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Waikikiamides A–C: Complex Diketopiperazine Dimer and Diketopiperazine–Polyketide Hybrids from a Hawaiian Marine Fungal Strain *Aspergillus sp.* FM242

Fuqian Wang,

Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawai'i 96720, United States; Department of Pharmacy, Wuhan No. 1 Hospital, Wuhan 430022, China

Ariel M. Sarotti,

Instituto de Química Rosario (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario 2000, Argentina

Guangde Jiang,

Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development, College of Pharmacy, University of Florida, Gainesville, Florida 32610, United States

José C. Huguet-Tapia,

Department of Plant Pathology, University of Florida, Gainesville, Florida 32611, United States

Shao-Liang Zheng,

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, United States

Xiaohua Wu,

Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawai'i 96720, United States

Chunshun Li,

Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawai'i 96720, United States

Yousong Ding,

Corresponding Authors: Yousong Ding – yding@cop.ufl.edu, **Shugeng Cao** – scao@hawaii.edu.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01411.

Experimental procedures and spectroscopic data for all new compounds (PDF)

Accession Codes

CCDC 1994346–1994347 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c01411

Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development, College of Pharmacy, University of Florida, Gainesville, Florida 32610, United States

Shugeng Cao
Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawai'i 96720, United States; Cancer Biology Program, University of Hawaii Cancer Center, Honolulu, Hawai'i 96813, United States

Abstract

Waikikiamides A-C (**1–3**), structurally complex diketopiperazine derivatives, and putative biogenic precursors, (+)-semivioxanthin (**4**), notoamide F (**5**), and (−)-notoamide A (**6**), were isolated from *Aspergillus* sp. FM242. **1** and **2**, bearing a hendecacyclic ring system, represent a novel skeleton. **3** features the first unique heterodimer of two notoamide analogs with an N–O-C bridge. Compounds **1** and **3** exhibit antiproliferative activity with IC_{50} values in the range of 0.56 to 1.86 μM. The gene clusters mined from the sequenced genome support their putative biosynthetic pathways.

Graphical Abstract

Natural products (NPs) have played a critical role in drug discovery.[1] Marine-derived materials, for example, sponges, coral, bacteria, and fungi, are well recognized as a unique but less explored source for the discovery of structurally novel and biologically active secondary metabolites.[2] For example, the antitumor agents ET-743,[3] salinosporamide A,[4] didemnin A,[5] norhalichondrin A,[6] and dolastatin 10[7] and the antituberculosis agent ilamycin E[8] were discovered from different marine origins. These clinically valuable agents propel the continuous investigation of marine NPs for new drug discovery.

Aspergillus is a large genus that is well recognized to produce a wide array of bioactive chemicals.[9] Indeed, the *Aspergillus* Secondary Metabolites Database (A2MDB) currently records 807 unique compounds produced by different *Aspergillus* species.[9] We have previously identified a unique benzodiazepine circumdatin M from *Aspergillus* sp. FM242 that was isolated from a sample collected at Waikiki beach in Oahu, Honolulu, Hawaii in May 2014.[10] In our ongoing research on this marine fungal strain, we isolated three novel complex prenylated indole diketopiperazine (DKP) derivatives, waikikiamides A-C (**1–3**), along with their potential biosynthetic precursors (+)-semivioxanthin (**4**), notoamide F (**5**), and (−)-notoamide A (**6**). Compounds **1** and **2** bear a hendecacyclic ring system formed from a prenylated indole DKP and polyketide **4**, representing an unprecedented skeleton, whereas **3** was the first prenylated indole DKP heterodimer with an N-O-C bridge. Herein

we report the isolation, structure elucidation, proposed biosynthetic pathways, and biological evaluation of waikikiamides A-C (**1–3**).

Waikikiamide A (**1**, Figure 1) was isolated as an optically active powder with a molecular formula of $C_{41}H_{39}N_3O_9$ (*m/z* 718.2743 $[M + H]^+$, Calcd for $C_{41}H_{40}N_3O_9$ 718.2759) that was established by high-resolution electrospray ionization mass spectrometry (HRESIMS) and required 24 degrees of unsaturation. The nuclear magnetic resonance (NMR)-based structural analysis of **1** was challenging due to the low H/C ratio. The correlation spectroscopy (COSY) spectrum of **1** showed five short spin systems: CH_2-CH_2-CH_2 in ring A, CH_2-CH in ring C, CH?CH (aromatic) in ring F, CH?CH (olefinic) in ring G, and CH_2-CH-CH_3 in ring A' (Figure 2). In the heteronuclear multiple bond correlation (HMBC) spectrum of **1**, correlations from H-2 to C-4; from H-21 to C-6, C-20, C-22, and C-23; from H-5 to C-4 and C-25; and from H_3-27/H_3-28 to C-6 and C-8 could establish rings A-D in fragment I, as shown in Figure 2. On the contrary, HMBCs from H-18 to C-10, C-16, and C-20; from H-17 to C-11 and C-19; from H-12 to C-10, C-14, and C-16; and from H_3-29/ H_3-30 to C-13 and C-14 indicated that rings F and G were a 5,6-disubstututed 2,2-dimethyl-2*H*-chromene. Although there was no HMBC to determine the connectivity of the three nitrogen atoms in fragment I, we assumed that ring E was a penta-substituted pyrrole 1-oxide and rings B/C had a 2,5-diazabicyclo[2.2.2]octane-3,6-dione core. The detailed NMR analysis revealed that fragment I of **1** was a Pro-Trp 2,5-DKP with two isoprenyl groups, which was the same as (+)-avrainvillamide, except for the 20- and 21-positions. The highly aromatic character of another fragment (II) was apparent from the presence of only **4** sp^3 carbons, including **1** methoxy and the CH_2-CH-CH_3 spin system, and 11 sp^2 carbons, including 9 nonprotonated and 2 tertiary aromatic carbons. When using DMSO-d_6 and pyridine-d_5 as solvents, the HMBC analysis clearly demonstrated the correlation from 10'-OH to C-9a', C-10', and C-10a'. NMR analysis further revealed that fragment II, a 3',7',8',9',10'-pentasubstituted benzoisochroman-**1**-one, was similar to (+)-semivioxanthin (**4**), except for the 8'- and 9'-positions, which was also isolated in this study. The connectivity between C-21 and C-8' was established on the basis of HMBCs from H-21 to C-6, C-20, C-22, C-23, C-7', C-8', and C-9'. According to the molecular formula of **1**, one additional ring and an oxygen atom had to be assigned. Because only the valences at C-20 and C-9' remained unsatisfied, the additional ring and oxygen could result only from an ether linkage between C-20 and C-9'. Hence, the planar structure of **1** (Figure 2) was determined, as shown.

In the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **1** (Figure 2), H-6 correlated to 26-NH, and H-21 showed correlations to 7'-OMe and H-18. These data indicated that H-6 and 26-NH had the same orientation, whereas H-21 and the C20-C19 bond had opposite orientations. To further determine the relative configuration of fragment I, we undertook quantum calculations of NMR shifts for correlation with experimental data, one leading strategy to facilitate the structural elucidation of complex molecules.[11] The continuous advances in the field enable the *in silico* predictions to complement the standard NMR spectroscopy nicely, emerging as a new tool when dealing with challenging architectures featuring a low H/C ratio.[12] Among the different methods of data correlation,[13] the DP4+ probability stands out for its good performance over a wide variety of systems.[14]

The diastereoisomers resulting from varying the configurations at C-6, C-20, and C-21 were generated, leading to eight structures (**1a** to **1h**, SI Section S10) including **1a** (all β H-6, 26-NH, C-19-C-20, and H-21) and **1h** (β H-6 and 26-NH; α C-19-C-20 and H-21). After a conformational sampling at the Merck molecular force field (MMFF) level (using both Spartan and Macromodel), the NMR shifts of all conformers found with a 10 kcal/mol cutoff for each configuration were computed at the PCM/ mPW1PW91/6–31+G**//B3LYP/6–31G* level.[14] Among all of the isomers, **1h** showed the closest match with the experimental values, demonstrating the smallest CMAE (corrected mean absolute error) values of 1.2 and 0.09 ppm for carbon and proton data, respectively (SI Section S14). Therefore, the DP4+ quantum calculations strongly supported **1h** to represent the real structure of fragment I (>99.9% overall probability), in good agreement with the NOESY correlations observed for waikikiamide A (**1**). Connecting the relative configurations of the fragment I and fragment II (C-3' position) was, on the contrary, much more difficult given the large separation of both stereoclusters by a plane aromatic core. Given the challenge to obtain suitable crystals for X-ray analysis, we computed the NMR shifts of 3'*epi*-**1h** in an attempt to suggest a sound full stereochemical assignment of **1**. As expected, the NMR shifts of 3'*epi*-**1h** were similar to those computed for **1h**, with slightly larger CMAE values (1.26 and 0.093 ppm vs 1.21 and 0.087 ppm, respectively), and DP4+ values favored **1h** in moderate probability (80.7%) (SI Section S16). Hence, the structure including the relative configuration of compound **1** was proposed, as shown.

To define the absolute configuration of **1**, the time-dependent density functional theory-electronic circular dichroism (TDDFT-ECD) method was applied to the most likely candidates (**1h** and 3'*epi*-**1h**).[15] The ECD spectra experimentally collected (Figure 3) showed positive Cotton effects at 225 and 275 nm and a strong negative Cotton effect at ~350 nm. The ECD calculations were carried out with the most stable conformers found for **1h** and 3'*epi*-**1h** at the B3LYP/6–31G* level. The Boltzmann-weighted ECD spectra of both **1h** candidates matched well with the experimental ECD spectrum (Figure 3), allowing us to assign the absolute configuration of **1**, as shown. The calculated ECD spectrum of 3'*epi*-**1h** (SI Section S26) was almost identical to that of **1h**, suggesting that the ECD spectrum seems to be governed mainly by the handedness of fragment I. Similar results were obtained at other levels of theory (including ωB97XD and CAM-B3LYP).

Waikikiamide B (**2**) had a molecular formula of $C_{41}H_{39}N_3O_8$ (*m/z* 702.2845 $[M + H]^+$, Calcd for $C_{41}H_{40}N_3O_8$ 702.2810), with one oxygen less than that of **1**. The detailed NMR analysis revealed that **2** was almost the same as **1**, except that the oxygen at the 9-position in **1** was missing in **2**. It is also supported by the chemical shift of C-8 (186.5) (SI Sections S27 and S54).[16] Its NOESY correlations and CD spectrum were almost the same as those of **1** (SI Sections S50, S56, and S59). Hence, the structure of **2** was determined, as shown.

The HRESIMS data of compound **3** provided a molecular formula of $C_{54}H_{62}N_6O_{11}$ (*m/z* 971.4545 $[M + H]^+$, Calcd for $C_{54}H_{63}N_6O_{11}$ 917.4549). NMR analyses revealed that **3** was also composed of two fragments (I and III). Fragment I was assigned as the prenylated indole DKP notoamide B, whereas fragment III was 12,13-dihydro-13-hydroxy-12-methoxy-notoamide G (SI Section S61). There was no HMBC between these

two fragments, which precluded the connectivity of fragments I and III of the molecule. Suitable crystals (0.16 × 0.10 × 0.08 mm) for an X-ray crystallographic study were obtained from acetonitrile at room temperature after repeated recrystallization. The final structure including the connectivity of fragments I and III, and the absolute configuration of **3** were then unambiguously determined by single-crystal X-ray diffraction (Figure 4, CCDC 1994347) with Flack(x) = 0.02(6), Hooft(y) = 0.02(5), p3(true) = 1.000, p3(false) = 0.3×10^{-82}, and p3(racemic twin) = 0.9×10^{-20} using PLATON/ BIJVOETPAIR.[17]

The structures of known compounds **4** ((+)-semivioxanthin)[18] and **5** (notoamide F (21-methoxy stephacidin A))[19] (SI Section S6, CCDC 1994346; 0.18 × 0.12 × 0.10 mm crystals were obtained from methanol at room temperature) and **6** ((−)-notoamide A)[20] were identified by the comparison of their physical data with the reported values in the literature and X-ray analysis.

Multiple prenylated indole DKP dimers (e.g., stephacidin B[21] and waikialoids A and B[22]) as well as the hybrids of DKP and polyketide (e.g., variecolortines A-C[23] and versicoamides F-H[24]) have been previously reported. To our knowledge, compound **3** was the first DKP heterodimer with an N-O-C bridge. Furthermore, compounds **1** and **2** represent a rare class of fungal secondary metabolites, which are hybrids of DKPs and polycyclic aromatic polyketides.

The biosynthesis of waikikiamides A and B (**1** and **2**) can be derived from the polyketide (+)-semivioxanthin (**4**) and the prenylated indole DKP (+)-avrainvillamide and aspergamide B, respectively, whereas the coupling of notoamide B and a derivative of notoamide G can produce compound **3** (Scheme 1). Co-isolation of **4–6** together with the LC/MS detection of notoamides R and G supported the proposed biosynthetic pathway of waikikiamides. Indeed, (+)-stephacidin A is a known common biosynthetic intermediate of (+)-avrainvillamide, aspergamide B, and notoamides[25] (SI Section S4). Furthermore, the genome of *Aspergillus* sp. FM242 was sequenced and assembled to provide 36.6 Mbp (395 contigs, 49.1% GC). The antiSMASH analysis revealed a gene cluster highly similar to the notoamide (*not*) cluster known in other fungal species and can be responsible for the biosynthesis of multiple prenylated indole DKPs for the production of waikikiamides.[25–27] It is of note that the N-O-C bridge in **3** is a unique feature among known dimeric DKPs, and the enzymatic formation of this bridge remains unstudied. Compared with known notoamide gene clusters,[26,27] the cluster identified in this work encodes five additional proteins: WaiA1, WaiA2, WaiB, WaiC, and WaiD (Figure 5). Among them, WaiD is predicted to be a benzoate 4-monooxygenase P450, whereas WaiC is an uncharacterized 740-amino-acid protein. The vast catalytic versatility of P450s[28] and the unknown function of WaiC make them potential candidates for the formation of the N-O-C bridge (Scheme 1). The sequenced genome also carries eight predicted polyketide synthases but only one nonreducing polyketide synthase (*NRPKS, SvioA*) at a different chromosomal region withthe *not* cluster. The set of SvioA, an *O*-methyltransferase (SvioM), and a short-chain dehydrogenase/reductase (SvioC) can synthesize the naphtho-α-pyrone semivioxanthin (**4**) from one acetyl-CoA and six malonyl-CoAs, whereas the pathway-specific laccase (SvioL) may fuse **4** with (+)-avrainvillamide and aspergamide B[29] to produce **1** and **2**, respectively (Scheme 1, Figure 5).

SvioL homologues are known to catalyze phenol coupling in the biosynthesis of polyketide dimers.[29]

Compounds **1–6** were evaluated for their antiproliferative activity against four different types of cancer cell lines, HT1080 (a human fibrosarcoma cell line), PC3 (a human prostatic tumor cell line), Jurkat (an immortalized T lymphocyte cell line), and A2780 (a human ovarian cancer cell line) (Table 1). Interestingly, compounds **1** and **3**, each with an N-O bond, were active with IC_{50} values between 0.56 and 1.86 μM, whereas compound **2** without an N-O bond and monomers (**4–6**) were inactive at 20 and 40 μM (the highest concentration tested) for **2** and **4–6**, respectively. The more rigid DKP-polyketide hybrid (**1**) was more active than the heterodimer of two DKPs (**3**).

In summary, three novel complex compounds (**1–3**) were isolated from *Aspergillus* sp. FM242 and characterized as either DKP-polyketide hybrids (**1** and **2**) or a heterodimer of two DKPs (**3**). The hybrids of DKP and polyketide (**1** and **2**) are quite rare secondary metabolites, and the DKP heterodimer (**3**) with an N-O-C bridge is unprecedented. Clearly, to determine the structure of **3** without X-ray analysis was impossible. The discovery of **1–3** expands the chemical diversity of the known DKP scaffolds and furnishes intriguing templates for synthetic and biosynthetic chemists. Furthermore, **1**, a DKP-polyketide hybrid with a nitrogenated methoxy group, showed the most antiproliferative activity, which may attract wide attention from cancer biologists, biochemists, medicinal chemists, and pharmacologists for new anticancer drug discovery and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

(1). (a)Newman DJ; Cragg GMJ Nat. Prod. 2016, 79, 629–661.(b)Cragg GM; Newman DJ Biochim. Biophys. Acta, Gen. Subj 2013, 1830, 3670–3695.(c)Mathur S; Hoskins C Biomed. Rep. 2017, 6, 612–614. [PubMed: 28584631]

(2). (a)Blunt JW; Carroll AR; Copp BR; Davis RA; Keyzers RA; Prinsep MR Nat. Prod. Rep. 2018, 35, 8–53 [PubMed: 29335692] (b)Deshmukh SK; Prakash V; Ranjan N Front. Microbiol. 2018, 8 (2536), 1–24. [PubMed: 29403461] (c)Blunt JW; Copp BR; Keyzers RA; Munro MH; Prinsep MR Nat. Prod. Rep. 2016, 33, 382–431. [PubMed: 26837534] (d)Blunt JW; Copp BR; Keyzers RA; Munro MH; Prinsep MR Nat. Prod. Rep. 2015, 32, 116–211. [PubMed: 25620233]

(3). Damia G; Silvestri S; Carrassa L; Filiberti L; Faircloth GT; Liberi G; Foiani M; D'Incalci M Int. J. Cancer 2001, 92, 583–588. [PubMed: 11304695]

(4). Feling RH; Buchanan GO; Mincer TJ; Kauffman CA; Jensen PR; Fenical W Angew. Chem., Int. Ed. 2003, 42, 355–357.

(5). Crampton SL; Adams EG; Kuentzel SL; Li LH; Badiner G; Bhuyan BK Cancer. Res. 1984, 44, 1796–1801. [PubMed: 6713383]

(6). Uemura D; Takahashi K; Yamamoto T; Katayama C; Tanaka J; Okumura Y; Hirata YJ Am. Chem. Soc. 1985, 107, 4796–4798.

(7). Schwartsmann G; da Rocha AB; Berlinck RG; Jimeno J Lancet Oncol. 2001, 2, 221–225. [PubMed: 11905767]

(8). Ma J; Huang H; Xie Y; Liu Z; Zhao J; Zhang C; Jia Y; Zhang Y; Zhang H; Zhang T; Ju J Nat. Commun. 2017, 8, 391. [PubMed: 28855504]

(9). Vadlapudi V; Borah N; Yellusani KR; Gade S; Reddy P; Rajamanikyam M; Vempati LNS; Gubbala SP; Chopra P; Upadhyayula SM; Amanchy R Sci. Rep. 2017, 7, 7325. [PubMed: 28779078]

(10). Wang F; Hu Z; Li C; Wu X; Cao S Tetrahedron Lett. 2019, 60, 1724–1726.

(11). Lodewyk MW; Siebert MR; Tantillo D Chem. Rev. 2012, 112, 1839–1862. [PubMed: 22091891]

(12). Kutateladze AG; Krenske EH; Williams CM Angew. Chem., Int. Ed. 2019, 58, 7107–7112.

(13). Grimblat N; Sarotti AM Chem. - Eur. J. 2016, 22, 12246–12261. [PubMed: 27405775]

(14). Grimblat N; Zanardi MM; Sarotti AM J. Org. Chem. 2015, 80, 12526–12534. [PubMed: 26580165]

(15). Li XC; Ferreira D; Ding Y Curr. Org. Chem. 2010, 14, 1678–1697. [PubMed: 24729741]

(16). Li F; Zhang Z; Zhang G; Che Q; Zhu T; Gu Q; Li D Org. Lett. 2018, 20, 1138–1141. [PubMed: 29400467]

(17). Spek AL Acta Crystallogr., Sect. D: Biol. Crystallogr. 2009, 65 (Pt 2), 148–155.

(18). (a)Zeeck A; Ruß P; Laatsch H; Loeffler W; Wehrle H; Zähner H; Holst H Chem. Ber. 1979, 112, 957–978.(b)Yada H; Sato H; Toshima H; Deura M; Ichihara A Biosci., Biotechnol., Biochem. 2001, 65, 484–486. [PubMed: 11302197]

(19). Tsukamoto S; Kato H; Samizo M; Nojiri Y; Onuki H; Hirota H; Ohta TJ Nat. Prod. 2008, 71, 2064–2067.

(20). Kato H; Yoshida T; Tokue T; Nojiri Y; Hirota H; Ohta T; Williams RM; Tsukamoto S Angew. Chem., Int. Ed. 2007, 46, 2254–2256.

(21). Qian-Cutrone J; Huang S; Shu Y; Vyas D; Fairchild C; Menendez A; Krampitz K; Dalterio R; Klohr SE; Gao QJ Am. Chem. Soc. 2002, 124, 14556–14557.

(22). Wang X; You J; King JB; Powell DR; Cichewicz RH J. Nat. Prod. 2012, 75, 707–715. [PubMed: 22400916]

(23). Zhong W; Wang J; Wei X; Chen Y; Fu T; Xiang Y; Huang X; Tian X; Xiao Z; Zhang W; Zhang S; Long L; Wang F Org. Lett. 2018, 20, 4593–4596. [PubMed: 30011219]

(24). Liu L; Wang L; Bao L; Ren J; Bahadur Basnet B; Liu R; He L; Han J; Yin W; Liu H Org. Lett. 2017, 19, 942–945. [PubMed: 28181808]

(25). Klas KR; Kato H; Frisvad JC; Yu F; Newmister SA; Fraley AE; Sherman DH; Tsukamoto S; Williams RM Nat. Prod. Rep. 2018, 35, 532–558. [PubMed: 29632911]

(26). Ding Y; Wet J. R. d.; Cavalcoli J; Li S; Greshock TJ; Miller KA; Finefield JM; Sunderhaus JD; McAfoos TJ; Tsukamoto S; Williams RM; Sherman DH J. Am. Chem. Soc. 2010, 132, 12733–12740. [PubMed: 20722388]

(27). Li S; Srinivasan K; Tran H; Yu F; Finefield JM; Sunderhaus JD; McAfoos TJ; Tsukamoto S; Williams RM; Sherman DH MedChemComm 2012, 3, 987–996. [PubMed: 23213353]

(28). (a)Zhang X; Li S Nat. Prod. Rep. 2017, 34, 1061–1089. [PubMed: 28770915] (b)Rudolf JD; Chang CY; Ma M; Shen B Nat. Prod. Rep. 2017, 34, 1141–1172. [PubMed: 28758170] (c)Podust LM; Sherman DH Nat. Prod. Rep. 2012, 29, 1251–1266. [PubMed: 22820933]

(29). (a)Fürtges L; Obermaier S; Thiele W; Foegen S; Müller M ChemBioChem 2019, 20, 1928–1932. [PubMed: 30868712] (b)Su J; Fu J; Wang Q; Silva C; Cavaco-Paulo A Crit. Rev. Biotechnol. 2018, 38, 294–307. [PubMed: 28738694]

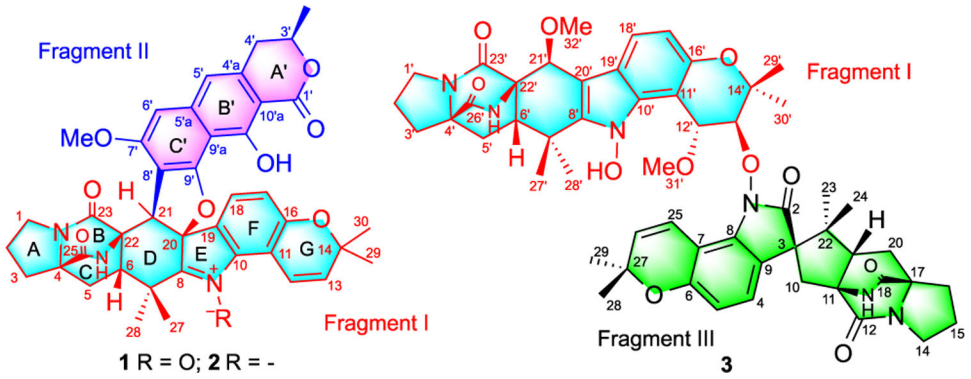


Figure 1.
Chemical structures of compounds **1–3**

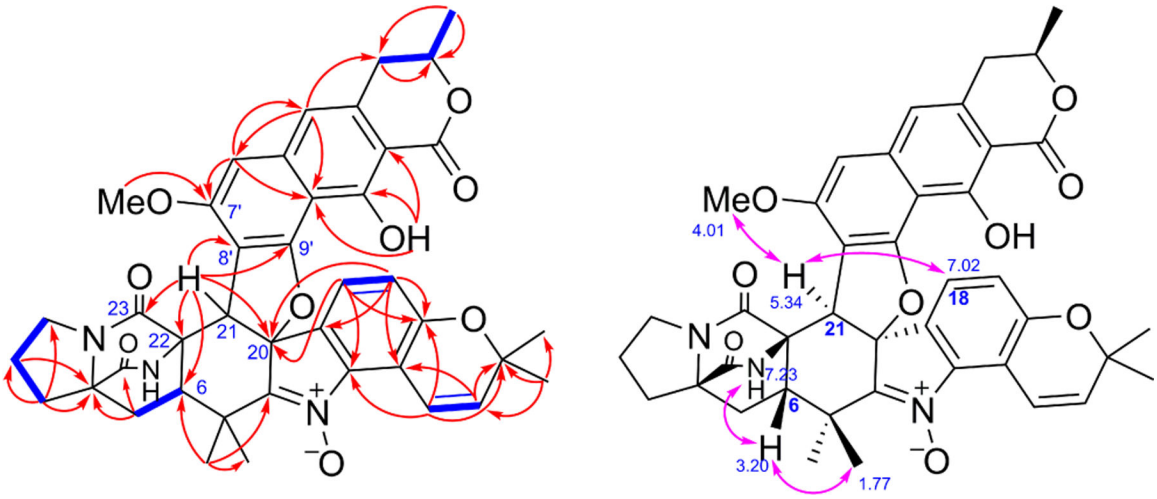


Figure 2.

Key COSY correlations (bold, blue) and HMBCs (single-headed, red) of **1.** Key NOESY correlations (double-headed, pink) of **1.**

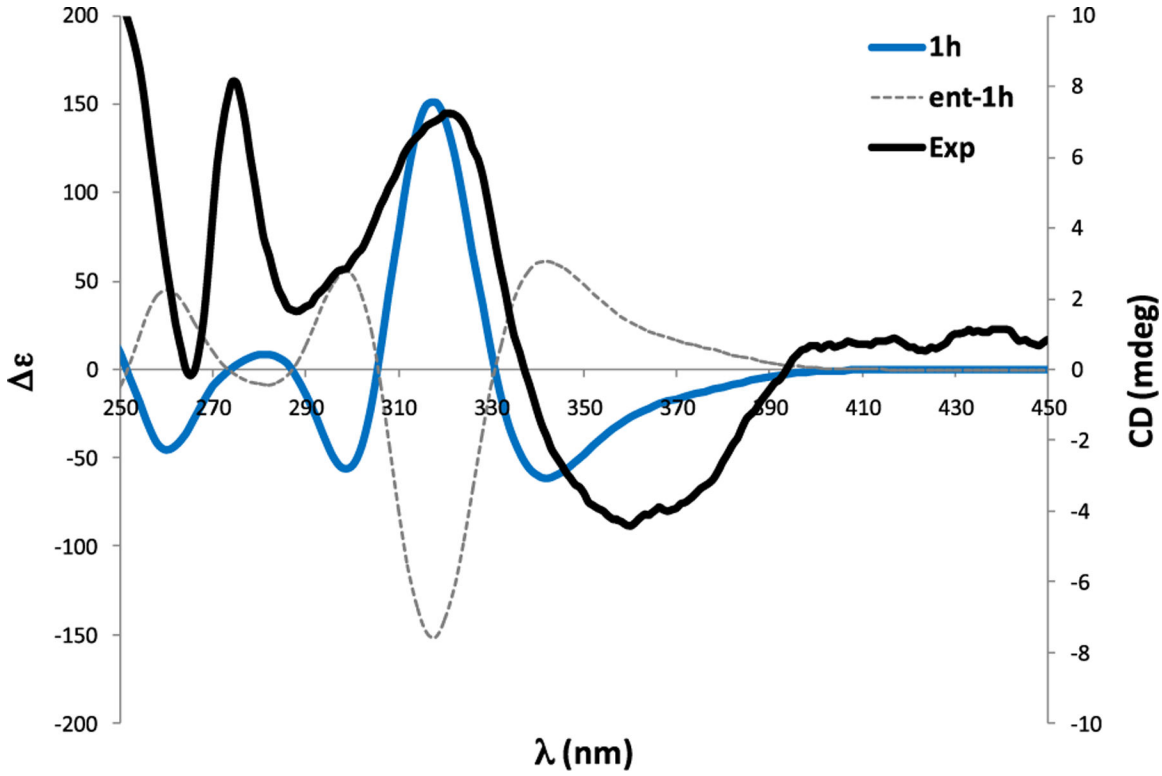


Figure 3.
Experimental and calculated ECD of **1**.

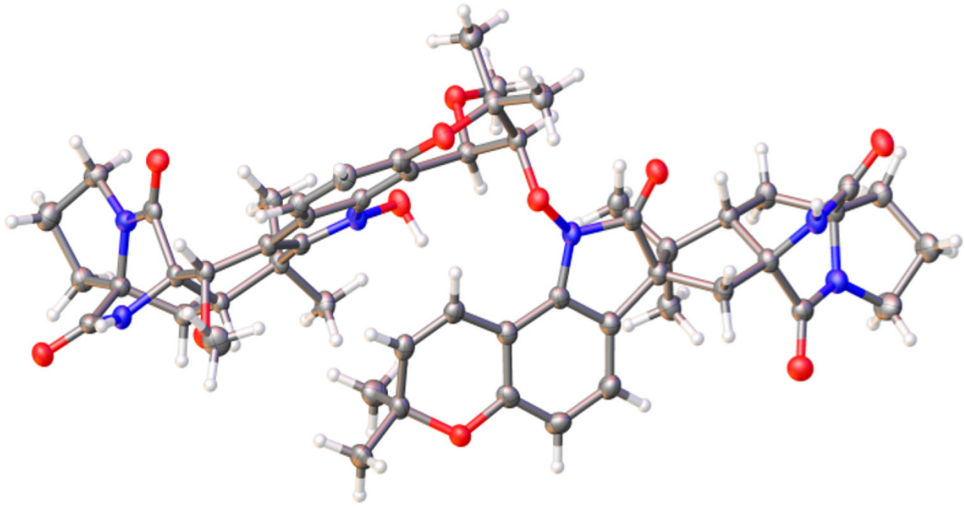


Figure 4.
X-ray crystal structure of **3**.

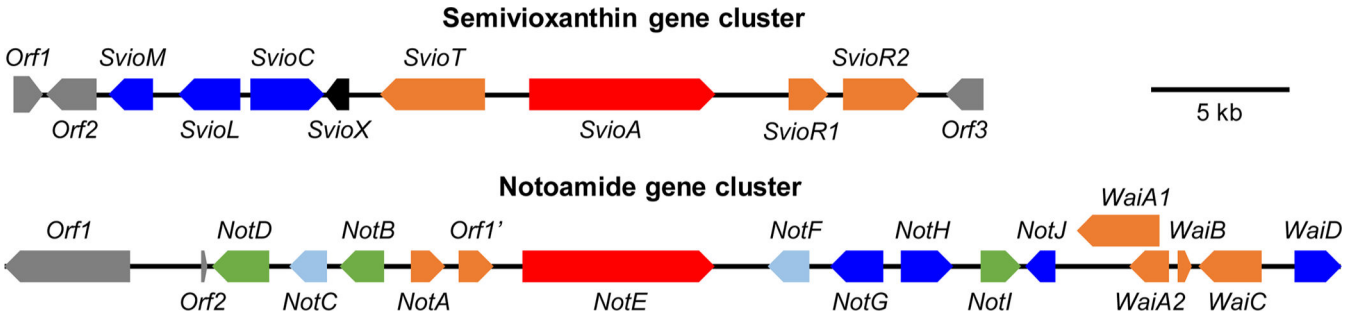
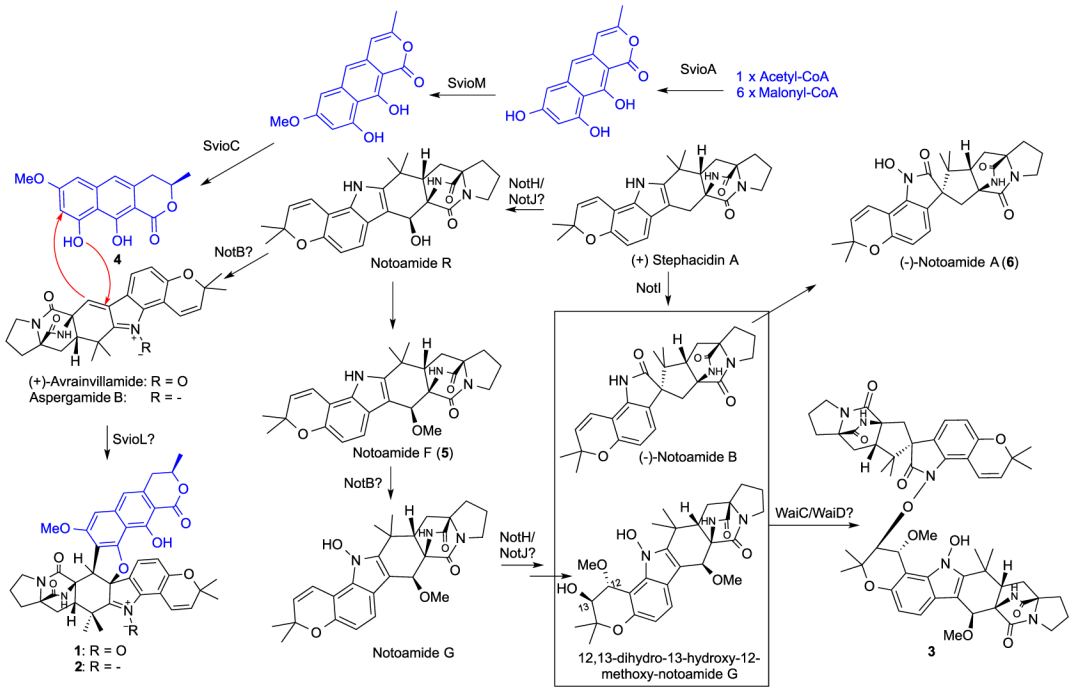


Figure 5.

Waikikiamide (*wai*) biosynthetic gene cluster derived from the complete sequencing and bioinformatic mining of the *Aspergillus* sp. FM 242 genome. The mined *wai* cluster comprises two separate subclusters, the semivioxanthin (*svio*) gene cluster (~34 kb, Genbank accession number: MT457560) and the notoammide (*not*) gene cluster (~47 kb, Genbank accession number: MT457561), located at two different chromosomal regions. The *svio* cluster (top) contains all genes required for the biosynthesis of semivioxanthin, and the putative laccase SvioL may catalyze the enzymatic fusion of semivioxanthin and (+)-avrainvillamide or aspergamide B to produce waikikiamides A and B (**1** and **2**). A total of 11 proteins encoded by genes from the *not* cluster (bottom) share high amino acid sequence similarities with those of the *not* cluster from notoamide-producing *Aspergillus* sp. MF297–2 and can be responsible for the biosynthesis of notoamides (+)-avrainvillamide and aspergamide B via the common biosynthetic intermediate (+)-stephacidin A. The *not* cluster from *Aspergillus* sp. FM292 also encodes an uncharacterized protein (WaiC) and an additional P450 (WaiD), which could be the candidates for the formation of a N-O-C bridge between (−)-notoamide B and 12,13-dihydro-13-hydroxy-12-methoxy-notoamide G to produce waikikiamide C (**3**).



Scheme 1.
Proposed Biosynthetic Pathways of 1 –3

Table 1.

Antiproliferative Activity (IC_{50}) of Compounds 1 and 3 (μM)[a]

cell lines	1	3	taxol
HT1080	0.519	1.135	0.0075
PC3	1.855	1.805	0.0136
Jurkat	0.62	1.79	0.0087
A2780S	0.78	1.127	0.0081

[a]Note: all in triplicate.