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# Aspergilones A and B, two benzylazaphilones with an unprecedented carbon skeleton from the gorgonian-derived fungus *Aspergillus* sp.

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ABSTRACT

Two novel benzylazaphilone derivatives with an unprecedented carbon skeleton, aspergilone A (**1**), and its symmetrical dimer with a unique methylene bridge, aspergilone B (**2**), have been isolated from the culture broth of a marine-derived fungus *Aspergillus* sp. from a gorgonian *Dichotella gemmacea*. Their structures and relative stereochemistries of **1** and **2** were elucidated using a combination of NMR spectroscopy and X-ray crystallography. Compound **1** not only exhibited in vitro selective cytotoxicity but also showed potent antifouling activity.

Marine-derived fungi have proved to be a promising source of bioactive metabolites and a growing number of marine fungi have been reported to produce novel bioactive compounds.[1,2] As part of a program to discover new bioactive secondary metabolites from marine-derived fungi in the South China Sea,[3–5] the EtOAc extract of the culture broth of a marine-derived fungus *Aspergillus* sp.[6,7] isolated from a gorgonian *Dichotella gemmacea* exhibited significant cytotoxicity against A-549 human lung carcinoma cell line. Chemical investigation of the bioactive extract led to the discovery of two new azaphilone derivatives, aspergilones A and B (**1** and **2**) (Fig. 1), which possess an unprecedented carbon skeleton. Herein we report the isolation, structural determination and biological activity of these new compounds.

HPLC analysis of the EtOAc extract of the fungus *Aspergillus* sp. showed the presence of the evident UV absorption spectra ($\lambda_{max}$ 199 and 323 nm). The extract was subjected to column chromatography on silica gel and Sephadex LH-20 successively. Further separation and purification using semi-preparative HPLC led to the isolation of compounds **1** and **2**.

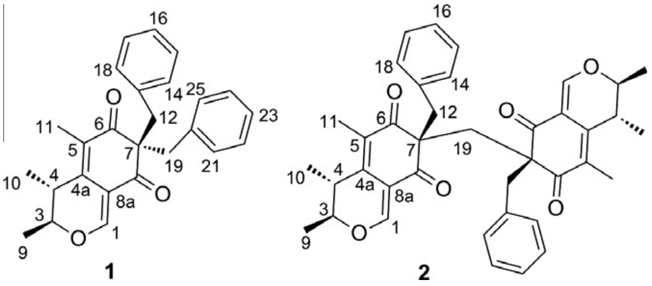

**Figure 1.** Structures of compounds **1** and **2**.

Aspergilone A (**1**)[8] was isolated as a colorless solid with the molecular formula of $C_{26}H_{26}O_3$ (14° of unsaturation) using HRESIMS (obsd $[M+H]^+$ at $m/z$ 387.1974, calcd 387.1960). This molecular formula was also supported by both $^1H$ and $^{13}C$ NMR spectral data (Table 1). In its $^1H$ NMR spectrum, ten aromatic proton signals between $\delta_H$ 7.11 and 6.82 indicated that the presence of two mono-substituted benzene rings. An

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**Table 1**
NMR data (acetone-$d_6$) of **1**[a]

| Position | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) | HMBC |
|---|---|---|---|
| 1 | 155.1, –C | 7.48, s | C-3, C-4a, C-8, C-8a |
| 2 | – | – | |
| 3 | 79.3, CH | 4.32, dq (6.6, 0.8) | C-1, C-4a, C-9 |
| 4 | 30.1, CH | 2.58, dq (7.0, 0.8) | C-4a, C-5, C-8a, C-9, C-10 |
| 4a | 145.2, –C | – | |
| 5 | 126.5, –C | – | |
| 6 | 200.3, –C | – | |
| 7 | 66.5, –C | – | |
| 8 | 199.3, –C | – | |
| 8a | 111.8, –C | – | |
| 9 | 18.0, $CH_3$ | 0.71, d (6.6) | C-3, C-4, |
| 10 | 16.9, $CH_3$ | 0.55, d (7.0) | C-3, C-4, C-4a |
| 11 | 9.5, $CH_3$ | 1.59, s | C-4a, C-5, C-6, C-8a |
| 12 | 47.0, $CH_2$ | 3.28, d (12.0)<br>3.14, d (12.0) | C-6, C-7, C-13, C-14, C-16, C-19 |
| 13 | 138.0, –C | – | |
| 14/18 | 130.5, CH | 6.89–6.87, m | C-13, C-15/17, C-19 |
| 15/17 | 127.2, CH | 7.11–7.06, m | C-13, C-14/18 |
| 16 | 128.6, CH | 7.11–7.06, m | C-14/18, C-15/17 |
| 19 | 45.6, $CH_2$ | 3.36, d (12.6)<br>3.22, d (12.6) | C-7, C-8, C-12, C-20, C-21, C-23 |
| 20 | 137.6, –C | – | |
| 21/25 | 130.3, CH | 6.84–6.82, m | C-19, C-20, C-22/24 |
| 22/24 | 127.1, CH | 7.11–7.06, m | C-20, C-21/25 |
| 23 | 128.8, CH | 7.11–7.06, m | C-21/25, C-22/24 |

[a] Measured at 600 MHz ($^1H$) and 150 MHz ($^{13}C$).

olefinic proton signal at $\delta_H$ 7.48, two methine signals at $\delta_H$ 4.32 (dq, $J$ = 6.6, 0.8 Hz), 2.58 (dq, $J$ = 7.0, 0.8 Hz) and three methyl groups at $\delta_H$ 1.59 (s), 0.71 (d, $J$ = 6.6 Hz) and 0.55 (d, $J$ = 7.0 Hz) were observed. Moreover, two methylenes were characterized by two AB doublets at $\delta_H$ 3.28 (d, $J$ = 12.0 Hz) and 3.14 (d, $J$ = 12.0 Hz), and at $\delta_H$ 3.36 (d, $J$ = 12.6 Hz) and 3.22 (d, $J$ = 12.6 Hz). The $^{13}C$ NMR spectral data of **1** showed two ketonic carbonyl carbons ($\delta_C$ 200.3 and 199.3), four olefinic carbons, and 12 aromatic carbon signals assignable to two mono-substituted benzene rings, three methyl groups including one olefinic methyl, two methylenes, two methine, and one quaternary carbon. The 14° of unsaturation inherent in the molecular formula of **1**, coupled with data showing the presence of two carbonyls, four olefinic carbons, and 12 aromatic carbons assigned to two benzene rings, indicated that aspergilone A (**1**) possesses another two rings. A further analysis of its NMR data ($^1H$ and $^{13}C$ NMR, $^1H$–$^1H$ COSY and HMBC spectra) of **1** revealed the presence of an azaphilone structure.[9] The correlations between H-3 and H-4, $H_3$-9; H-4 and $H_3$-10 in the $^1H$–$^1H$ COSY spectrum revealed the $CH_3$–CH–CH–$CH_3$ subunit in **1**. In the HMBC spectrum, two benzyl groups attached to C-7 were confirmed by the correlations from $H_2$-12 to C-6, C-7 and from $H_2$-19 to C-7, C-8, respectively. The structure of **1** as shown in Figure 1 was established. Considering the exceptionally high field shifts of $H_3$-9 (0.71) and $H_3$-10 (0.55) which were strongly shielded by the ring current of benzene, the sites of the protons of the two methyl groups (9-$CH_3$ and 10-$CH_3$) should lie above the two benzene ring plane, respectively. Finally, the above-mentioned deduction and the relative stereochemistry of aspergilone A (**1**) were unambiguously confirmed by single-crystal X-ray analysis as shown in Figure 2. The molecular structure of **1** showed that the methyl groups of 9-$CH_3$ and 10-$CH_3$ lie exactly above the plane of the corresponding benzene rings. Then the relative configurations of two chiral centers were (3$S^*$, 4$R^*$).

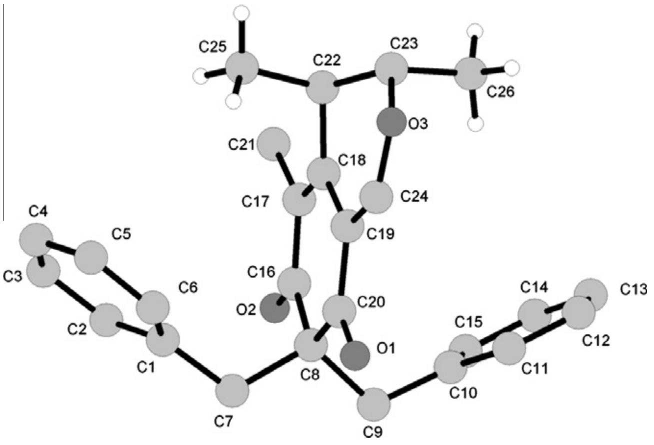

**Figure 2.** Molecular structure of aspergilone A (**1**).

Aspergilone B (**2**)[10] was isolated as a colorless solid. HREIMS analysis of **2** gave an $[M]^+$ ion at $m/z$ 604.2820 which is consistent with a molecular formula of $C_{39}H_{40}O_6$ (calcd for $C_{39}H_{40}O_6$, 604.2819) with 20° of unsaturation. The $^1H$ NMR and $^{13}C$ NMR data (Table 2) of **2** were very similar to those of **1**. The most noticeable difference in the $^1H$ NMR spectrum of **2** was that all $^1H$ NMR signals of benzylazaphilone showed double integrating intensity except for the additional methylene group, suggesting that **2** was a symmetrical dimer of **1**, connected at C-7 via a methylene bridge (C-19). The HMBC correlations from $H_2$-12 to C-19 and from $H_2$-19 to C-7, C-8 confirmed that the position of the methylene group was at C-7. Accordingly, the planar structure of aspergilone B (**2**) was determined by comparison of its spectral data with those of **1**. Additional evidence that confirmed the structure of **2** came from the EIMS experiment that illustrated prominent fragment ions in support of the structure (Fig. 3). The relative configurations of C-3 and C-4 in compound **2** were assigned as identical to **1** on the basis of the $^1H$–$^1H$ coupling constants and interpretation of 1D NOE experiment. However, the relative configuration of C-7 could not be confirmed because of lacking NOE correlations for $H_2$-12 and $H_2$-19. A more detailed comparison of the $^1H$ NMR data of compound **2** with that of **1** revealed that the methyl signal ($H_3$-10) signal shifted downfield to $\delta_H$ 1.07 (0.52 ppm difference), suggesting that there was no effect of the ring current of benzene over it. While the other methyl signal ($H_3$-9) in **2** shifted much more upfield ($\delta_H$ 0.48) compared to that of **1** indicated that this methyl was further influenced by the aromatic anisotropic shielding effect of the only one benzene. The above data together with the structure of **1** strongly suggested that the protons of 9-$CH_3$ with the conspicuously high field shifts should locate at the same side with the benzene ring plane, so that the relative configurations of **2** could be determined as 3$S^*$, 4$R^*$ and 7$S^*$.

Aspergilones A and B (**1** and **2**) were the latest examples of azaphilone derivatives with the benzyl group and aspergilone B (**2**) is also the first azaphilone dimer with a unique methylene bridge. Although numerous natural dimers, including flavonoids,[11] macrolides,[12] alkaloids,[13] anthraquinones,[14] xanthones[15] and naphthopyranones[16], have been reported to date, the methylene bridge is highly unusual among natural dimers. The co-isolation of aspergilones A and B (**1** and **2**) suggests that **2** could be biosynthesized by two molecules of debenzylaspergilone A and one molecule of formaldehyde in the fungus. The discovery of aspergilone B (**2**) also provides additional evidence that there may be a formaldehyde biosynthetic system in nature.[17–19]

Compound **1** was found to exhibit in vitro selective cytotoxicity toward HL-60 human promyelocytic leukemia, MCF-7 human breast adenocarcinoma and A-549 human lung carcinoma cell lines with $IC_{50}$ values of 3.2, 25.0 and 37.0 μg/mL, respectively.[20] However, compound **2** was found to be relatively noncytotoxic ($IC_{50}$ >50 μg/mL) against these three tumor cell lines.

**Table 2**
NMR data ($CDCl_3$) of **2**[a]

| Position | $\delta_C$, mult. | $\delta_H$ (*J* in Hz) | HMBC |
|---|---|---|---|
| 1/1′ | 154.8, –C | 7.53, s | C-3/3′, C-4a/4a′, C-8/8′, C-8a/8a′ |
| 2/2′ | – | – | – |
| 3/3′ | 78.6, CH | 4.22, dq (6.6, 0.6) | – |
| 4/4′ | 34.7, CH | 2.44, dq (6.6, 0.6) | C-4a/4a′, C-9/9′, C-10/10′ |
| 4a/4a′ | 143.7, –C | – | – |
| 5/5′ | 125.4, –C | – | – |
| 6/6′ | 200.1, –C | – | – |
| 7/7′ | 62.8, –C | – | – |
| 8/8′ | 198.4, –C | – | – |
| 8a/8a′ | 111.0, –C | – | – |
| 9/9′ | 17.7, $CH_3$ | 0.48, d (6.6) | C-3/3′, C-4/4′ |
| 10/10′ | 18.0, $CH_3$ | 1.07, d (6.6) | C-3/3′, C-4/4′, C-4a/4a′ |
| 11/11′ | 9.4, $CH_3$ | 1.66, s | C-4a/4a′, C-5/5′, C-6/6′, C-8a/8a′ |
| 12/12′ | 50.9, $CH_2$ | 3.09, d (12.6)<br>3.01, d (12.6) | C-6/6′, C-7/7′, C-8/8′, C-13/13′, C-14/14′, C-18/18′, C-19 |
| 13/13′ | 135.6, –C | – | – |
| 14/14′, 18/18′ | 130.0, CH | 6.88–6.86, m | C-15/15′, C-17/17′ |
| 15/15′, 17/17′ | 127.7, CH | 7.04–7.01, m | C-13/13′, C-14/14′ |
| 16/16′ | 126.7, CH | 7.02, m | C-14/14′, C-15/15′ |
| 19 | 43.8, $CH_2$ | 3.07, s | C-7/7′, C-8/8′ |

[a] Measured at 600 MHz ($^1H$) and 150 MHz ($^{13}C$).

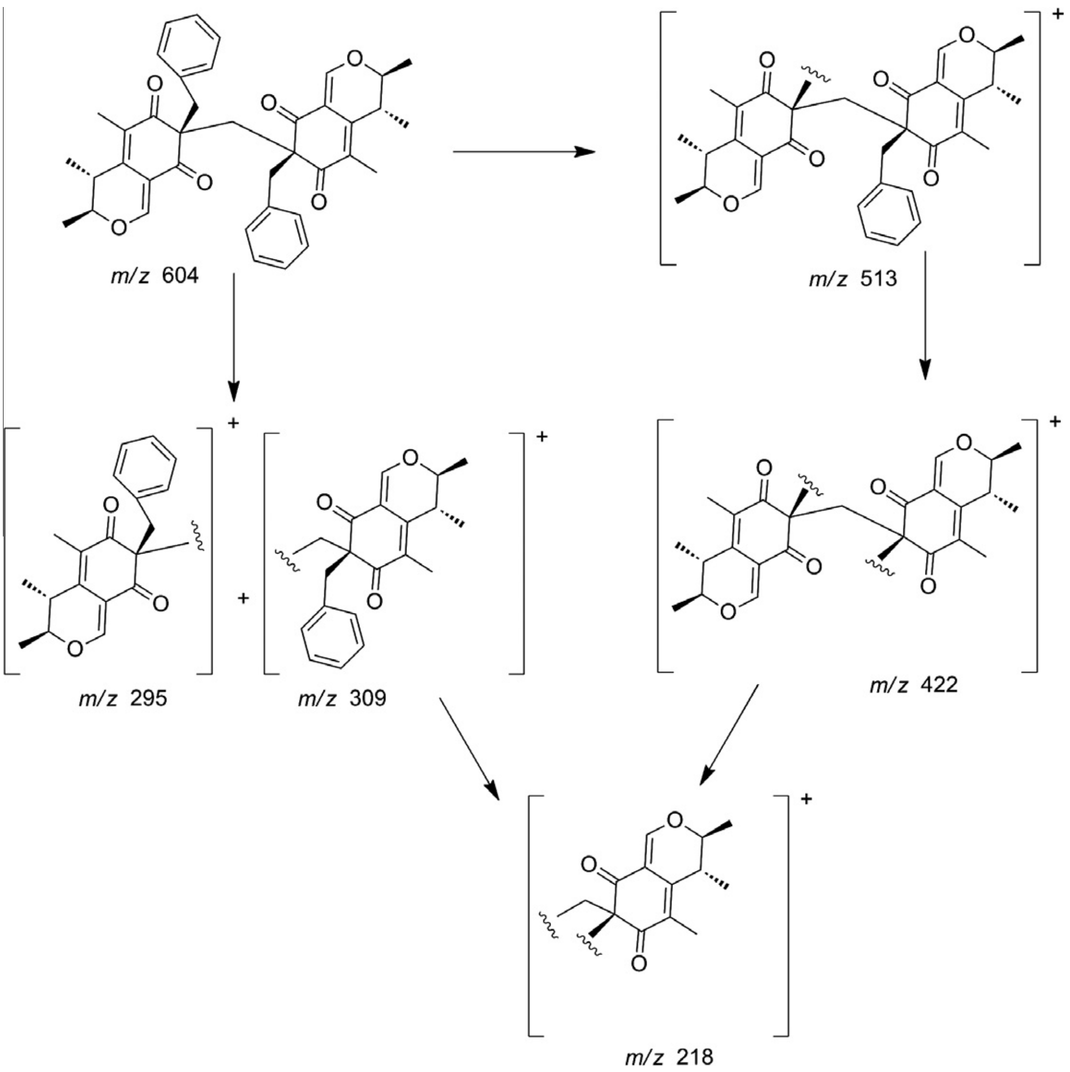

**Figure 3.** Fragment ions of aspergilone B (**2**) in the EIMS experiment.

Compound **1** also exhibited potential antifouling activity against the larval settlement of barnacle *Balanus amphitrite* at nontoxic concentration with $EC_{50}$ value of 7.68 μg/mL, which was lower than the standard requirement of an $EC_{50}$ of 25 μg/mL established by the U.S. Navy program as an efficacy level for natural antifouling agents.[21]

## Acknowledgment

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.005.

## References and notes

1. Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2006**, *23*, 26.
2. Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143.
3. Wen, L.; Cai, X. L.; Xu, F.; She, Z. G.; Chan, W. L.; Vrijmoed, L. L. P.; Gareth, J. E. B.; Lin, Y. C. *J. Org. Chem.* **2009**, *74*, 1093.
4. Shao, C. L.; Wang, C. Y.; Gu, Y. C.; Wei, M. Y.; Pan, J. H.; Deng, D. S.; She, Z. G.; Lin, Y. C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3284.
5. Wei, M. Y.; Wang, C. Y.; Liu, Q. A.; Shao, C. L.; She, Z. G.; Lin, Y. C. *Mar. Drugs* **2010**, *8*, 941.
6. Fungal material: The marine-derived fungus *Aspergillus* sp. was isolated from a piece of tissue from the inner part of the freshly gorgonian *D. gemmacea* (GX-WZ-20080034), which was collected from the South China Sea in September, 2008. The strain was deposited in the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, PR China, with the access code ZJ-2008001. Thirty liters of the fungal strain was cultivated in liquid medium (10.0 g of glucose, 2.0 g of yeast extract, 2.0 g of peptone in 1 L of seawater, in a 1.0 L Erlenmeyer flasks each containing 400 mL of culture broth) at 27 °C without shaking for 30 days.
7. Extraction and isolation: The fungal cultures were filtered through cheesecloth, and the filtrate (30.0 L) was extracted with EtOAc (2 × 30.0 L). The organic extracts were concentrated in vacuo to yield a yellow oily residue (2.50 g). This extract was chromatographed on a silica gel column using a stepwise gradient of petroleum ether–EtOAc to afford 8 fractions (Fractions 1–8). Fraction 2 (0.35 g) was isolated by column chromatography on silica gel eluted with petroleum ether–EtOAc (8:2), and then subjected to Sephadex LH-20 eluted with mixtures of petroleum ether–$CHCl_3$–MeOH (2:1:1) to obtain compound **1** (5.6 mg). Repeated chromatography of Fraction 3 (0.18 g) using Sephadex LH-20 eluted with mixtures of $CHCl_3$–MeOH (1:1) and petroleum ether–$CHCl_3$–MeOH (2:1:1), then by reversed-phase HPLC using a semi-preparative C18 (Kromasil 7 μm, 10 × 25 mm) column coupled with a Waters 2996 photodiode array detector, at a flow rate of 2.0 mL/min (1:1 MeOH–$H_2O$) obtained compound **2** (1.2 mg).
8. Aspergilone A (**1**): a colorless crystal; $[\alpha]_D^{25}$ +18.9 (*c* 0.65, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 199.3, 323.2 nm; IR (KBr) $\nu_{max}$ 2972, 2912, 2860, 1672, 1606, 1560, 1447, 1295, 1235, 1155, 744 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 600 MHz), and $^{13}C$ NMR (acetone-$d_6$, 150 MHz), see Table 1; EIMS *m*/*z* 386 $[M]^+$, 295, 91; FABMS *m*/*z* 387 $[M+H]^+$, 295; ESIMS *m*/*z* $[M+H]^+$ 387; HRESIMS *m*/*z* $[M+H]^+$ 387.1974 (calcd for $C_{26}H_{26}O_3$, 387.1960). Crystallizes in orthorhombic, space group *P*2(1)2(1)2(1) with $a = 10.3292(11)$ Å, $b = 13.7223(14)$ Å, $c = 15.3534(16)$ Å, $\alpha = \beta = \gamma = 90.00°$, $C_{26}H_{26}O_3$, $M_r = 386.47$, $V = 2176.2(4)$ $Å^3$, $Z = 4$, $D_c = 1.180$ g/$cm^3$, $F(0\,0\,0) = 824$, $\mu = 0.076$ $mm^{-1}$, the final $R = 0.0437$ and $wR = 0.1159$ for 16280 observed reflections ($I > 2\sigma(I)$). The paragraph crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 738790).
9. Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M.; Asakawa, Y. *Chem. Pharm. Bull.* **1994**, *42*, 2397.
10. Aspergilone B (**2**): a colorless crystal; $[\alpha]_D^{25}$ +11.6 (*c* 0.11, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 199.3, 323.2 nm; $^1H$ NMR ($CDCl_3$, 600 MHz), and $^{13}C$ NMR ($CDCl_3$, 150 MHz), see Table 2; EIMS *m*/*z* 604 $[M]^+$, 513, 485, 421, 422, 407, 309, 295, 296, 277, 264, 236, 218, 219, 91; ESIMS *m*/*z* 605.2 $[M+H]^+$, 627.3 $[M+Na]^+$; HREIMS *m*/*z* $[M]^+$ 604.2820 (calcd for $C_{39}H_{40}O_6$, 604.2819), 513.2273 (calcd for $C_{32}H_{33}O_6$, 513.2272); HRESIMS *m*/*z* $[M+H]^+$ 605.2910 (calcd for $C_{39}H_{41}O_6$, 605.2903).
11. Ariyasena, J.; Baek, S.-H.; Perry, N. B.; Weavers, R. T. *J. Nat. Prod.* **2004**, *67*, 693.
12. Rao, M. R.; Faulkner, D. J. *J. Nat. Prod.* **2002**, *65*, 386.
13. Kanokmedhakul, S.; Kanokmedhakul, K.; Yodbuddee, D.; Phonkerd, N. *J. Nat. Prod.* **2003**, *66*, 616.
14. Agusta, A.; Ohashi, K.; Shibuya, H. *Chem. Pharm. Bull.* **2006**, *54*, 579.
15. Isaka, M.; Palasarn, S.; Kocharin, K.; Saenboonrueng, J. *J. Nat. Prod.* **2005**, *68*, 945.
16. Santos, L. C.; Piacente, S.; Pizza, C.; Albert, K.; Dachtler, M.; Vilegas, W. *J. Nat. Prod.* **2001**, *64*, 122.
17. Wu, X. Y.; Liu, X. H.; Lin, Y. C.; Luo, J. H.; She, Z. G.; Li, H. J.; Chan, W. L.; Antus, S.; Kurtan, T.; Elsasser, B.; Krohn, K. *Eur. J. Org. Chem.* **2005**, 4061.
18. Yao, G. M.; Ding, Y.; Zuo, J. P.; Wang, H. B.; Wang, Y. B.; Ding, B. Y.; Chiu, P.; Qin, G. W. *J. Nat. Prod.* **2005**, *68*, 392.
19. Ito, H.; Kasajima, N.; Tokuda, H.; Nishino, H.; Yoshida, T. *J. Nat. Prod.* **2004**, *67*, 411.
20. Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622.
21. Thiyagarajan, V.; Harder, T.; Qiu, J. W.; Qian, P. Y. *Mar. Biol. (Berl.)* **2003**, *143*, 543.