K. L. Vik, B. Moe, R. Lier, E. M. Bardal, T. I. L. Nilsen, *Cent. Eur. J. Public Health* **2014**, *24*, 924–929.

[4] E. Chorin, A. Hassidim, M. Hartal, O. Havakuk, N. Flint, T. Ziv-Baran, Y. Arbel, *Am. J. Hypertens.* **2015**, *28*, 1157–1163.

[5] E. E. Calle, R. Kaaks, *Nat. Rev. Cancer* **2004**, *4*, 579–591.

[6] World Health Organization, Obesity and overweight, **2015**; http://www.who.int/mediacentre/factsheets/fs311/en/; Accessed September 15, 2015.

[7] Y. Xia, C. M. Kelton, J. J. Guo, B. Bian, P. C. Heaton, *Obesity* **2015**, *23*, 1721–1728.

[8] Food and Drug Administration, FDA announces withdrawal of fenfluramine and dexfenfluramine (fen-phen), **1997**; http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm179871.htm; Accessed September 15, 2015.

[9] Food and Drug Administration, FDA Drug Safety Communication: Completed safety review of Xenical/Alli (orlistat)and severe liver injury **2010**; http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm213038.htm; Accessed September 15, 2015.

[10] a) D. B. S. Brashier, A. K. Sharma, N. Dahiya, S. K. Singh, A. Khadka, *J. Pharmacol. Pharmacother.* **2014**, *5*, 175–178; b) L. Costantino, D. Barlocco, *Future Med. Chem.* **2015**, *7*, 315–336.

[11] a) N. G. Heatley, M. A. Jennings, H. W. Florey, *Br. J. Exp. Pathol.* **1947**, *28*, 35–46; b) F. Comer, F. McCapra, I. Qureshi, A. Scott, *Tetrahedron* **1967**, *23*, 4761–4768; c) F. W. Comer, F. McCapra, I. H. Qureshi, J. Trotter, A. I. Scott, *Chem. Commun. (London)* **1965**, 310–311.

[12] W. A. Ayer, M. H. Saeedighomi, *Can. J. Chem.* **1981**, *59*, 2536–2538.

[13] G. Li, F. Liu, L. Shen, H. Zhu, K. Zhang, *J. Nat. Prod.* **2011**, *74*, 296–299.

[14] a) M. Isaka, U. Srisanoh, W. Choowong, T. Boonpratuang, *Org. Lett.* **2011**, *13*, 4886–4889; b) Q. Y. Qi, L. Bao, J. W. Ren, J. J. Han, Z. Y. Zhang, Y. Li, Y. J. Yao, R. Cao, H. W. Liu, *Org. Lett.* **2014**, *16*, 5092–5095; c) Q. Y. Qi, J. W. Ren, L. W. Sun, L. W. He, L. Bao, W. Yue, Q. M. Sun, Y. J. Yao, W. B. Yin, H. W. Liu, *Org. Lett.* **2015**, *17*, 3098–3101.

[15] For our studies on *Boreostereum vibrans*, see: a) H. P. Chen, Z. Z. Zhao, R. H. Yin, X. Yin, T. Feng, Z. H. Li, K. Wei, J. K. Liu, *Nat. Prod. Bioprospect.* **2014**, *4*, 271–276; b) M. Y. Jiang, F. Wang, X. L. Yang, L. Z. Fang, Z. J. Dong, H. J. Zhu, J. K. Liu, *Chem. Pharm. Bull.* **2008**, *56*, 1286–1288; c) M. Y. Jiang, L. Zhang, Z. J. Dong, Z. L. Yang, Y. Leng, J. K. Liu, *Chem. Pharm. Bull.* **2010**, *58*, 113–116; d) D. Z. Liu, F. Wang, T. G. Liao, J. G. Tang, W. Steglich, H. J. Zhu, J. K. Liu, *Org. Lett.* **2006**, *8*, 5749–5752; e) G. Q. Wang, K. Wei, L. Zhang, Z. H. Li, Q. A. Wang, J. K. Liu, *J. Asian Nat. Prod. Res.* **2014**, *16*, 447–452; f) G. Q. Wang, K. Wei, T. Feng, Z. H. Li, L. Zhang, Q. A. Wang, J. K. Liu, *J. Asian Nat. Prod. Res.* **2012**, *14*, 115–120; g) G. Q. Wang, K. Wei, Z. H. Li, T. Feng, J. H. Ding, Q. A. Wang, J. K. Liu, *J. Asian Nat. Prod. Res.* **2013**, *15*, 950–955.

[16] a) K. Wei, G. Q. Wang, X. Bai, Y. F. Niu, H. P. Chen, C. N. Wen, Z. H. Li, Z. J. Dong, Z. L. Zuo, W. Y. Xiong, J. K. Liu, *Nat. Prod. Bioprospect.* **2015**, *5*, 129–157; b) P. J. Zhao, Y. L. Yang, L. Du, J. K. Liu, Y. Zeng, *Angew. Chem. Int. Ed.* **2013**, *52*, 2298–2302; *Angew. Chem.* **2013**, *125*, 2354–2358.

[17] a) F. Cardoso-Martínez, J. M. de La Rosa, A. R. Díaz-Marrero, J. Darias, L. D'Croz, C. Cerella, M. Diederich, M. Cueto, *Eur. J. Org. Chem.* **2015**, 2256–2261; b) C. Almeida, N. Part, S. Bouhired, S. Kehraus, G. M. Koenig, *J. Nat. Prod.* **2011**, *74*, 21–25; c) N. Bjarnholt, B. L. Møller, *Phytochemistry* **2008**, *69*, 1947–1961; d) P. Moya, M. Castillo, E. Primo-Yúfera, F. Couillaud, R. Martínez-Máñez, M.-D. Garcerá, M. A. Miranda, J. Primo, R. Martínez-Pardo, *J. Org. Chem.* **1997**, *62*, 8544–8545; e) L. Calcul, W. D. Inman, A. A. Morris, K. Tenney, J. Ratnam, J. H. McKerrow, F. A. Valeriote, P. Crews, *J. Nat. Prod.* **2010**, *73*, 365–372; f) K. Shindo, Y. Yamagishi, Y. Okada, H. Kawai, *J. Antibiot.* **1994**, *47*, 1072–1074; g) T. Hertiani, R. Edrada-Ebel, S. Ortlepp, R. W. M. van Soest, N. J. de Voogd, V. Wray, U. Hentschel, S. Kozytska, W. E. G. Mueller, P. Proksch, *Bioorg. Med. Chem.* **2010**, *18*, 1297–1311; h) T. Ogamino, S. Nishiyama, *J. Synth. Org. Chem. Jpn.* **2005**, *63*, 583–593; i) P. Amade, M. Mallea, N. Bouaicha, *J. Antibiot.* **1994**, *47*, 201–207; j) M. Hashimoto, T. Komori, T. Kamiya, *J. Am. Chem. Soc.* **1976**, *98*, 3023–3025; k) M. Hashimoto, T. Komori, T. Kamiya, *J. Antibiot.* **1976**, *29*, 890–901; l) H. Yagi, S. Matsunaga, N. Fusetani, *Tetrahedron* **1993**, *49*, 3749–3754.

[18] R. A. Aungst, Jr., C. Chan, R. L. Funk, *Org. Lett.* **2001**, *3*, 2611–2613.

[19] M. De Zotti, S. Bobone, A. Bortolotti, E. Longo, B. Biondi, C. Peggion, F. Formaggio, C. Toniolo, A. Dalla Bona, B. Kaptein, L. Stella, *Chem. Biodiversity* **2015**, *12*, 513–527.

[20] P. M. Dewick in *Medicinal Natural Products: A Biosynthetic Approach*, John Wiley & Sons, Hoboken, **2009**, pp. 20–21, 474–478.

[21] a) W. L. Kelly, C. A. Townsend, *J. Am. Chem. Soc.* **2002**, *124*, 8186–8187; b) C. A. Townsend, G. M. Salituro, *J. Chem. Soc. Chem. Commun.* **1984**, 1631–1632.

[22] A. Bénarouche, V. Point, F. Carrière, J. F. Cavalier, *Biochimie* **2014**, *101*, 221–231.

[23] a) M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589–601; b) J. J. Zhang, X. W. Yang, J. Z. Ma, X. Liu, L. X. Yang, S. C. Yang, G. Xu, *Nat. Prod. Bioprospect.* **2014**, *4*, 73–79.

[24] The percent yield of these final compounds were based on the weight of total extract.

Received: October 14, 2015
Published online on January 13, 2016

