ORGANIC LETTERS

2013
Vol. 15, No. 9
2168–2171

# Isolation and Photoinduced Conversion of 6-*epi*-Stephacidins from *Aspergillus taichungensis*

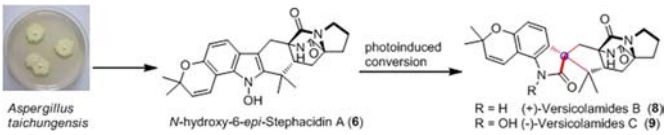
**Shengxin Cai, Yepeng Luan, Xianglan Kong, Tianjiao Zhu, Qianqun Gu, and Dehai Li***

*Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao, 266003, P. R. China*

*dehaili@ouc.edu.cn*

**Received March 14, 2013**

## ABSTRACT




**Three prenylated indole alkaloids with a rare *anti* bicyclo-[2.2.2]diazaoctane core ring (5−7) were isolated from *Aspergillus taichungensis*. The structures including absolute configurations were elucidated based on NMR, X-ray, and CD methods. (+)-Versicolamides B and C (8−9) which contain a spiro-center, together with seven analogues (7, 10−15), were isolated as photoinduced conversion products of 6. Biological evaluation indicated that 6 and 7 exhibited significant cytotoxicities with $IC_{50}$ values in the low micromolar range.**

Prenylated indole alkaloids including brevianamides, paraherquamides, malbrancheamides, stephacidins, asperparalines, versicolamides, avrainvillamide, marcfortines, and sclerotiamide, which all contain the bicyclo-[2.2.2]diazaoctane core ring, have been isolated mainly from *Aspergillus* spp. and *Penicillium* spp.[1] This family is biosynthetically derived from tryptophan, a cyclic amino acid, and either one or two isoprene units.[2] Due to their complex structures and diverse bioactivities, including antitumor, insecticidal, and calmodulin inhibition, this family has become an interesting target for synthetic, biosynthetic and bioactivity studies.[1h,2,3]

Biomimetic total synthesis of (+)-stephacidin A (**1**) and (−)-notoamide B (**2**) has been carried out by employing an intramolecular Diels−Alder (IMDA) reaction *via* notoamide E (**3**);[3d] however feeding and incorporation experiments strongly suggested that **3** was not the specific precursor leading to **1** and **2**.[1h] Recently, Williams and co-workers confirmed that notoamide T (**4**) could be converted to **1** and **2**[4] and also demonstrated the

(1) (a) Whyte, A. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **1996**, *59*, 1093–1095. (b) Sugie, Y.; Hirai, H.; Inagaki, T.; Ishiguro, M.; Kim, Y.; Kojima, Y.; Sakakibara, T.; Sakemi, S.; Sugiura, A.; Suzuki, Y.; Brennan, L.; Duignan, J.; Huang, L. H.; Sutcliffe, J.; Kojima, N. *J. Antibiot.* **2001**, *54*, 911–916. (c) Qian-Cutrone, J.; Huang, S.; Shu, Y.; Vyas, D.; Fairchild, C.; Menendez, A.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Gao, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556–14557. (d) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2254–2256. (e) Tsukamoto, S.; Kato, H.; Samizo, M.; Nojiri, Y.; Onuki, H.; Hirota, H.; Ohta, T. *J. Nat. Prod.* **2008**, *71*, 2064–2067. (f) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3573–3577. (g) Tsukamoto, S.; Kawabata, T.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1297–1300. (h) Tsukamoto, S.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *J. Am. Chem. Soc.* **2009**, *131*, 3834–3835. (i) Tsukamoto, S.; Umaoka, H.; Yoshikawa, K.; Ikeda, T.; Hirota, H. *J. Nat. Prod.* **2010**, *73*, 1438–1440. (j) Li, S. *Nat. Prod. Rep.* **2010**, *27*, 57–78. (k) Finefield, J. M.; Frisvad, J. C.; Sherman, D. H.; Williams, R. M. *J. Nat. Prod.* **2012**, *75*, 812–833.

(2) Finefield, J. M.; Kato, H.; Greshock, T. J.; Sherman, D. H.; Tsukamoto, S.; Williams, R. M. *Org. Lett.* **2011**, *13*, 3802–3805.

(3) (a) Wulff, J. E.; Herzon, S. B.; Siegrist, R.; Myers, A. G. *J. Am. Chem. Soc.* **2007**, *129*, 4898–4899. (b) Artman, G. D., III; Grubbs, A. W.; Williams, R. M. *J. Am. Chem. Soc.* **2007**, *129*, 6336–6342. (c) Wulff, J. E.; Siegrist, R.; Myers, A. G. *J. Am. Chem. Soc.* **2007**, *129*, 14444–14451. (d) Greshock, T. J.; Grubbs, A. W.; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2262–2265. (e) Miller, K. A.; Tsukamoto, S.; Williams, R. M. *Nat. Chem.* **2009**, *1*, 63–68. (f) Ding, Y.; Wet, J. R.; Cavalcoli, J.; Li, S. Y.; Greshock, T. J.; Miller, K. A.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Tsukamoto, S.; Williams, R. M.; Sherman, D. H. *J. Am. Chem. Soc.* **2010**, *132*, 12733–12740. (g) Baran, P. J.; Hafensteiner, B. D.; Ambhaikar, N. B.; Guerrero, C. A.; Gallagher, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 8678–8693. (h) Myers, A. G.; Herzon, S. B.; Wulff, J. E.; Siegrist, R.; Svenda, J.; Zajac, M. A. *PCT int. Appl.* WO 2006102097 A2, 20060928 2006.

(4) Sunderhaus, J. D.; McAfoos, T. J.; Finefield, J. M.; Kato, H.; Li, S.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. *Org. Lett.* **2013**, *15*, 22–25.

10.1021/ol400694h 
**Published on Web 04/03/2013**

biotransformation from **1** to **2** in feeding experiments.[2] To the best of our knowledge, biosynthetic studies of this family has mostly focused on compounds with the *syn*-relative configuration in the bicyclo-[2.2.2]diazaoctane core ring. In addition, the origin of the fascinating enantio-divergence observed ((±)-stephacidins A, (±)-notoamides B, and (±)-versicolamides B) and the *syn*- and *anti*-relative configurations have not been fully elucidated. A combination of genome mining, proteomics, and biotransformation experiments aimed at elucidating these questions is currently underway.[2,3]

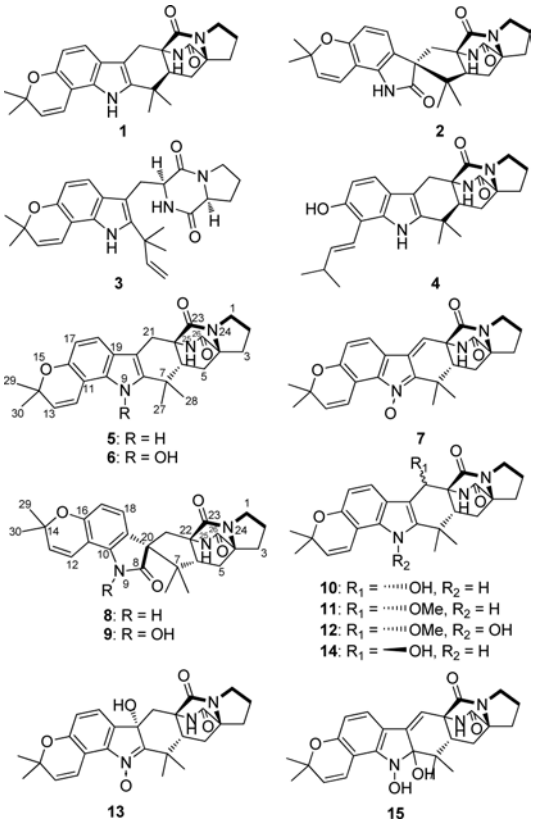



**Figure 1.** Chemical structures of **1**–**15**.

In our continued search for new anticancer natural products from fungi, an extract from *Aspergillus taichungensis* ZHN-7-07 exhibited strong *in vitro* cytotoxicity against the murine leukemia P388 cell line. A series of *p*-terphenyls,[5] aspergilazine A,[6] and brevianamide F were isolated from this stain cultured in a liquid medium. When the medium was varied to rice, three prenylated indole alkaloids with the bicyclo-[2.2.2]diazaoctane core ring, 6-*epi*-stephacidin A (**5**), *N*-hydroxy-6-*epi*-stephacidin A (**6**), and 6-*epi*-avrainvillamide (**7**), were identified by chemical investigation of the extract along with 3-*epi*-notoamide C.[1h] The structures and absolute configurations were elucidated by detailed analysis of MS, NMR, CD, and X-ray data (Supporting Information, SI). Interestingly, **5**–**7** all contained the rare *anti* relative configuration as (+)-versicolamide B and no compounds with *syn* configuration were isolated. The *syn*/*anti* relationship refers to the relative configuration between the C6–C7 and C4–N24 bonds (6-*epi*-stephacidin A numbering). Until now, only three cases with the *anti* relative configuration, (±)-versicolamides B and chrysogenamide A, have been reported.[1f,g,7] (±)-Versicolamides B were isolated from two different cultures of *Aspergillus sp*. as minor metabolites, the major metabolites of which had the *syn* configuration.[1f,g]

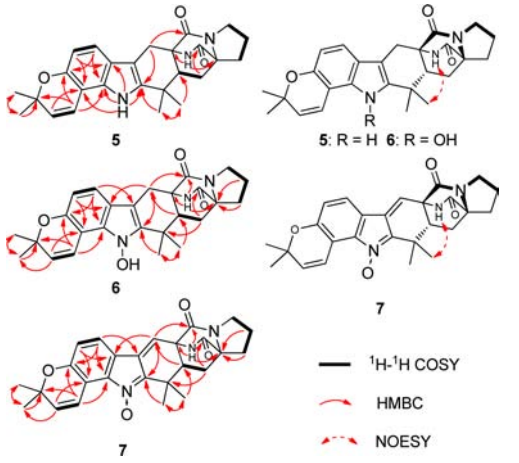



**Figure 2.** Selected $^1H-^1H$ COSY, HMBC, and NOESY correlations of **5**–**7**.

Compound **5**[8] was isolated as a colorless block crystal. Based on the HRESIMS $[M + H]^+$ ion at *m*/*z* 432.2299 (calcd 432.2287), the molecular formula was established as $C_{26}H_{29}N_3O_3$ indicating 14 degrees of unsaturation. Careful analysis of 1D NMR (Tables S1 and S2, SI) together with the HMQC spectrum revealed the presence of two amide/carbonyl carbons, eight aromatic carbons, two carbons for double bond, four quaternary carbons, one methine, five methylenes, and four methyls. With seven degrees of unsaturation accounted for the eight aromatic carbons, two olefinic carbons, and two carbonyls, there must be seven rings to meet the 14 degrees of unsaturation. Further detailed 1D and 2D NMR spectral analysis led to the elucidation of the planar structure of **5**, the same as stephacidin A (**1**), although there was an obvious difference between the $^{13}C$ chemical shifts of C-6 of these two compounds ($\delta_C$ 46.2 for **5**, $\delta_C$ 49.2 for **1**)[1c,9] implying a

(5) Cai, S.; Zhou, H.; Kong, X.; Zhu, T.; Li, D.; Gu, Q. *J. Nat. Prod.* **2011**, *74*, 1106–1110.

(6) Cai, S.; Kong, X.; Wang, W.; Zhou, H.; Zhu, T.; Li, D.; Gu, Q. *Tetrahedron Lett.* **2012**, *53*, 2615–2617.

(7) Lin, J.; Wen, J.; Zhu, T.; Fang, Y.; Gu, Q.; Zhu, W. *J. Antibiot.* **2008**, *62*, 81–85.

(8) Colorless block crystal; $[\alpha]^{25}_D$ 27.0 (*c* 0.26, $CH_2Cl_2$/MeOH 1:1); IR (KBr) $\nu_{max}$ 3443, 3262, 2972, 2936, 1692, 1668, 1456, 1406, 1253, 1187, 1117, 1058, 729 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (see Tables S1 and S2, SI); HRESIMS $[M + H]^+$ *m*/*z* 432.2299 (calcd 432.2287).

(9) Stephacidin A (**1**) was isolated from another fungus and was confirmed by NMR and CD spectra.

difference in the stereostructure of these two compounds. The complete structure of **5** was further confirmed by X-ray crystallographic analysis (Figure 3), revealing the *anti*-relative configuration as shown in Figure 1.

Many synthetic and biosynthetic studies of stephacidin A (**1**) and its analogues have been developed.[1h,2,3] The epimer, 6-*epi*-stephacidin A (**5**), which bears the *anti*-relative configuration, has been synthesized and has been suggested as a natural precursor of (+)-versicolamide B,[1f,10] but until now no biosynthetic study regarding the *anti*-relative configuration compounds has been made due to the shortage of this kind of compound, and also the rarity of the fungal sources that can produce them.

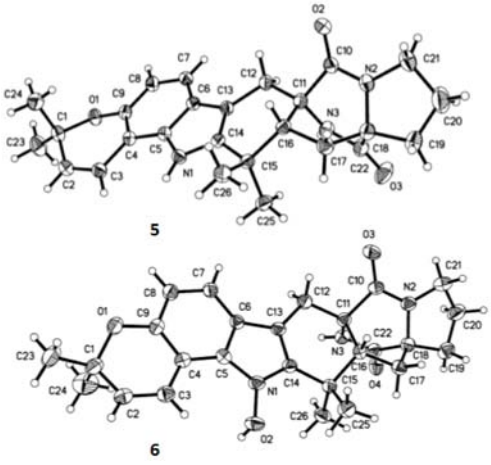



**Figure 3.** X-ray crystal structures for compounds **5** and **6**.

The 1D NMR spectra of **6**[11] (Tables S1 and S2, SI) bore a striking resemblance to compound **5**, except for the chemical shift of one exchangable proton ($\delta_H$ 10.82 for **6** and $\delta_H$ 10.47 for **5**) and the difference of HRESIMS ($[M + H]^+$: 432.2299 for **5**; $[M - H]^-$: 446.2085 for **6**), thus **6** was an oxidized analogue of **5**. The complete structure and *anti*-relative configuration of **6** were further determined by $^1H-^1H$ COSY and HMBC correlations (Figure 2) and X-ray analysis (Figure 3).

The molecular formula of 6-*epi*-avrainvillamide (**7**)[12] was determined as $C_{26}H_{27}N_3O_4$ based on an HRESIMS $[M + H]^+$ ion at $m/z$ 446.2076 (calcd 446.2080). The biggest differences between the 1D NMR spectra of **7** and **5** (Tables S1 and S2, SI) were the absence of the resonances for one methylene ($\delta_H$, 3.58, 2.72; $\delta_C$, 28.1) and 1-NH ($\delta_H$, 10.47) and the presence of one $sp^2$ methine ($\delta_H$, 7.05; $\delta_C$, 121.3) assigned as C-21. Based on intensive analysis of both 1D and 2D NMR spectra (Tables S1 and S2, SI, Figure 2), the structure of **7** was elucidated.

The absolute configurations of **5**–**7** were elucidated by CD spectra comparison with that of brevianamide B (Figure 4). Williams reported that the Cotton effect at 200–250 nm arising from an n–$\pi^*$ transition of the diketopiperazine amide bonds is diagnostic for the bicyclo-[2.2.2]diazaoctane ring system.[1d,13] Thus the absolute configurations of **5**–**7** were assigned as 4*S*, 6*R*, 22*S*.

In this study, we found that **6** was unstable when exposed to air and light. Compound **6** was dissolved in DMSO and drops of methanol were added, and then the solution was placed under UV light. After 3 days, (+)-versicolamides B and C (**8**–**9**), which contain a spiro-center, together with seven analogues (**7**, **10**–**15**) were purified from the photoconversion reaction mixture (Figure 1). Conversely, **5** was stable under the same conditions. The structures of **8**–**14** were determined by a combination of spectroscopic methods (for NMR, MS, and CD spectra, see SI). Due to the minor amount and instability of **15**, only $^1H$ NMR was obtained. Although the data were limited, the structure could be proposed upon comparison of the $^1H$ NMR data with other compounds obtained in this study.

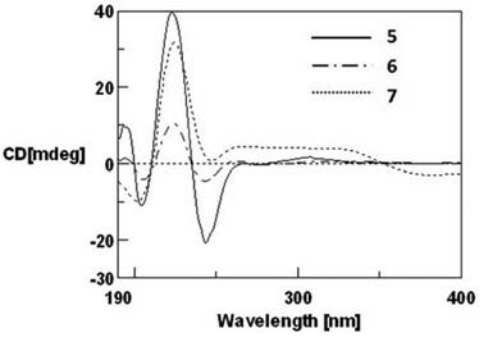



**Figure 4.** CD spectra of compounds **5**–**7**.

A proposal for the mechanism of the conversion of **6** is shown in Scheme 1. Since the UV irradiation could accelerate the decomposition reaction, the initiation is likely due to a free radical reaction. As shown in Scheme 1, the N–OH bond of **6** was homolytically cleaved with UV light, and then intermediate **16** was furnished by the subsequent rearrangement and recombination with •OH, further oxidation, and elimination affording compound **13** and intermediate **17**, respectively. Catalyzed by acid, compounds **10**, **11**, and **14** were produced from the reaction of **17** with methanol or $H_2O$ (i). Similarly, compound **8** was formed first by pinacol rearrangement and then addition with $H_2O$ (ii). Alternatively, **17** underwent oxidation to give compound **7**, which was further catalyzed by *hv* to form a structure containing oxaziridine (intermediate **18**),

(10) Greshock, T. J.; Williams, R. M. *Org. Lett.* **2007**, *9*, 4255–4258.

(11) Colorless block crystal; $[\alpha]^{25}_D$ 28.6 (*c* 0.2, $CH_2Cl_2$/MeOH 1:1); IR (KBr) $\nu_{max}$ 3439, 3192, 2972, 2923, 1680, 1668, 1438, 1409, 1360, 1205, 1117, 1021, 726 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (see Tables S1 and S2, SI); HRESIMS $[M - H]^-$ $m/z$ 446.2085 (calcd 446.2080).

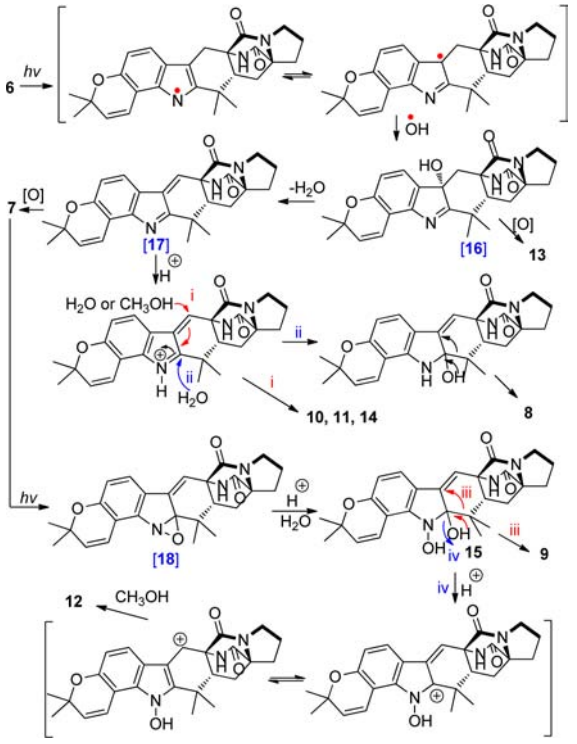
(12) Pale yellow amorphous solid; $[\alpha]^{25}_D$ 97.8 (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ 3416, 3241, 2988, 2925, 2875, 1692, 1406, 1265, 1191, 1112 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR (see Tables S1 and S2, SI); HRESIMS $[M + H]^+$ $m/z$ 446.2076 (calcd 446.2080).

(13) Williams, R. M.; Kwast, E.; Coffman, H.; Glinka, T. *J. Am. Chem. Soc.* **1989**, *111*, 3064–3065.

and **15** was obtained from **18** reacting with $H_2O$ under acidic conditions. Compound **15** could undergo pinacol rearrangement to give **9** (iii), or generate carbocation intermediate (iv), and the following charge rearrangement and addition of methanol furnished **12**.

In order to find analogues like the photoinduced conversion products and clarify the biosynthesis of the *anti* configured alkaloids, detailed observation and analysis have been carried out by employing *A. taichungensis*. The fungal isolate was cultured on rice, with subsequent metabolite profiles analyzed daily by HPLC-UV and then HPLC-MS from the fourth day. *N*-Hydroxy-6-*epi*-stephacidin A (**6**) appeared on the fourth day, and 6-*epi*-stephacidin A (**5**) and 6-*epi*-avrainvillamide (**7**) appeared on the ninth day; after 10 days, there were no further obvious changes (SI). In particular, **8**–**15**, which were obtained from the photoinduced conversion, and any other analogues were targeted through the HPLC and HPLC-MS data; unfortunately, no detectable peaks with related UV and MS were found.

**Scheme 1.** Proposed Conversion Mechanism for **7**–**15** from **6**




Biological evaluation of **5**–**11** and **13**–**14** using both SRB[14] and MTT[15] methods showed that **6** and **7** exhibited significant cytotoxicity with $IC_{50}$'s of 4.45 and 1.88 $\mu$M against HL-60 and 3.02 and 1.92 $\mu$M v. A-549, respectively. No other compounds showed significant cytotoxicity. Compound **7** shares the same planar structure with avrainvillamide which can bind to the nuclear chaperone nucleophosmin, a proposed oncogenic protein that is overexpressed in many different human tumors, to inhibit the growth of the cancer cell lines. The antiproliferative activity of stephacidin B arises from its prior dissociation to form avrainvillamide.[3a,c] In our experiment, **6** and **7** functioned equivalently to inhibit the growth of the two tested cell lines, the reason for this might be that **6** can be transformed to **7** and then act as avrainvillamide does in the cancer cell lines. Biological evaluation of compounds **12** and **15** was hindered by the small quantities of material available.

In conclusion, three prenylated indole alkaloids (**5**–**7**) containing the rare *anti* bicyclo-[2.2.2]diazaoctane core ring were isolated from *A. taichungensis* ZHN-7-07. Biological evaluation indicated that **6** and **7** had significant cytotoxicities against HL-60 and A-549 cell lines. In the past few years, the stephacidin/notoamide family, which has the unique bicyclo-[2.2.2]diazaoctane core ring, has attracted the attention of a number of synthetic chemists and has stimulated many interesting studies concerning the biogenesis of this core ring structure.[2,3] Among them, the confirmation of chemical transformation and bioconversion from (±)-stephacidin A with a *syn*-relative configuration to (±)-notoamide B by Williams and co-workers is particularly notable.[2] Here we report the photoinduced conversion from *N*-hydroxy-6-*epi*-stephacidin A (**6**) with an *anti*-relative configuration to (+)-versicolamides B and C (**8**–**9**) for the first time. The photoconversion of **6** was useful in understanding the origin of this family of alkaloids containing the spiro ring system such as **8** and **9**. In addition, the metabolite profiles revealed **8** and **9** were not present, suggesting that our fungal strain lacks the face-selective enzyme responsible for the conversion from 6-*epi*-stephacidin A (**5**) or *N*-hydroxy-6-*epi*-stephacidin A (**6**) to **8**–**9**.

**Acknowledgment.** This work was financially supported by the National Natural Science Fundation of China (No. 41176120), the National High Technology Research and Development Program of China (No. 2013AA092901), the Program for New Century Excellent Talents in University (No. NCET-12-0499), the Public Projects of State Oceanic Administration (No. 2010418022-3), the Promotive Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (No. BS2010HZ027), and the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0944). We also thank Dr. R. A. Keyzers (Victoria University of Wellington) for helping with the manuscript preparation.

**Note Added after ASAP Publication.** References 1k, 3g, and 3h were missing in the version published ASAP April 3, 2013, the correct version reposted April 16, 2013.

**Supporting Information Available.** Detailed isolation procedure and spectrospic data. This material is available free of charge via the Internet at http://pubs.acs.org.

(14) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

(15) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

The authors declare no competing financial interest.