Organic LETTERS

Letter

pubs.acs.org/OrgLett

Curvulamine, a New Antibacterial Alkaloid Incorporating Two Undescribed Units from a *Curvularia* Species

Wen Bo Han,[†,‡,&] Yan Hua Lu,[§,&] Ai Hua Zhang,[†,‡] Gao Fei Zhang,[†] Ya Ning Mei,[∥] Nan Jiang,[⊥] Xinxiang Lei,[#] Yong Chun Song,[†] Seik Weng Ng,[▽,⊗] and Ren Xiang Tan*[,†]

[†]Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, China

[‡]State Key Laboratory of Elemento-organic Chemistry, Nankai University, Tianjin 300071, China

[§]State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai 200237, China

[∥]Department of Clinical Laboratory, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

[⊥]School of Pharmacy, Nanjing Medical University, Nanjing 210029, China

[#]College of Chemistry & Materials Engineering, Wenzhou University, Wenzhou 325035, China

[▽]Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

[⊗]Chemistry Department, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah, Saudi Arabia

Supporting Information

ABSTRACT: The white croaker (*Argyrosomus argentatus*) derived *Curvularia* sp. IFB-Z10 produces curvulamine as a skeletally unprecedented alkaloid incorporating two undescribed extender units. Curvulamine is more selectively antibacterial than tinidazole and biosynthetically unique in the new extenders formed through a decarboxylative condensation between an oligoketide motif and alanine.

The possibility that humans might eventually win the "war" against human pathogenic microorganisms becomes more remote owing to the rapidly developed pathogens' resistance to the prescribed antibiotics.[1] Bacterial pathogens such as *Streptococcus* sp., *Bacteroides* sp., and *Peptostreptococcus* sp. are emerging (or re-emerging) as growing threats to human health in many regions of the world.[2−4] Accordingly, there is an urgent need for novel reagents to combat infections by these pathogens. Novel agents with potent bioactivities have recently been found in microbes that grow in a relatively harsh marine environment, characterized by high salinity, scarce nutrients, and high osmotic and hydraulic pressures.[5−10] Among the richest sources of these compounds are marine symbionts that coexist with symptomless fish, sponges, algae, and soft corals;[9] this does reflect the fact that genomes of the symbionts have the ability to undergo rapid and dynamic change, as they coevolve with their hosts.[11,12]

To local fishers' observation, white croaker (*Argyrosomus argentatus*) is infrequently infected by microbial pathogens that may readily infect other fishes in the same aquatic habitat, and it usually remains healthy after feeding on infected or dead prey. One possible explanation for these observations is that the gut flora of white croaker includes symbiont(s) that provide antimicrobial defense, as observed in other competitive marine species.[13,14] Our previous study reported isolation and characterization of antifungal compounds from the fungal symbiont *Myrothecium* sp. Z16 that derived from white croaker.[15] Encouraged by the discovery, the endogenous microbes isolated from white croaker were recultivated, and the ethyl acetate (EtOAc) extracts derived from the cultures were analyzed by ^{1}H NMR, LC−MS, and bacterial inhibition assays. Interestingly, the extract from *Curvularia* sp. IFB-Z10 displayed potent antibacterial activity because of the fungal biosynthesis of the novel alkaloid named curvulamine (**1**), which are active against *Veillonella parvula, Streptococcus* sp., *Bacteroides vulgatus*, and *Peptostreptococcus* sp. with minimum inhibitory concentrations (MICs) at 0.37 μM.

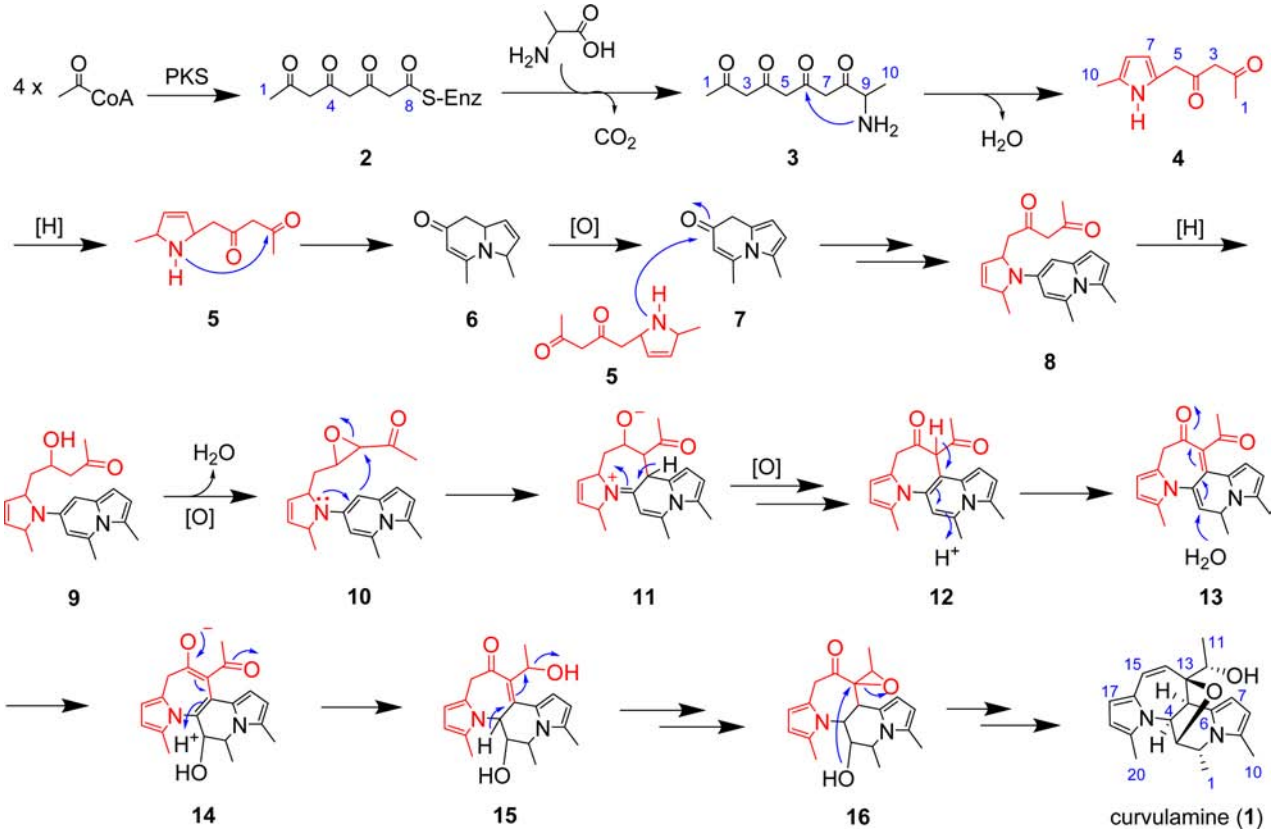
Chromatographic fractionation of the EtOAc extract derived from the culture of *Curvularia* sp. IFB-Z10 gave a dinitrogenated alkaloid named curvulamine (**1**). Compound **1** possesses the novel carbon skeleton constructed from 1-(5-methyl-1*H*-pyrrol-2-yl)pentane-2,4-dione (**4**) and 3,5-dimethylindolizin-7(8*H*)-one (**7**) through an unexpected combination of inter- and intramolecular additions (Scheme 1).

Curvulamine (**1**) has the molecular formula $C_{20}H_{24}N_2O_2$, which in its Na^+-liganded form, corresponds to a peak at m/z 347.1733 in its high-resolution electrospray ionization mass

Received: September 1, 2014
Published: October 6, 2014

dx.doi.org/10.1021/ol502572g | *Org. Lett.* 2014, 16, 5366−5369

Scheme 1. Possible Biosynthetic Pathway to 1: Involvement of Two New Oligoketide Extender Units 4 and 7



spectrometry (HR-ESI-MS). This compares with calculated *m*/*z* 347.1730 for $C_{20}H_{24}N_2O_2Na$. A pair of 2,5-disubstituted pyrrole nuclei was indicated by the doublets at δ_H 6.12 and 5.94 (J = 3.5 Hz) and δ_H 5.93 and 5.91 (J = 3.0 Hz) in the ^{1}H NMR spectrum of **1** (Table S1, Supporting Information). Two more doublets at δ_H 5.72 and 6.45 correspond to a *cis*-disubstituted vinyl group (substructure **a**) with the coupling constant magnitude (J = 12.0 Hz) suggesting the inclusion of the double bond in an acyclic chain or a seven-membered (or larger) ring.[16] The assignments in the ^{1}H NMR spectrum of **1** were confirmed by its ^{1}H−^{1}H COSY, which identified two more coupling sequences: the 1-substituted ethyl and 1,2,3,4-tetrasubstituted pentyl groups (substructures **c** and **e**) and the 2-methylation of two pyrrole moieties (substructures **d** and **f**). The two pyrrolic proton doublets at δ_H 5.94 and 5.93 showed allylic couplings with two broadened methyl singlets at δ_H 2.28 and 2.30, respectively. The ^{13}C NMR spectrum of **1** also revealed an oxygenated quaternary carbon (substructure **b**) resonating at δ_C 89.3, which supports the existence of substructures in this alkaloid. Compound **1** was further analyzed by HSQC, ROESY, and HMBC experiments, leading to the unequivocal assignment of all ^{1}H and ^{13}C NMR signals. With the index of hydrogen deficiency of the molecular formula in mind, the formulated connection of **a**−**f** was defined by the key HMBC correlations, namely H-15 to C-13 and C-17, H-14 to C-5 and C-12, H-5 to C-7, H-4 to C-19, and H-2 to C-4 and C-9 (Figure 1 and Table S1, Supporting Information). The ether linkage between C-3 and C-13 was demonstrated by the HMBC cross-peak from H-3 to C-13. On the basis of the molecular formula, the presence of a 12-hydroxy group is postulated, although the 12-methine had unexpected resonances at δ_H 2.67 and δ_C 70.2. Inspection of the molecular

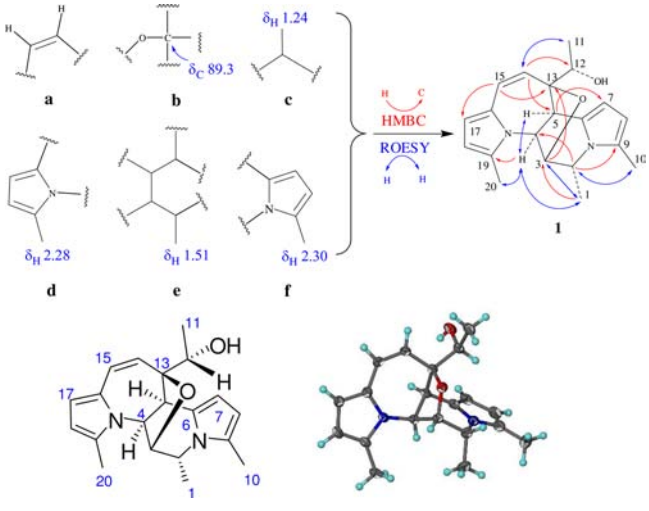


Figure 1. Substructures **a**−**f** in curvulamine (**1**) (upper) and its stereochemical structure (lower).

model pinpointed that H-12 of **1** was oriented toward the diamagnetic area of the pyrrole ring. This situation was previously discerned with daeschol A and dalesconols A−C, whose H-21 signals are upfield shifted significantly, owing to the positioning of the protons in the diamagnetic area of benzene rings.[6,7]

The relative stereochemical configuration of all chiral carbons but C-12 was determined from the ROESY correlations of **1** (Figure 1). Curvulamine (**1**) crystallizes from methanol as a monosolvate, $C_{20}H_{24}N_2O_2{\cdot}CH_3OH$. Room-temperature diffraction measurements were performed on a Bruker APEX DUO

dx.doi.org/10.1021/ol502572g | *Org. Lett.* 2014, 16, 5366−5369

diffractometer equipped with Cu radiation up to a $2\theta_{max}$ of 130°. The structure refined on 1741 out of 1843 $I \geq 2\sigma(I)$ reflections to an R index of 0.037; 947 Friedel pairs were merged (Figure 1).

Curvulamine (**1**) was shown to be antibacterial against patients-derived pathogens *Veillonella parvula*, *Actinomyces israelii*, *Streptococcus* sp., *Peptostreptococcus* sp., and *Bacteroides vulgatus* with the minimal inhibition concentrations (MICs) at 0.37 μM. In particular, curvulamine (**1**) was tested to be more potent than tinidazole (Table 1), a coassayed antibacterial drug

Table 1. Antibacterial Activity of 1 (MICs in μM)

pathogens	curvulamine (**1**)	tinidazole[a]
Veillonella parvula	0.37	0.49
Actinomyces israelii	>10	>10
Streptococcus sp.	0.37	1.01
Bacteroides vulgatus	0.37	2.02
Peptostreptococcus sp.	0.37	2.02

[a]Positive control.

prescribed in clinic.[17,18] Moreover, the antibacterial action of **1** seems more selective than tinidazole, which is also antifungal,[19] since the new alkaloid displayed at 10 μM no fungicidal activity against target fungi *Alternaria solani* ATCC11078, *Botrytis cinerea* ATCC20599, *Fusarium coeruleum* ATCC20088, *F. graminearum* ATCC20329, *F. moniliforme* ATCC90250, *Phytophthora capsici* ATCC 42668, *Rhizotonia cerealis* ATCC42606, *R. solani* ATCC 76090, and *Sclerotinia sclerotiorum* ATCC56844. In addition, we have patented the acetylcholinesterase inhibitory action of curvulamine.[20,21]

The potency and selectivity of curvulamine (**1**) in the antimicrobial action motivated us to extend our curiosity to the biosynthesis of the unusual curvulamine framework in the fungus. The structural feature of **1** facilitated our postulation that it might have been derived from the undescribed extender units **4** and **7** (Scheme 1).

To clarify whether the extender units originated from acetate-derived polyketides, *Curvularia* sp. IFB-Z10 was regrown separately in the presence of sodium [1-^{13}C]- and [2-^{13}C]-acetates. Fungal cultures were extracted, extracts fractionated, and the curvulamine products analyzed by ^{13}C NMR. When cultured with addition of [1-^{13}C]-acetate, curvulamine (**1**) displayed enriched ^{13}C signals of C-2, C-4, C-6, C-8, C-12, C-14, C-16, and C-18 (product **1a**; Figure 2); when cultured with exposure to [2-^{13}C]-acetate, compound **1** showed ^{13}C enrichments at C-1, C-3, C-5, C-7, C-11, C-13, C-15, and C-17 (product **1b**; Figure 2). However, no ^{13}C-labeling was observed at C-9, C-10, C-19 or C-20 in products **1a** and **1b** resulting from the [1-^{13}C]- or [2-^{13}C]-acetate supplemented cultures, respectively. This suggests that these carbons could be derived from another as yet unknown precursor.

The reaction mechanism of aminolevulinate synthase from fungi and animals[22,23] may be relevant in the incorporation of the four carbons into curvulamine molecule through the C−C formation between acyl CoA thioester and α-carbon of amino acid with concomitant release of carbon dioxide as discerned in the synthesis of aminolevulinate under the enzymatic catalysis.[24,25] By analogy, the four ^{13}C-acetate unlabellable carbons of curvulamine might derive from alanine. This hypothesis was tested by culturing *Curvularia* sp. IFB-Z10 in the presence of [2,3-^{13}C]-alanine followed by ^{13}C NMR analysis of the main curvulamine product **1**. As expected, ^{13}C

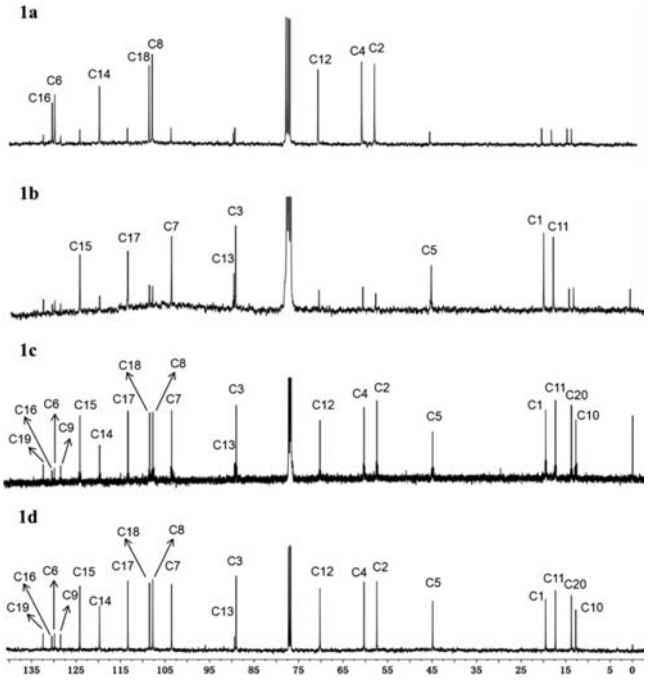


Figure 2. ^{13}C enrichment at specific positions of **1**. *Curvularia* sp. IFB-Z10 was grown in the presence of [1-^{13}C]- and [2-^{13}C]-acetates, and [2,3-^{13}C]-alanine, respectively, and extracts were analyzed by the ^{13}C NMR spectra of labeled products **1a**, **1b**, **1c** and nonlabeled product **1d**.

was enriched in C-9, C-10, C-19, and C-20 in product **1c** (Figure 2). However, a remarkable isotope enrichment was also observed in other resonance peaks because [2,3-^{13}C]-alanine is metabolized in fungi into [1,2-^{13}C]- acetate,[26] which happens to be the precursor for synthesis of product **1c**. This suggests as well that the alanine metabolism of this fungus is similar or identical to that of *Saccharomyces cerevisiae*.[26] Furthermore, ^{13}C-labeled product **1c** displayed the expected long-range ^{13}C−^{13}C couplings (Tables S2 and S3, Supporting Information).

D-Cycloserine is an inhibitor of serine palmitoyltransferase (SPT), a member of α-oxoamine synthase (AOS) that utilizes a pyridoxal 5′-phosphate (PLP) cofactor in catalyzing the Claisen-like condensation between an amino acid and an acyl CoA substrate.[27] *Curvularia* sp. IFB-Z10 was therefore recultured in the presence of D-cycloserine at 1.0 mM. As expected, alkaloid **1** disappeared in the D-cycloserine exposed fermentation (Figure S1, Supporting Information). By analogy, plumbagin, an inhibitor of 8-amino-7-oxononanoate synthase (AONS, another member of AOS),[28] was also shown to inhibit the production of **1** at 0.5 mM. On the basis of these enzyme inhibition tests, an AOS member is most presumably involved in the Claisen-like condensation between alanine and the tetraketide acyl CoA substrate in the fungus (Figure S1, Supporting Information). According to these data, the biosynthetic pathway of **1** was postulated in Scheme 1. As earlier key steps, an oligoketide-CoA (**2**) was assumed to be transformable into **3** through its Claisen-type condensation with alanine.[24,25] Compound **3** might undergo successive intramolecular additions to generate new extenders **4** and **7**.[29,30] Reduction of **4** gave **5** which coupled to **7** to afford **8**. Compound **1** might have produced by further derivation of **8** via presumed intermediates **9**−**16** (Scheme 1).

dx.doi.org/10.1021/ol502572g | *Org. Lett.* 2014, 16, 5366−5369

In conclusion, this work describes a novel alkaloid with potent antibacterial activity from a marine-derived *Curvularia* sp. IFB-Z10 and clarifies in particular the involvement of an oligoketide−alanine hybridization in its biosynthetic pathway according to the isotope-feeding experiments and enzyme inhibition tests. The unexpected carbon architecture of curvulamine is derived uniquely from undescribed extender units 1-(5-methyl-1*H*-pyrrol-2-yl)pentane-2,4-dione (**4**) and 3,5-dimethylindolizin-7(8*H*)-one (**7**) (Scheme 1). The work may add a novel starting molecule for new antibacterial drugs, which are urgently needed in the global "war" against human pathogenic bacteria. Meanwhile, the new extender-based framework opens collectively interesting topics such as the efficient generation and programed organization of nitrogenated extender units for novel bioactive alkaloids and biomimetic syntheses of the skeletally unprecedented compounds that may lead to new biology and biomedicine.

■ ASSOCIATED CONTENT

Supporting Information

General methods and details of isolation of metabolite, 1D and 2D NMR spectra, crystallographic file (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rxtan@nju.edu.cn.

Author Contributions

[&]These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The work was cofinanced by the MOST (2013AA092901), NSFC grants (81121062, 21132004, and 21072092).

■ REFERENCES

(1) D'Costa, V. M.; King, C. E.; Kalan, L.; Morar, M.; Sung, W. W. L.; Schwarz, C.; Froese, D.; Zazula, G.; Calmels, F.; Debruyne, R.; Golding, G. B.; Poinar, H. N.; Wright, G. D. *Nature* **2011**, *477*, 457−461.

(2) Kumar1, S.; Fox, B.; Owston, M.; Hubbard, G. B.; Dick, E. J., Jr *J. Med. Primatol.* **2012**, *41*, 266−277.

(3) Snydman, D. R.; Jacobus, N. V.; McDermott, L. A.; Golan, Y.; Hecht, D. W.; Goldstein, E. J. C.; Harrell, L.; Jenkins, S.; Newton, D.; Pierson, C.; Rihs, J. D.; Yu, V. L.; Venezia, R.; Finegold, S. M.; Rosenblatt, J. E.; Gorbach, S. L. *Clin. Infect. Dis.* **2010**, *50*, S26−33.

(4) Jarrya, J.; Shekherb, M.; Imperatoa, M.; Michela, P. *Clin. Res. Hepatol Gastroenterol.* **2011**, *35*, 765−767.

(5) Fang, W.; Ji, S.; Jiang, N.; Wang, W.; Zhao, G. Y.; Zhang, S.; Ge, H. M.; Xu, Q.; Zhang, A. H.; Zhang, Y. L.; Song, Y. C.; Zhang, J.; Tan, R. X. *Nat. Commun.* **2012**, *3*, 1039.

(6) Zhang, Y. L.; Zhang, J.; Jiang, N.; Lu, Y. H.; Wang, L.; Xu, S. H.; Wang, W.; Zhang, G. F.; Xu, Q.; Ge, H. M.; Ma, J.; Song, Y. C.; Tan, R. X. *J. Am. Chem. Soc.* **2011**, *133*, 5931−5940.

(7) Zhang, Y. L.; Ge, H. M.; Zhao, W.; Dong, H.; Xu, Q.; Li, S. H.; Li, J.; Zhang, J.; Song, Y. C.; Tan, R. X. *Angew. Chem., Int. Ed.* **2008**, *47*, 5823−5826.

(8) Wang, B.; Waters, A. L.; Sims, J. W.; Fullmer, A.; Ellison, S.; Hamann, M. T. *Microbiol Ecol.* **2013**, *65*, 1068−1075.

(9) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2013**, *30*, 237−323.

(10) Mei, W. L.; Zheng, B.; Zhao, Y. X.; Zhong, H. M.; Chen, X. L.; Zeng, Y. B.; Dong, W. H.; Huang, J. L.; Proksch, P.; Dai, H. F. *Mar. Drugs* **2012**, *10*, 1993−2001.

(11) McCutcheon, J. P.; Moran, N. A. *Nat. Rev. Microbiol.* **2012**, *10*, 13−26.

(12) Raffaele, S.; Kamoun, S. *Nat. Rev. Microbiol.* **2012**, *10*, 417−430.

(13) Kwan, J. C.; Donia, M. S.; Han, A. W.; Hirose, E.; Haygood, M. G.; Schmidt, E. W. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20655−20660.

(14) Lauritano, C.; Procaccini, G.; Lanora, A. *Mar. Environ. Res.* **2012**, *76*, 22−31.

(15) Liu, J. Y.; Huang, L. L.; Ye, Y. H.; Zou, W. X.; Guo, Z. J.; Tan, R. X. *J. Appl. Microb.* **2006**, *100*, 195−202.

(16) Shen, L.; Jiao, R. H.; Ye, Y. H.; Wang, X. T.; Xu, C.; Song, Y. C.; Zhu, H. L.; Tan, R. X. *Chem.—Eur. J.* **2006**, *12*, 5596−5602.

(17) Wust, J. *Antimicrob. Agents Chemother.* **1977**, *11*, 631−637.

(18) Manes, G.; Balzano, A. *Expert Rev. Anti-Infect. Ther.* **2004**, *2*, 695−705.

(19) Rani, N.; Sharma, A.; Gupta, G. K.; Singh, R. *Mini-Rev. Med. Chem.* **2013**, *13*, 1626−1655.

(20) Tan, R. X.; Zhang, G. F.; Song, Y. C.; Cui, J. T.; Wang, W. CN 102329735 B, 2013.

(21) Tan, R. X.; Han, W. B.; Mei, Y. N.; Zhang, A. H.; Zhang, G. F. CN 103408557 A, 2013.

(22) Misumi, K.; Sugiura, T.; Yamaguchi, S.; Mori, T.; Kamei, I.; Hirai, H.; Kawagishi, H.; Kondo, R. *Biosci. Biotechnol. Biochem.* **2011**, *75*, 178−180.

(23) Hunter, G. A.; Ferreira, G. C. *Cell. Mol. Biol.* **2009**, *55*, 102−110.

(24) Turbeville, T. D.; Zhang, J. S.; Adams, W. C.; Hunter, G. A.; Ferreira, G. C. *Arch. Biochem. Biophys.* **2011**, *511*, 107−117.

(25) Zhang, W. J.; Bolla, M. L.; Kahne, D.; Walsh, C. T. *J. Am. Chem. Soc.* **2010**, *132*, 6402−6411.

(26) Yu, S. L.; An, Y. J.; Yang, H. j.; Kang, M. S.; Kim, H. Y.; Wen, H.; Jin, X.; Kwon, H. N.; Min, K. J.; Lee, S. K.; Park, S. *J. Proteome Res.* **2013**, *12*, 1619−1627.

(27) Lowther, J.; Yard, B. A.; Johnson, K. A.; Carter, L. G.; Bhat, V. T.; Raman, M. C. C.; Clarke, D. J.; Ramakers, B.; McMahon, S. A.; Naismith, J. H.; Campopiano, D. J. *Mol. Biosyst.* **2010**, *6*, 1682−1693.

(28) Choi, J. S.; Park, N. J.; Lim, H. K.; Ko, Y. K.; Kim, Y. S.; Ryu, S. Y.; Hwang, I. T. *Pestic. Biochem. Physiol.* **2012**, *103*, 166−172.

(29) Judd, W. R.; Ban, S.; Aubé, J. *J. Am. Chem. Soc.* **2006**, *128*, 13736−13741.

(30) Freeman, D. B.; Furst, L.; Condie, A. G.; Stephenson, C. R. J. *Org. Lett.* **2012**, *14*, 94−97.