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# Acanthomanzamines A−E with New Manzamine Frameworks from the Marine Sponge *Acanthostrongylophora ingens*

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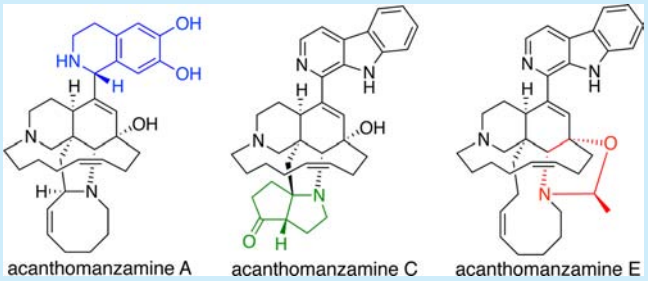
Supporting Information

**ABSTRACT:** Five new manzamine alkaloids, acanthomanzamines A−E, were isolated from the marine sponge *Acanthostrongylophora ingens*. Acanthomanzamines A and B are the first examples, containing a tetrahydroisoquinoline instead of a β-carboline in manzamine-related alkaloids. Acanthomanzamine C contains a hexahydrocyclopenta[*b*]-pyrrol-4(2*H*)-one ring that may be converted from an eight-membered ring in manzamine A. Acanthomanzamines D and E have an additional oxazolidine and 2-methyloxazolidine rings, respectively, which fuse to the manzamine skeleton.




Manzamines are structurally interesting alkaloids that are isolated from marine sponges and are characterized by a fused tetra- or pentacyclic ring system that is attached to a β-carboline moiety. More than 80 manzamine-related alkaloids have so far been identified following the discovery of manzamine A (**6**),[1] including the alkaloids with novel carbon frameworks, e.g., two dimers designated kauluamine[2] and *neo*-kauluamine,[3] manzamine C,[4] nakadomarin A,[5] ma'eganedin A,[6] manadomanzamine A,[7] zamamidine A,[8] acantholactone,[9] zamamiphidin A,[10] and acantholactam,[11] together with their various biological activities,[12] such as inhibitory activity against the proteasome, an intracellular proteolytic complex.[12e] In the search for drug leads from natural sources, we detected an extract from the marine sponge *Acanthostrongylophora ingens* that exhibited inhibitory activity against the proteasome. The purification of a small-molecular inhibitor of the proteasome from the extract of this sponge afforded five new alkaloids, designated acanthomanzamines A−E (**1**−**5**), and the known alkaloid, manzamine A (**6**) (Figure 1). In the present study, we described the isolation, structural determination, and biological activities of **1**−**5**.

The sponge *A. ingens* was collected in Mantehage, Indonesia, and extracted with EtOH. The EtOH extract was evaporated, and the residual aqueous solution was extracted with EtOAc and then *n*-BuOH. Purification of the fractions by $SiO_2$ and ODS column chromatographies and HPLC afforded five new alkaloids **1**−**5**.

Acanthomanzamine A (**1**) had the molecular formula $C_{34}H_{47}N_3O_3$, and this was determined by HRFABMS and $^{13}C$ NMR (Supporting Information, Table S1) spectrometry. The $^1H$ NMR spectrum (Supporting Information, Table S1) showed four singlet signals at δ 6.58 (H-5), 6.32 (H-8), 5.11 (H-11), and 4.78 (H-1), a triplet signal at δ 5.32 (H-33), and four multiplet signals at δ 6.11 (H-32), 5.45 (2H, H-15 and H-16), and 4.65 (H-34) in the lower field, whereas the higher field signals were similar to those of **6**. This spectral feature strongly indicated that **1** was a manzamine-related alkaloid that contained another heterocyclic ring instead of the β-carboline moiety in **6**. An analysis of 2D NMR spectra, including COSY, HSQC, and HMBC, confirmed that **1** contained the same pentacyclic ring system as that in **6**. The residual structure, designated as partial structure ***a*** shown in Figure 2, was estimated to be composed of $C_9H_{10}NO_2$. The COSY spectrum of this structure revealed the connection of two methylene signals at δ 2.87 (2H, m, $H_2$-4) and 3.09 (m, H-3)/3.46 (m, H-3) (Figure 2). HMBC correlations from $\delta_H$ 3.09/3.46 ($H_2$-3) to $\delta_C$ 55.6 (C-1) indicated that these carbons were connected through a nitrogen atom based on their carbon chemical shifts. Other HMBC correlations from $\delta_H$ 2.87 ($H_2$-4) to $\delta_C$ 114.9 (C-5), 121.4 (C-8a), and 123.7 (C-4a), from $\delta_H$ 6.32 (H-8) to $\delta_C$ 55.6 (C-1), 123.7 (C-4a), and 145.2 (C-6), and from $\delta_H$ 6.58 (H-5) to $\delta_C$ 121.4 (C-8a) and 144.0 (C-7) showed the presence of a 1,2,3,4-tetrahydroisoquinoline-6,7-diol moiety.

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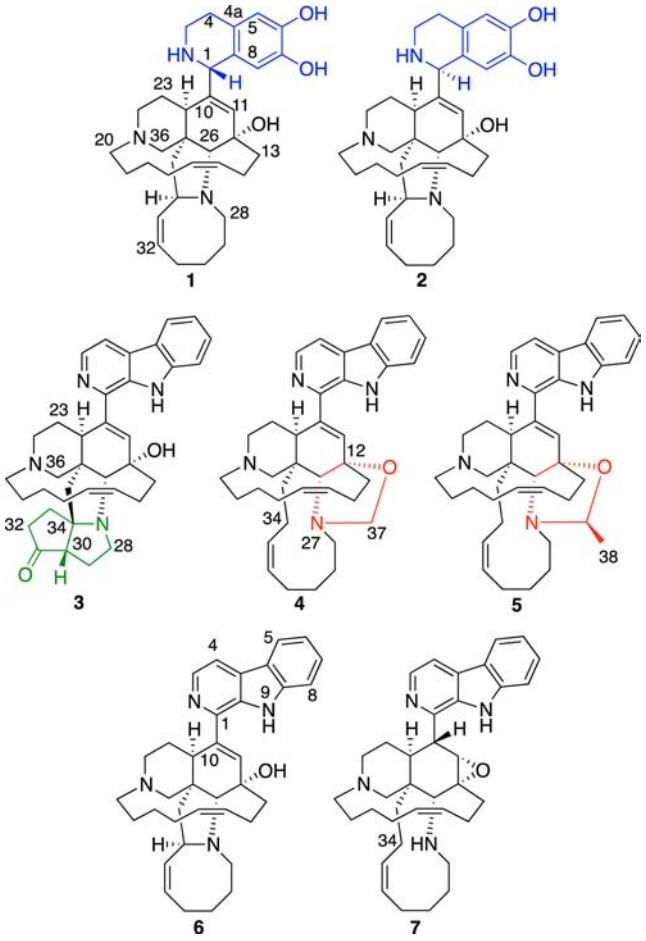

**Figure 1.** Structures of acanthomanzamines A–E (**1**–**5**) and manzamines A and B (**6** and **7**). The partial structures of **1** and **2**, shown in blue, indicate the (1*S*)- and (1*R*)-tetrahydroisoquinoline moieties, respectively, that of **3**, shown in green, indicates the hexahydrocyclopenta[*b*]pyrrol-4(2*H*)-one moiety, while those of **4** and **5**, shown in red, indicate the oxazolidine and 2-methyloxazolidine moieties, respectively.

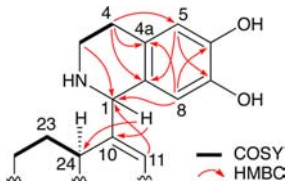


**Figure 2.** COSY and key HMBC correlations of the partial structure ***a*** in **1**.

The connection between C1 and C10 was implied by HMBC correlations from $\delta_H$ 4.78 (H-1) to $\delta_C$ 38.2 (C-24) and 139.2 (C-10) and from $\delta_H$ 5.11 (H-11) to $\delta_C$ 55.6 (C-1).

Acanthomanzamine B (**2**) had the same molecular formula as **1**. An analysis of 2D NMR spectra (Supporting Information, Table S1) indicated that **2** had the same structure as **1**, except for the configuration of C-1. NOE experiments of **1** and **2** exhibited no correlation and, therefore, their C-1 configurations could not be determined. We then calculated the theoretical ECD spectra for both 1*R*- and 1*S*-configurations (Figure 3) by a standard calculation procedure (Supporting Information).[13] Conformational searches for the 1*R*- and 1*S*-configurations were performed on the basis of a molecular mechanical method by CONFLEX7

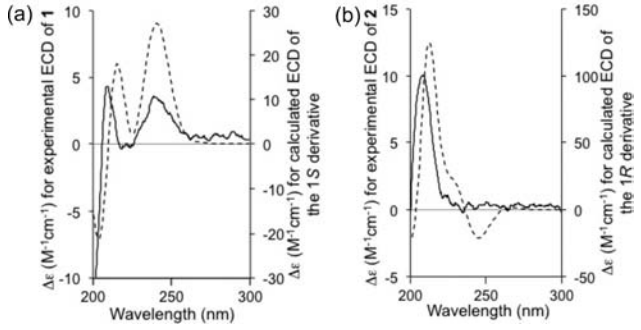


**Figure 3.** Experimental and calculated ECD spectra in MeOH: (a) experimental ECD of **1** (solid line) and calculated ECD of the 1*S* derivative (dashed line); (b) experimental ECD of **2** (solid line) and calculated ECD of the 1*R* derivative (dashed line).

with the MMFF94S force field. The *R*- and *S*-isomers gave as many as 11450 and 10126 conformers, respectively, whereas only eight conformers showed populations over 1% for both isomers. Therefore, these eight stable conformers for each configuration were further optimized on Gaussian 09 (Revision A.02) by the density functional theory (DFT) method at the B3LYP/6-31G(d) level in the presence of MeOH with a polarizable continuum model (PCM). The internal energies obtained were corrected with vibrational frequencies for all computed conformers. The most stable four and five conformers, which covered 93.7 and 91.9% of the populations for the 1*R*- and 1*S*-configurations, respectively, were subjected to the time-dependent density functional theory (TDDFT) calculations at the B3LYP/TZVP level in the presence of MeOH with PCM. The resultant rotational strengths were converted into Gaussian-type curves and summed to give the theoretical ECD spectrum for each conformer. The calculated ECD spectra were finalized by considering the Boltzmann distributions of the conformers (Figure 3), which implied that **1** and **2** had 1*S*- and 1*R*-configurations, respectively.

Acanthomanzamine C (**3**) had the molecular formula $C_{36}H_{42}N_4O_2$. An analysis of 2D NMR spectra indicated the presence of a fused 6-, 6-, 5-, and 13-membered ring system connected to the *β*-carboline moiety, which showed that modifications existed in the 8-membered ring in **6**. Compared to **6**, the NMR spectra (Supporting Information, Table S2) showed the presence of a ketone carbon ($\delta$ 219.0, C-31) and quaternary carbon ($\delta$ 77.0, C-34), the latter of which was connected to a heteroatom, and the absence of the two olefin carbons (C-32 and C-33) observed in **6**. HMBC correlations from $\delta_H$ 3.16/3.28 ($H_2$-28) and 1.97/2.09 ($H_2$-33) to $\delta_C$ 77.0 (C-34) and from $\delta_H$ 2.05/2.33 ($H_2$-29), 2.51 (H-30), and 1.97/2.09 ($H_2$-33) to $\delta_C$ 219.0 (C-31) (Figure 4) revealed the presence of the bicyclo ring system N27–C34 fused to the five-membered ring C25–C26–N27–C34–C35 in **6**, which was supported by HMBC correlations from $\delta_H$ 2.16/2.22 ($H_2$-35) to $\delta_C$ 36.6 (C-

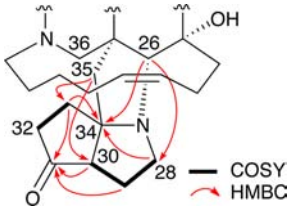


**Figure 4.** Key COSY and key HMBC correlations of **3**.


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33) and 58.5 (C-30) and from $\delta_H$ 3.64 (H-26) to $\delta_C$ 56.8 (C-28) and 77.0 (C-34). The configurations at C-30 and C-34 were estimated based on NOE correlations. Among the four possible structures **3a**-**3d** (Figure 5a), the possibility of the *trans*-

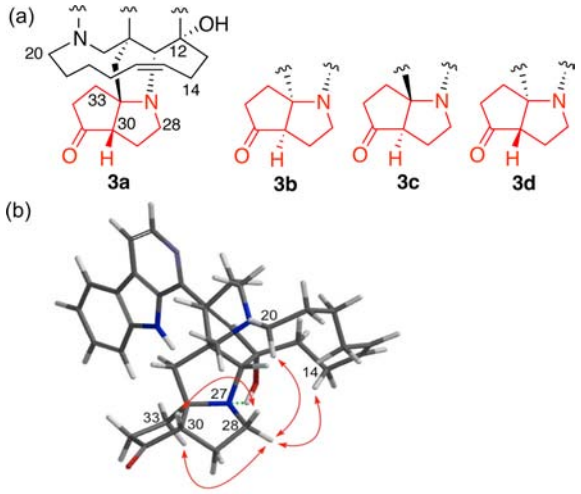
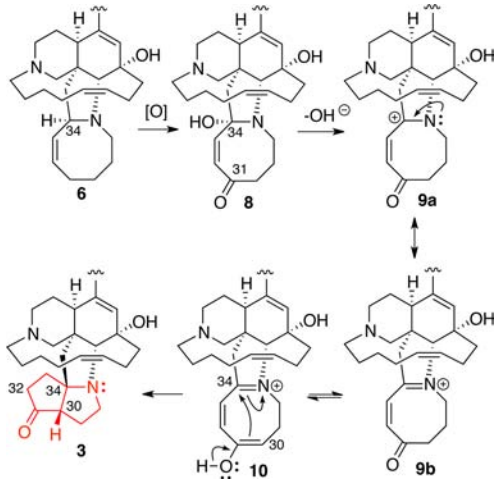



**Figure 5.** Study on the configuration of **3**: (a) four possible structures of **3** (**3a**, **3b**, **3c**, and **3d**); (b) observed NOE correlations in the energy-minimized conformation of **3a**, calculated using Spartan'14 (Wave function, Inc.). Red arrows, NOE correlations; green dashed line, a hydrogen bond.

configured ring, **3c** or **3d**, was excluded because of the observed NOE correlation between H-28 and H-33 (Figure 5b). NOE correlations from H-28 to H-14 and H-20 indicated that the structure **3a** was preferable to that of **3b** (Supporting Information, Figure S28). The proposed mechanism of formation of **3** is shown in Scheme 1. After the oxidation

**Scheme 1. Possible Biogenetic Pathway from 6 to 3**




catalyzed by enzyme at C-34 and C-31, **6** would be converted to **9a** with a carbocation stabilized by the tertiary nitrogen. Through a keto−enol tautomerization (**9a**/**9b** and **10**) a new link between C30 and C34 would be generated to afford **3**.

Acanthomanzamine D (**4**) had the molecular formula $C_{37}H_{46}N_4O$, containing one $CH_2$ unit more than that of **6**, and this was determined by HRFABMS. An analysis of 2D NMR spectra showed the presence of a tetracyclic ring system connected to the $\beta$-carboline moiety in manzamine B (**7**)[14] (Figure 1) together with an isolated methylene group [$\delta_H$ 4.19 (d) and 4.36 (br s) ($H_2$-37)/$\delta_C$ 84.7 (C-37)] (Supporting Information, Table S3). HMBC correlations from $H_2$-37 to C-12 ($\delta_C$ 79.8) and C-28 ($\delta_C$ 58.6) showed that the methylene group was located between the oxygen atom at C-12 and the nitrogen atom (N-27), and this was supported by the low-field chemical shift of C-37 (Figure 6). On the other hand, HRFABMS of

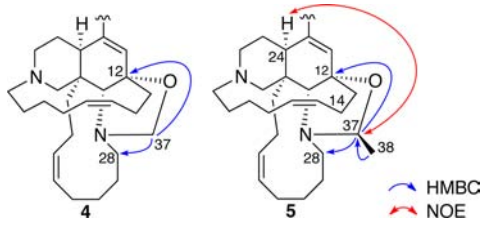



**Figure 6.** Key HMBC and NOE correlations of **4** and **5**.

acanthomanzamine E (**5**) revealed the molecular formula $C_{38}H_{48}N_4O$, which contained one $CH_2$ unit more than that of **4**. The presence of an ethylidene group instead of the methylene group in **4** was revealed by the COSY and HMBC correlations, $\delta_H$ 1.29 (3H, d, $J$ = 5.7 Hz, $H_3$-38)/$\delta_C$ 87.9 (C-37) and $\delta_H$ 4.67 (br q, $J$ = 5.7 Hz, H-37)/$\delta_C$ 78.7 (C-12) and 51.9 (C-28) (Figure 6) (Supporting Information, Table S3). An NOE correlation between H-37 ($\delta_H$ 4.67) and H-24 ($\delta_H$ 3.13) unambiguously established the 37*S*-configuration. Thus, **4** and **5** contained additional oxazolidine and 2-methyloxazolidine rings, respectively, which were fused to the skeleton of **7**.

Manzamine-related alkaloids exhibit various biological activities, including cytotoxic,[12a] antimicrobial,[12b] antimalarial,[12c] antiviral,[12d] antiinflammatory,[12d] antiatherosclerotic,[12e] and insecticidal[12f] activities, together with proteasome inhibitory activity.[12g] Using the compounds isolated in this study,[15] we tested their cytotoxic activities and two inhibitory activities against the proteasome as well as the accumulation of the cholesterol ester in macrophages (Table 1). The cytotoxicities of

**Table 1. Biological Activities**

| compd | cytotoxicity $IC_{50}$[a] ($\mu$M) | inhibition of the proteasome $IC_{50}$[b] ($\mu$M) | inhibition of the accumulation of the cholesterol ester[c] (%) |
|---|---|---|---|
| **1** | 4.2 | 4.1 | 48 |
| **2** | 5.7 | 7.8 | 73 |
| **4** | 15 | 0.63 | 73 |
| **5** | >20 | 1.5 | 61 |
| **6** | 13 | 2.0 | 80 |

[a]Tested against HeLa cells. [b]Tested against the chymotrypsin-like activity of the proteasome. [c]Tested at a concentration of 20 $\mu$M.

**1** and **2** in HeLa cells were more potent than those of **4**−**6**, which indicated that the presence of 1,2,3,4-tetrahydroisoquinoline-6,7-diol accelerated cytotoxicity. Proteasome inhibitory activity was measured against the chymotrypsin-like activity of the proteasome, and the compounds containing $\beta$-carboline, i.e., **4**−**6**, exhibited more potent proteasome inhibitory activity than those containing 1,2,3,4-tetrahydroisoquinoline-6,7-diol, i.e., **1** and **2**. We recently reported that **6** inhibited the accumulation of the cholesterol ester in macrophages[12e] and, in the present study, the isolated compounds (**1**, **2**, **4**, and **5**) were also found to inhibit this accumulation to the similar extents to **6**, with 48−73% inhibition being observed at 20 $\mu$M. On the other hand,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acantholactam, which was previously isolated by us[11] and in which the eight-membered ring in manzamine-related alkaloids was broken, exhibited the above three biological activities at much lower levels than the other manzamine-related alkaloids. Taking into consideration that the presence of the 1,2,3,4-tetrahydroisoquinoline-6,7-diol ring instead of the $\beta$-carboline moiety reduced the inhibitory activity against the proteasome, together with our previous result on acantholactam, the intact eight-membered ring and presence of $\beta$-carboline appear to be required for the proteasome inhibitory activity of manzamine-related compounds.

Although more than 80 manzamine-derived alkaloids have been isolated to date, acanthomanzamines A (**1**) and B (**2**) are the first compounds to contain the 1,2,3,4-tetrahydroisoquinoline-6,7-diol moiety instead of the $\beta$-carboline moiety. Acanthomanzamine C (**3**) contained the hexahydrocyclopenta[*b*]pyrrol-4(2*H*)-one ring system instead of the eight-membered ring in manzamine A (**6**). Acanthomanzamines D (**4**) and E (**5**) additionally had the oxazolidine and 2-methyloxazolidine rings, respectively, both of which fused to the framework of manzamine B (**7**). Manzamines typically possess 36 carbons accommodated in a tetra- or pentacyclic ring system connected to the $\beta$-carboline moiety. Ma'eganedin A[6] was the first compound to contain the insertion of a methylene group between N-2 and N-27 in **7**. In the cases of **4** and **5**, methylene and ethylidene groups, respectively, were inserted between N-27 and the oxygen atom at C-12. Thus, in the present study, we succeeded in isolating five manzamine congeners with novel structures.

## ■ ASSOCIATED CONTENT

### Supporting Information

Full experimental details and 1D and 2D NMR spectra of **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

‡A.F. and H.K. contributed equally.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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(15) Compound **3** has not been subjected to the three biological tests due to its limited amount available.


## ■ NOTE ADDED AFTER ASAP PUBLICATION

Figure 1 contained errors in the version published ASAP on June 26, 2014; the correct version reposted on July 10, 2014.