Bioactive compounds from coral reef invertebrates*

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Abstract: Manzamine A (1) known as a cytotoxin exhibited potent antimalarial activity with 40% recovery 60 days after single intraperitoneal (i.p.) injection against mice infected with the malaria parasite Plasmodium berghei. Pachastrisamine (6), a simple derivative of a sphingosine, isolated from a sponge, Pachastrissa sp. showed a high level of cytotoxicity. New sesquiterpene carbonimidic dichlorides and related aldehydes (8–12) have been isolated as cytotoxic constituents of the sponge Stylotella aurantium and shown to have moderate cytotoxicity. Cyclic undecapeptides, barangamides A–D (13–16), have been isolated from the sponge Theonella swinhoei. Their structures were established by spectroscopic analysis and chemical reaction.

Marine invertebrates have been recognized as an important source of bioactive compounds having medicinal potential. Although no major therapeutic drugs have yet been developed from the sea, several compounds have so far entered clinical trials as anticancer drugs. Apart from human medicines, the research on marine natural products in the last three decades has also brought to the discoveries of many chemically and biologically interesting molecules. Some of them, e.g., kainic acid, okadaic acid, tetrodotoxin, manoalide, palytoxin, etc., have become indispensable tools in biochemical research and played significant roles in the recent advancement of life sciences. In our search for bioactive compounds from coral reef invertebrates we have focused mainly on cytotoxic compounds and found some unique compounds [1]. In this paper we report some recent results including antimalarial activity of manzamine A and new cytotoxic metabolites from sponges.

ANTIMALARIAL ACTIVITY OF MANZAMINES

Manzamines are complex alkaloids isolated from marine sponges. Since the first report of manzamine A in 1986 [2], some 40 related compounds have been described from more than a dozen species of sponges [3]. Manzamine A (1) was initially described as a cytotoxic compound exhibiting IC₅₀ 0.07 μg/mL against P388 mouse leukemia cells. The unique structure of the manzamines has attracted a number of synthetic chemists to challenge total synthesis [3]. Two total syntheses of manzamine A have recently been reported [4].

More recently, manzamine A was shown to have potent antimalarial activity in an assay against rodent malaria parasite Plasmodium berghei in vivo [5]. In our collaboration, Kara and coworkers at the National University of Singapore tested manzamine A (1), manzamine F (2), and (–)-8-hydroxymanzamine A (3, supplied by M. T. Hamann) along with the existing antimalarial drugs, chloroquine and artemisinin, with mice infected with P. berghei. A test sample was administered by i.p. injection to infected mice on day 2 at doses ranging from 50 to 1000 μM/kg, and number of surviving mice were

*Presented at the 22nd IUPAC International Symposium on the Chemistry of Natural Products, São Carlos, Brazil, 3–8 September 2000. Other presentations are published in this issue, pp. 549–626.

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counted after days 4, 6, 10, 15, 25, and 60. All control mice died by day 4. In the case of manzamine A at a dose of 100 µM/kg, numbers of surviving mice were 5 (100%) on day 10, 4 (80%) on day 15, and 2 (40%) on days 25 and 60. That is, 40% of mice recovered 60 days after single injection. However, in the case of chloroquine at doses of 100 and 50 µM/kg, which gave the best results, all were alive on day 6 but dead on day 10. Artemisinin at a dose of 1000 µM/kg attained better result than chloroquine with 1 (20%) complete recovery on day 60 and 5 (100%) survivals on day 10. (−)-8-Hydroxymanzamine A (3) also exhibited significant effect with 100% survival on day 10 but none on day 15 at a dose of 100 µM/kg. Manzamine F (2) showed no life-prolonging effect. Antimalarial effects were also observed by oral administration of oil suspensions of both compounds 1 and 3. It is interesting to note the difference in the activity and structures of the manzamines tested. It appears that the functionality on the 8-membered ring of the manzamines is important for the activity.

CYTOTOXIC SPHINGOSINE DERIVATIVE

In our collaboration, Natori and coworkers at Kirin Brewery, Co. discovered a series of unusual α-galactoceramides, agelasphines (e.g., 4), from the sponge Agelas mauritianus collected in Okinawa [6]. These compounds showed good in vivo antitumor activity with T/C 160–190% against B16 melanoma and potent immunostimulatory activity in a mixed lymphocyte reaction assay, while they were not cytotoxic. Extensive studies on the structure–activity relationship led the Kirin scientists to select a synthetic cerebroside coded KRN 7000 (5) which is now under clinical trials as an anticancer drug.

Recently, we found a somewhat related compound, pachastrisamine (6), a sphingosine derivative, from a sponge, Pachastrissa sp. Compound 6 displayed potent cytotoxicity with IC₅₀ 0.01 µg/mL against P388, A-549, HT-29, and MEL 28 tumor cell lines. The structure of 6, [α]₀ +18.4°, was determined by spectroscopic data and the absolute stereochemistry by application of the modified Mosher’s
method [7] on its MTPA amides. It is closely related to a known synthetic compound [8], but the stereochemistry is different. Obviously, 6 is a biosynthetic product formed from the corresponding sphingosine.

**CYTOTOXIC SESQUITERPENES**

Terpenoids containing an isocyano or isothiocyanate group are abundantly known from marine sponges of the order Halichondrida, while those having the related functional group carbonimidic dichloride (-NCCl₂) are rare. Since the first report of carbonimidic dichlorides by Faulkner in 1977 [9], only about a dozen sesquiterpenes with this uncommon functionality have so far been reported from three species of sponges and one nudibranch. In addition to their rarity we are interested in the biogenesis of the functional group and in their biological activity, such as antifouling activity [10] and cytotoxicity [11].

We recently described two new cytotoxic carbonimidic dichlorides, reticulidins A (7) and B isolated from the nudibranch *Reticulidia fungia* [11]. In a subsequent study we isolated three new congeners (8–10) and two related aldehydes (11, 12) together with six previously known carbonimidic dichlorides including reticulidins from the sponge *Stylotella aurantium* collected in Okinawa. Except for 10, which was not tested, all new compounds showed moderate cytotoxicity as shown in Table 1. The aldehydes 11 and 12 could either be catabolic products or biosynthetic precursors of corresponding carbonimidic dichlorides. At this point we have no evidence to determine their biosynthetic relationship.

![Chemical structures](image)

**Table 1** Cytotoxicity (IC₅₀ µg/mL) of sesquiterpenes 7–12.

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**CYCLIC PEPTIDES**

The sponge *Theonella swinhoei* has been described as a source of a variety of secondary metabolites including macrolides [12] and cyclic peptides [13]. When we examined the constituents of a cytotoxic sponge which turned out to be *T. swinhoei*, collected at Baranglompo Island, Indonesia, we obtained several new cyclic peptides and cyclic depsipeptides together with previously known related compounds. Here we discuss only the cyclic undecapeptides, barangamides A–D (13–16), which posed us a problem of determining the correct sequence.
Peptidic nature of barangamide A (13), C₅₆H₉₇N₁₁O₁₂, was shown by NMR data (e.g., 11 C = 0 signals at δ162–176). The presence of eleven carbonyls and no other double bonds required the compound to have one ring, indicating the peptide to be cyclic. NMR analysis revealed the nature and the sequence of the eleven residues. Stereochemistry of each residue was identified by acid hydrolysis and by performing the Marfey analysis on the hydrolysate. Thus, the residues were β-Ala (×3), L-Ala, D-allo-Ile, L-Me-Ile, D-allo-Me-Ile, D-Leu (×2), L-Thr, and L-Me-Val. Although the gross sequence could be determined by NMR data, the remaining problem was how to distinguish the two isomeric methyl isoleucines (L-Me-Ile, D-allo-Me-Ile) in the sequence. This problem was solved by detailed analysis of coupling constants and NOE around the two residues. Authentic samples of isoleucine and allo-isoleucine exhibited small vicinal coupling constants (J = 4 Hz) for H-2 and H-3, while 13 showed large values (J = 10–10.5 Hz) for the corresponding protons (H6/H7, H40/H41) in the Me-Ile units. This indicated restricted rotation around the C6–C7 and C40–C41 axes and anti relationship of these protons. This observation together with NOE results clearly distinguished the two isomeric Me-Ile as shown below [14]. The structures of barangamides B–D (14–16) were similarly determined [15].

ACKNOWLEDGMENTS

We thank Dr. U. K. A. Kara and Mr. K. K. H. Ang, University of Singapore, for antimalarial assay and Dr. D. G. Gravalos and coworkers at PharmaMar, Spain, for cytotoxicity assay.

REFERENCES