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**Abstract**

Chromatographic separation of a crude extract obtained from the fungus *Aspergillus* sp., isolated from the Mediterranean sponge *Tethya aurantium*, yielded a new tryptophan derived alkaloid, 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-dihydrobenzo[*e*][1,4]diazepine-2,5-dione (**1**) and a new meroterpenoid, austalide R (**2**), together with three known compounds (**3**-**5**). The structures of the new compounds were unambiguously elucidated on the basis of extensive one and two-dimensional NMR ($^1$H, $^{13}$C, COSY, HMBC, and ROESY) and mass spectral analysis. Interestingly, the compounds exhibited antibacterial activity when tested against a panel of marine bacteria, with **1** selectively inhibiting *Vibrio* species and **2** showing a broad spectrum of activity. In contrast, no significant activity was observed against terrestrial bacterial strains and the murine cancer cell line L5178Y.

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## Introduction

Prevention or treatment of bacterial disease outbreaks in aquacultures is a major challenge facing this industry.[1] For instance, destructive infections caused by bacteria of the genus *Vibrio* and those causing necrotizing hepatopancreatitis (NHP) are the main diseases commonly affecting shrimp farms.[2] Hence, there is a great need for new antibiotics to combat such diseases and the resulting stock loss, especially with the development of bacterial resistance to traditionally used antibiotics.[1]

A potential source of novel antibacterial compounds are marine-derived fungi, which have attracted considerable attention in recent years.[3-5] They have been isolated from virtually every possible marine habitat, including inorganic matter, microbial communities, plants, invertebrates and vertebrates. In particular, sponges have yielded numerous fungal strains, which have been reported to produce a variety of pharmacologically active and structurally diverse metabolites.[3,6-11] The need of these organisms to adapt and survive in an environment that is significantly different from that of terrestrial organisms may have shaped their natural product patterns resulting in many cases in the production of unique secondary metabolites.[12-15]

The chemical profiles of both terrestrial and marine *Aspergillus* species have been studied by several research groups, and a vast diversity of secondary metabolites with novel structures and interesting biological activities was already elucidated.[16-25] In continuation of our previous studies on the sponge-derived *Aspergillus* sp. strain, isolated from the Adriatic Sea sponge *Tethya aurantium*,[6,25] two new compounds, 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-

dihydrobenzo[*e*][1,4]diazepine-2,5-dione (**1**) and austalide R (**2**), as well as the known

compounds 8-*O*-4-dehydrodiferulic acid (**3**), cytochalasin Z17 (**4**) and

dihydroisoflavipucine (**5**) (Figure 1), were now isolated and identified. All compounds

exhibited antibacterial activity against marine-derived strains, with **1** selectively

inhibiting *Vibrio* species and **2** showing a broad spectrum of activity, which may raise

the prospect of using such compounds as antifouling agents or to combat epizootics in
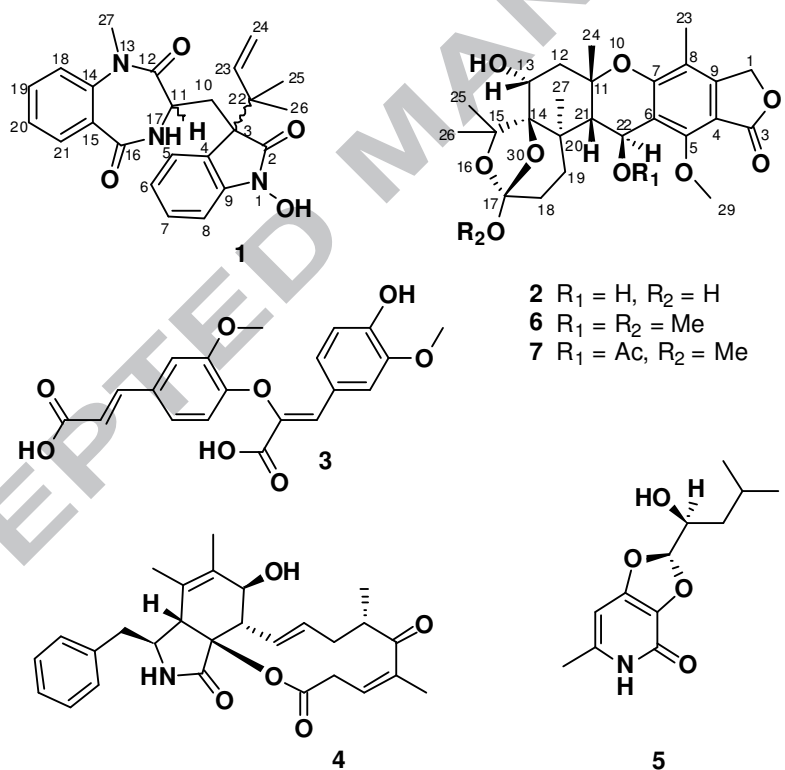
aquaculture in the future.



**Figure 1.** Structures of **1-7**.

**Results and Discussion**

The crude ethyl acetate extract of the fungus *Aspergillus* sp. was subjected to repeated column chromatography, followed by semi preparative HPLC separation, to afford two new compounds (**1** and **2**), along with three known compounds (**3**-**5**) (Figure 1). The molecular formula of **1** was established as $C_{24}H_{25}N_3O_4$ on the basis of the [M+H]$^+$ signal at *m/z* 420.1917 in the HRESIMS. The UV absorbance bands observed at $\lambda_{max}$ (MeOH) 214.1, 250.1 and 290.0 nm suggested the presence of an indoline chromophore.[26] The ^{1}H NMR and COSY spectra of **1** (Table 1) revealed the presence of eight aromatic protons corresponding to two ABCD spin systems resonating at $\delta_H$ 7.30, 6.90, 7.21, 6.73 ppm (H-5 to H-8, respectively) and at $\delta_H$ 7.24, 7.50, 7.18, 7.27 ppm (H-18 to H-21, respectively), an olefinic ABX spin system at $\delta_H$ 4.94/5.02 (H$_2$-24) and 6.07 (H-23) ppm, an aliphatic ABX spin system at $\delta_H$ 2.30/2.70 (H$_2$-10) and 2.90 (H-11) ppm, two geminal methyl groups at $\delta_H$ 0.98 ppm (H$_3$-25 and H$_3$-26), a nitrogen bearing methyl group at $\delta_H$ 3.15 ppm (H$_3$-27), and a NH group at $\delta_H$ 8.18 ppm (H-17). The ^{13}C NMR (Table 1) and DEPT spectra confirmed the presence of 24 carbon atoms in the structure of **1**, including one aliphatic and nine olefinic methine groups, one aliphatic and one olefinic methylene groups, three methyl groups, as well as two aliphatic and seven olefinic quaternary carbon atoms, the latter including three amide carbonyl carbons resonating at $\delta_C$ 172.3, 169.9, and 166.8 ppm (C-2, C-12, and C-16, respectively). Furthermore, analysis of the HMQC spectrum allowed the assignment of proton signals to the corresponding proton bearing carbon atoms.

**Table 1.** ^{1}H, ^{13}C NMR, COSY and HMBC data of **1** at 300 (^{1}H) and 100 (^{13}C) MHz

(DMSO-$d_6$, $\delta$ in ppm, $J$ in Hz)

| Position | $\delta_H$ | $\delta_C$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | 172.3 | | |
| 3 | | 54.7 | | |
| 4 | | 124.8 | | |
| 5 | 7.30 d (7.8) | 126.6 | 6 | 3, 7, 9 |
| 6 | 6.90 dt (0.1, 7.6) | 120.8 | 5, 7 | 4, 7, 8 |
| 7 | 7.21 t (7.7) | 128.0 | 6, 8 | 5, 8, 9 |
| 8 | 6.73 d (7.6) | 106.3 | 7 | 4, 6, 9 |
| 9 | | 142.7 | | |
| 10 | 2.30 dd (7.7, 14.9) | 28.9 | 11 | 2, 3, 4, 11, 12, 22 |
| | 2.70 dd (3.2, 14.9) | | | |
| 11 | 2.90 brm | 49.6 | 10, 17 | |
| 12 | | 169.9 | | |
| 13 | | | | |
| 14 | | 140.1 | | |
| 15 | | 128.2 | | |
| 16 | | 166.8 | | |
| 17 | 8.18 d (5.9) | | 11 | 10, 11, 15 |
| 18 | 7.24 d (8.3) | 121.6 | 19 | 15, 20 |
| 19 | 7.50 dt (1.7, 8.5) | 132.0 | 18, 20 | 14, 18, 21 |
| 20 | 7.18 t (8.4) | 125.1 | 19, 21 | 15, 18 |
| 21 | 7.27 d (8.3) | 128.8 | 20 | 14, 16, 19 |
| 22 | | 41.8 | | |
| 23 | 6.07 dd (10.8, 17.4) | 142.9 | 24 | 22, 25, 26 |
| 24 | 4.94 dd (0.1, 17.4) | 113.4 | 23 | 22, 23 |
| | 5.02 dd (0.1, 10.9) | | | |
| 25 | 0.98 s | 21.3 | | 3, 22, 23, 26 |
| 26 | 0.98 s | 22.5 | | 3, 22, 23, 25 |
| 27 | 3.15 s | 34.9 | | 12, 14 |

The identified spin systems of **1** were connected based on inspection of the HMBC spectrum (Table 1, Figure 2). Correlations of the tertiary methyl group protons H$_3$-27 ($\delta_C$ 34.9 ppm) to the amide carbonyl C-12 and to C-14 ($\delta_C$ 140.1 ppm), of H-18 to C-15 ($\delta_C$ 128.2 ppm) and C-20, of H-21 to C-14, C-16 and C-19, of the amide proton H-17 to C-10 ($\delta_C$ 28.9 ppm), C-11 ($\delta_C$ 49.6 ppm) and C-15, and of H$_2$-10 to C-11 and

C-12, established the 1-methyl-1,4-benzodiazepine-2,5-dione moiety of **1**. Further correlations of $H_2$-10 to C-2 ($\delta_C$ 172.3 ppm), C-3 ($\delta_C$ 54.7 ppm), C-4 ($\delta_C$ 124.8 ppm), and C-22 ($\delta_C$ 41.8 ppm), of H-5 to C-3, C-7 and C-9 ($\delta_C$ 142.7 ppm), and of H-8 to C-4, C-6 and C-9, corroborated the presence of an indolin-2-one moiety and revealed its connection with the 1,4-diazepine-2,5-dione ring through $CH_2$-10. The 2-methylbut-3-en-2-yl side chain was verified by correlations of both methyl groups $CH_3$-25 ($\delta_C$ 21.3 ppm ) and $CH_3$-26 ($\delta_C$ 22.5 ppm) to each other and to C-3, C-22, and C-23 ($\delta_C$ 142.9 ppm), of the olefinic proton H-23 to C-22, C-25 and C-26, and of $H_2$-24 ($\delta_C$ 113.4 ppm) to C-22 and C-23. The hydroxyl group was located at N-1 as the assignment of all other atoms was completed, and based on comparison of observed chemical shift values with those reported for similar 1-hydroxyindolin-2-one substructures.[27] Hence, **1** was determined as a novel metabolite with an unusual structural framework and named 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-dihydrobenzo[*e*][1,4]diazepine-2,5-dione. Attempts to determine the relative configuration of **1** by analysis of the ROESY spectrum failed due to free rotation around the methylene bridge $CH_2$-10.
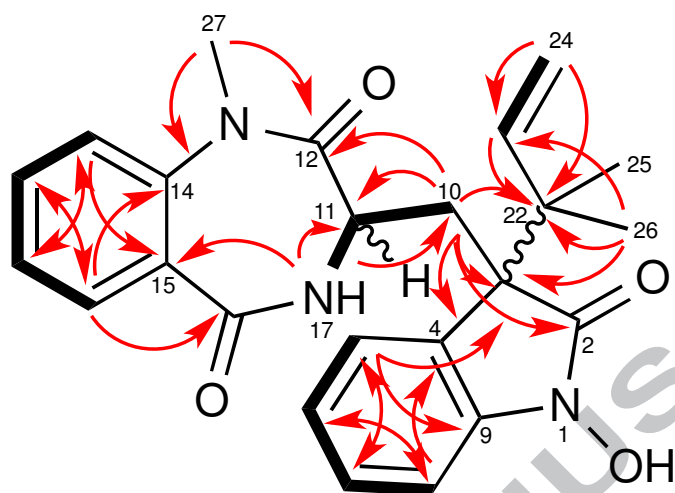
**Figure 2.** Key COSY ( ▬▬ ) and HMBC ( ⟶ ) correlations observed for **1**.

HRESIMS indicated the molecular formula $C_{25}H_{32}O_9$ for **2** in accordance with the [M+H]$^+$ signal at $m/z$ 477.2119. Its UV spectrum showed characteristic maxima of an austalide at $\lambda_{max}$ (MeOH) 222.6 and 268.5 nm.[28] The $^1$H and $^{13}$C NMR spectra (Table 2) revealed five methyl groups at $\delta_H$ ($\delta_C$) 0.79 (18.2), 1.29 (28.8), 1.32 (29.1), 1.43 (25.6) and 1.99 (10.5) ppm (CH$_3$-27, -24, -26, -25 and -23, respectively), and one methoxy group at $\delta_H$ 4.01 ($\delta_C$ 62.5) ppm (OCH$_3$-29). Additionally, four methylene groups were observed, including the oxygenated benzylic methylene group at C-1 ($\delta_H$ 5.25, $\delta_C$ 68.1 ppm), and three methine groups, two of which situated on oxygen-bearing carbon atoms based on their chemical shift values at $\delta_H$ ($\delta_C$) 3.89 (67.6) and 4.83 (59.5) ppm (CH-13 and CH-22, respectively).

**Table 2.** $^1$H, $^{13}$C NMR, COSY, HMBC and ROESY data of **2** ($\delta$ in ppm, $J$ in Hz)

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| Position | $\delta_H{}^a$ | $\delta_C{}^b$ | COSY$^a$ | HMBC$^a$ | ROESY$^c$ |
|---|---|---|---|---|---|
| 1 | 5.25 s | 68.1 | | 3,4,7,8,9 | |
| 3 | | 168.6 | | | |
| 4 | | 107.0 | | | |
| 5 | | 156.7 | | | |
| 6 | | 119.4 | | | |
| 7 | | 157.2 | | | |
| 8 | | 113.9 | | | |
| 9 | | 147.3 | | | |
| 11 | | 75.2 | | | |
| 12 | 2.01 d (4.7) | 42.8 | 13 | 11, 14 | |
| | 2.20 d (14.3) | | | | |
| 13 | 3.89 brs | 67.6 | 12 | | 26 |
| 13-OH | 4.62 brs | | | | |
| 14 | | 85.5 | | | |
| 15 | | 83.8 | | | |
| 17 | | 117.1 | | | |
| 17-OH | 7.04 brs | | | | |
| 18 | 1.50 m, 1.74 m | 30.6 | 19 | 17,20 | |
| 19 | 1.80 m, 1.93 m | 31.0 | 18 | 17 | |
| 20 | | 38.0 | | | |
| 21 | 2.34 brs | 46.3 | 22 | 6,11,19,20,22,24,27 | 24 |
| 22 | 4.83 d (2.8) | 59.5 | 21 | 6,7,11,20 | 27 |
| 22-OH | 5.20 brs | | | 6 | |
| 23 | 1.99 s | 10.5 | | 6,7,8,9 | |
| 24 | 1.29 s | 28.8 | | 11,12,21 | 21 |
| 25 | 1.43 s | 25.6 | | 14,15,26 | 27 |
| 26 | 1.32 s | 29.1 | | 14,15,25 | |
| 27 | 0.79 s | 18.2 | | 14,19,20,21 | 22,25 |
| 29 | 4.01 s | 62.5 | | 5 | |

$^a$ 300 MHz (DMSO-$d_6$)

$^b$ 75 MHz (DMSO-$d_6$)

$^c$ 600 MHz (MeOH-$d_4$)

Furthermore, three hydroxyl groups were observed at $\delta_H$ 4.62, 5.20 and 7.04 ppm (13-,
22-, and 17-OH, respectively). The $^{13}$C NMR (Table 2) and DEPT spectra confirmed
the presence of 25 carbon atoms in the structure, including 12 quaternary carbon
atoms. These data were in accordance with the data reported for austalide O,[6]

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previously isolated from the same fungal strain, suggesting that **2** has the same molecular skeleton as austalide O. Comparison of the NMR spectra of both compounds disclosed the disappearance of the methoxy group located at C-17 of austalide O in **2** and the appearance of a hydroxyl group ($\delta_H$ 7.04 ppm) instead. This was also consistent with the 14 amu decrease in the molecular weight of **2** compared to austalide O.[6] The structure of **2** was further confirmed by inspection of COSY, HMQC and HMBC spectra (Table 2). Based on the ROESY spectrum of **2**, comparison of the optical rotation values and the corresponding chirality centers of austalides M and O,[6] **2** was assigned to have (11*S*,13*R*,14*R*,20*R*,21*S*,22*S*) absolute configuration. Accordingly, **2** was characterized as a new natural product named austalide R.

The known compounds (**3**-**5**) were identified as 8-*O*-4-dehydrodiferulic acid,[29,30] cytochalasin Z17[31] and dihydroisoflavipucine,[32] respectively, by comparing their data (¹H and ¹³C NMR, MS and [$\alpha$]$_D$) with literature values. This is the first report of **4** and **5** from a sponge-derived fungus. Previous studies described the isolation of **4** from *A. terreus* and *A. flavipes* obtained from *Artemisia annua* and the mangrove plant *Acanthus ilicifolius*,[31,33] respectively, and of **5** from *Phoma* sp. isolated from *Salsola oppositifolia*.[32]

Compounds **1**-**5**, in addition to austalides M (**6**) and N (**7**) that were previously isolated from the same fungal strain,[6] were evaluated for their antibacterial activity against a panel of terrestrial and marine-derived bacteria, as well as for their cytotoxic activity against the murine cancer cell line L5178Y. All compounds showed

antibacterial activity against marine-derived strains (Table 3), at levels sometimes equivalent and/or lower than the positive control Seanine™ for *Vibrio harveyi*, *V. natriegens*, *Roseobacter litoralis*, *Pseudoalteromonas elyakovii*, *Halomonas aquamarina*, *Polaribacter irgensii* and *Shewanella putrefaciens*. Austalides **2** and **6** showed a broad spectrum of activity inhibiting 8 out of 11 tested strains at MIC values equal or inferior to the Seanine™'s ones (for *V. harveyi*, *R. litoralis*, *P. elyakovii*, *H. aquamarina*, *P. irgensii* and *S. putrefaciens*), whereas **7** inhibited only *V. natriegens* and *R. litoralis*. This indicates that a bulky substituent at C-22 may alter the spectrum of antibacterial activity. Furthermore, **1** exclusively inhibited *Vibrio* species, **4** exhibited selective and pronounced activity against *R. litoralis* (with a significantly lower MIC than Seanine™), and **5** displayed strong activity against *S. putrefaciens* and *V. natriegens*. In contrast, only **5** showed considerable activity against terrestrial *Staphylococcus aureus*. Furthermore, all compounds proved inactive against the murine cancer cell line L5178Y in the cytotoxicity assay. These data indicate that compounds **1-7** selectively inhibit marine-derived bacterial strains and lack cytotoxicity as judged from the cell line assay. This is of special interest as it may raise the prospect of using such compounds as antifouling agents or to combat epizootics in aquaculture in the future.

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**Table 3.** Results of antibacterial assay for **1**-**7** expressed as Minimum Inhibitory Concentrations (MIC)

| Bacterial strain | MIC [$\mu$g/mL][a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Seanine | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| Terrestrial | | | | | | | | |
| *Escherichia coli* | 1 | >10 | >10 | >10 | >10 | >10 | >10 | >10 |
| *Staphylococcus aureus* | 0.0001 | >10 | >10 | >10 | >10 | 0.001 | >10 | >10 |
| Marine | | | | | | | | |
| *Halomonas aquamarina* | 0.1 | >10 | 0.1 | >10 | >10 | >10 | 0.001 | >10 |
| *Polaribacter irgensii* | 1 | >10 | 0.1 | >10 | >10 | >10 | 0.01 | >10 |
| *Pseudoalteromonas elyakovii* | 0.1 | >10 | 0.1 | >10 | >10 | >10 | 0.001 | >10 |
| *Roseobacter litoralis* | 1 | >10 | 0.01 | 1 | 0.0001 | 10 | 0.001 | 0.01 |
| *Shewanella putrefaciens* | 1 | >10 | 0.1 | >10 | >10 | 0.001 | 0.001 | >10 |
| *Vibrio harveyi* | 1 | 1 | 0.1 | >10 | >10 | >10 | 0.001 | >10 |
| *V. natriegens* | 1 | 1 | >10 | >10 | 10 | 0.001 | 10 | 0.01 |
| *V. proteolyticus* | 0.01 | 0.1 | 0.1 | 0.1 | >10 | >10 | >10 | >10 |
| *V. carchariae* | 0.0001 | 0.1 | 0.01 | 1 | >10 | >10 | 0.01 | >10 |

[a] MIC values indicating the same or higher bioactivity than Seanine are highlighted.

**Acknowledgments**

**Supplementary data**

Supplementary data (experimental section and compound characterization) associated with this article can be found in the online version, at http://XXXXXXXXXXXXXX

## References

1. Holmström, K.; Gräslund, S.; Wahlström, A.; Poungshompoo, S.; Bengtsson, B.-E.; Kautsky, N. *Int. J. Food Sci. Tech.* **2003**, *38*, 255-266.

2. Roque, A.; Molina, A. A.; Bolán, M. C.; Gómez, G. B. *Int. J. Antimicrob. Ag.* **2001**, *17*, 383-387.

3. Rateb, M. E.; Ebel, R. *Nat. Prod. Rep.* **2011**, *28*, 290-344.

4. Saleem, M.; Ali, M. S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y. S. *Nat. Prod. Rep.* **2007**, *24*, 1142-1152.

5. Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143-163.

6. Zhou, Y.; Mándi, A.; Debbab, A.; Wray, V.; Schulz, B.; Müller, W. E. G.; Lin, W.; Proksch, P.; Kurtán, T.; Aly, A. H. *Eur. J. Org. Chem.* **2011**, 6009-6019.

7. Liu, H.; Edrada-Ebel, R. A.; Ebel, R.; Wang, Y.; Schulz, B.; Draeger, S.; Müller, W. E. G.; Wray, V.; Lin, W.; Proksch, P. *J. Nat. Prod.* **2009**, *72*, 1585-1588.

8. Liu, H.; Edrada-Ebel, R. A.; Ebel, R.; Wang, Y.; Schulz, B.; Draeger, S.; Müller, W. E. G.; Wray, V.; Lin, W. H.; Proksch, P. *Helv. Chim. Acta.* **2011**, *94*, 623-631.

9. Bringmann, G.; Lang, G.; Steffens, S.; Schaumann, K. *J. Nat. Prod.* **2004**, *67*, 311-315.

10. Lopez-Gresa, M. P.; Cabedo, N.; Gonzalez-Mas, M. C.; Ciavatta, M. L.; Avila, C.; Primo, J. *J. Nat. Prod.* **2009**, *72*, 1348-1351.

11. Daferner, M.; Anke, T.; Sterner, O. *Tetrahedron* **2002**, *58*, 7781-7784.

12. Debbab, A.; Aly, A. H.; Proksch, P. *Fungal Divers.* **2011**, *49*, 1-12.

13. Debbab, A.; Aly, A. H.; Lin, W. H.; Proksch, P. *Microb. Biotechnol.* **2010**, *3*,

544-563.

14. Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666-673.

15. Sang, U. L.; Yukihiro, A.; Dongho, L.; Jae-Hyuk, J.; Jong, S. A.; Hyuncheol, O. *J. Nat. Prod.* **2011**, *74*, 1284-1287.

16. Kito, K.; Ookura, R.; Yoshida, S.; Namikoshi, M.; Ooi, T.; Kusumi, T. *J. Nat. Prod.* **2007**, *70*, 2022-2025.

17. Zheng, J.; Xu, Z.; Wang, Y.; Hong, K.; Liu, P.; Zhu, W. *J. Nat. Prod.* **2010**, *73*, 1133-1137.

18. Trisuwan, K.; Rukachaisirikul, V.; Kaewpet, M.; Phongpaichit, S.; Hutadilok-Towatana, N.; Preedanon, S.; Sakayaroj, J. *J. Nat. Prod.* **2011**, *74*, 1663-1667.

19. Yurchenko, A. N.; Smetanina, O. F.; Kalinovsky, A. I.; Pivkin, M. V.; Dmitrenok, P. S.; Kuznetsova, T. A. *Russ. Chem. Bull.* **2010**, *59*, 852-856.

20. He, J.; Kithsiri, W. E. M.; Bashyal, B. P.; Zhan, J.; Seliga, C. J.; Liu, M. X.; Pierson, E. E.; Piesron, L. S.; Vanetten, H. D.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2004**, *67*, 1985-1991.

21. Li, G. Y.; Yang, T.; Luo, Y. G.; Chen, X. Z.; Fang, D. M.; Zhang, G. L. *Org. Lett.* **2009**, *11*, 3714-3717.

22. Du, L.; Zhu, T. J.; Liu, H. B.; Fang, Y. C.; Zhu, W. M.; Gu, Q. Q. *J. Nat. Prod.* **2008**, *71*, 1837-1842.

23. Cai, S.; Sun, S.; Zhou, H.; Kong, X.; Zhu, T.; Li, D.; Gu, Q. *J. Nat. Prod.* **2011**, *74*, 1106-1110.

24. Ingavat, N.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. *J. Nat. Prod.* **2011**, *74*,

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1650-1652.

25. Zhou, Y.; Debbab, A.; Mándi, A.; Wray, V.; Schulz, B.; Müller, W. E. G.; Kassack, M.; Lin, W.; Kurtán, T.; Proksch, P.; Aly, A. H. *Eur. J. Org. Chem.* **2013**, 894-906.

26. Kimura, Y.; Hamasaki, T.; Nakajima, H. *Tetrahedron Lett.* **1982**, *23*, 225-228.

27. McIver, A. L.; Deiters, A. *Org Lett.* **2010**, *12*, 1288-1291.

28. Horak, R. M.; Steyn, P. S.; Vleggaar, R. *J. Chem. Soc. Perkin Trans.* 1 **1985**, 363-367.

29. Bunzel, M.; Ralph, J.; Funk, C.; Steinhart, H. *Eur. Food Res. Technol.* **2003**, *217*, 128-133.

30. Funk, C.; Ralph, J.; Steinhart, H.; Bunzel, M. *Phytochemistry* **2005**, *66*, 363-371.

31. Lin, Z.; Zhang, G.; Zhu, T.; Liu, R.; Wei, H.; Gu, Q. *Helv. Chim. Acta.* **2009**, *92*, 1538-1544.

32. Loesgen, S.; Bruhn, T.; Meindl, K.; Dix, I.; Schulz, B.; Zeek, A.; Bringmann, G. *Eur. J. Org. Chem.* **2011**, 5156-5162.

33. Zhang, H.; Zhang, J.; Hu, S.; Zhang, Z.; Zhu, C.; Ng, S. W.; Tan, R. *Planta Med.* **2010**, *76*, 1616-1621.