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*Original Research Article*

# Isolation and Evaluation of Anti-Mycobacterial Activity of Alkaloid Compounds from Marine Invertebrate Sponge *Haliclona* sp.

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ABSTRACT

Marine invertebrate sponges are a rich source of new bioactive substances. The objective of this investigation was to isolate the anti-mycobacterium bioactive compounds from sponge *Haliclona* sp. A new alkaloid (1) was obtained through bioassay-guided purification together with eight previously known substances, including cyclostellettamines A (5), B (6), C (7), E (8), and F (9) and the haliclocyclamines A (2), B (3), and C. The structures of 1-9 were established applying spectroscopic analysis, including 1D NMR ($^{1}$H and $^{13}$C-NMR), 2D NMR (HMQC, HMBC, 1H-1H COSY), mass spectroscopy, IR, and UV. Furthermore, the findings revealed that the compounds exhibited inhibitory activity against *Mycobacterium smegmatis* at 10 µg/disc.

***Keywords***: Antimycobacterial, Structure elucidation, Alkaloid, Natural product, *Haliclona* sp.

## Introduction

*Mycobacterium tuberculosis* is an infectious bacteria that causes tuberculosis (TB). WHO estimated that in 2020 there would be 10.0 million new cases of TB and 1.3 million TB-related deaths.[1,2] *M. tuberculosis* strains that are at least resistant to rifampicin and isoniazid, the two crucial drugs used to treat TB, produce multidrug-resistant tuberculosis (MDR-TB). Globally in 2012, there are a total of 450,000 cases of MDR-TB with approximately 170,000 deaths. Delmanid is a medication that prevents the production of mycobacterial cell walls, and it has been authorized in the EU and Japan since 2018 for the treatment of MDR-TB. [3,4] Natural products have been the major sources of secondary metabolites, which has served as lead molecules for drug discovery in the past few decades.[5-7] During studies on anti-mycobacterium activity from marine invertebrate and terrestrial organisms,[8-11] it was found that the ethanolic extract of sponge *Haliclona* sp was active against *Mycobacterium smegmatis* at 50 µg/disc. Bioassay-guided fractionation of ethanolic extract from marine sponge *Haliclona* sp. yieded a new alkaloid (**1**) together with known compound (**2-9).** Therefore, this study aims to discuss the isolation of these compounds, as well as to determine their structure and anti-mycobacterial activity.

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## Materials and Methods

*General Experimental Procedures*

On a JEOL spectrometer, CD3OD's $^{1}$H and $^{13}$C NMR spectra were captured at 400 MHz for $^{1}$H and 100 MHz for $^{13}$C (H 3.31, C 49.0), after which HRFAB mass spectrometry was carried out using JEOL JMS-MS 700. The L-6200 system was used for preparative HPLC (Hitachi Ltd.), while UV was performed on Hitachi U-3310 UV.

*Animal Material*

The marine invertebrate sponge was discovered in 2013 by scuba diving in the sea off Manado, North Sulawesi, Indonesia. Dr. K. Ogawa (Z. Nakai Laboratory Japan) classified the samples (2013-147) as *Haliclona* sp. and they were subsequently preserved at the Laboratory of Natural Product, Tohoku Medical and Pharmaceutical University-Japan

*Extraction and Isolation*

The marine sponge sample (619.9 g) was sliced into small pieces, steeped in 70% ethanol three times, and partitioned with 200 mL of EtOAc to obtain 2.6 g of extract. The ethyl acetate extract was then separated into 7 fractions using a chromatographic column with ODS stationary phase (100 g) as well as MeOH and $H_2O$ mobile phases. To obtain compound 3, 190.5 mg of fraction 7 was purified using HPLC with 1.8 mg of MeOH:$H_2O$ solvent (65:35 v/v). HPLC was also used to separate 1.8 mg of 4 from 201.8 mg of fraction 6 using MeOH:$H_2O$ (65:35 v/v). Subsequently, 1.8 mg of 8 and 1.8 mg of 9 were isolated from 219.2 mg fraction 5 using the same solvent. The HPLC column used was the PEGASIL ODS.

*Anti-Mycobacterial Assay*

The disc diffusion method was used to conduct the anti-mycobacterial assay.[12,13] *M. smegmatis* strain NBRC 3207 was earned from laboratory culture stock stored at -80°C. A Middlebrook 7H9 broth medium containing 0.05% polysorbate 80 (BD), 0.5% glycerol, and 10% Middlebook OADC (BD) was used to cultivate the test bacteria for two days at 37°C. 1 mL of inoculum was poured into 100 mL Middlebook 7H9 agar medium at 40°C. Subsequently, isolated compounds in MeOH were placed on paper discs and then evaporated to remove

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MeOH. A plate containing the paper disc was incubated at 37°C for two days. MeOH served as the negative control, while streptomycin sulfate (2 µg/disc) was the positive control.[12,13]

**Results and Discussions**

Compound 1 was isolated and found to be a white solid with the chemical formula $C_{34}H_{54}N_2$ (m/z 489.4205 [M-H]+, -0.3 mmu). Furthermore, its $^1$H NMR spectrum exhibited signals of mutually coupled pyridine proton $\delta$ 8.90 (br s), 8.82 (d, $J$ = 5.6 Hz), 8.45 (d, $J$ = 7.6 Hz), 8.02 (t, $J$= 5.6 Hz) as well as $^{13}$C signals at $\delta$ 146.8, 145.6, 145.2, 143.4, and 129.1, as shown in Table 1.

The examination of the 2D NMR spectra showed a pair of 1, 3-disubstituted pyridinium units, which were replaced by methylene chains. $^1$H NMR at $\delta$ 1.33-1.23 indicated that the methylene unit constituted two di-substituted pyridine rings, as shown in Figure **2**.

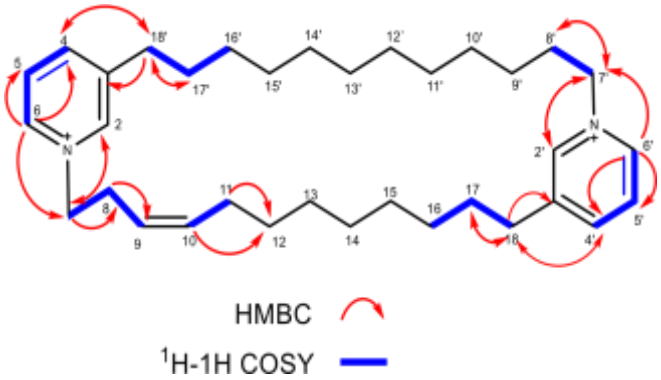
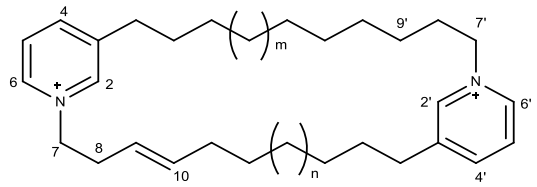
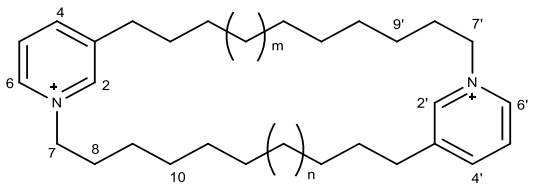



**Figure 2:** $^1$H-$^1$H COSY and HMBC correlations for **1**



**1** m= 3, n= 3
**2** m= 3, n= 5
**3** m= 5, n= 5



**4** m= 5, n= 6
**5** m= 3, n= 3
**6** m= 3 n= 4
**7** m= 4, n= 4
**8** m= 4, n= 5
**9** m= 5. n= 5

**Figure 1:** Structure of compounds **1-9** from marine invertebrate sponge *Haliclona* sp.

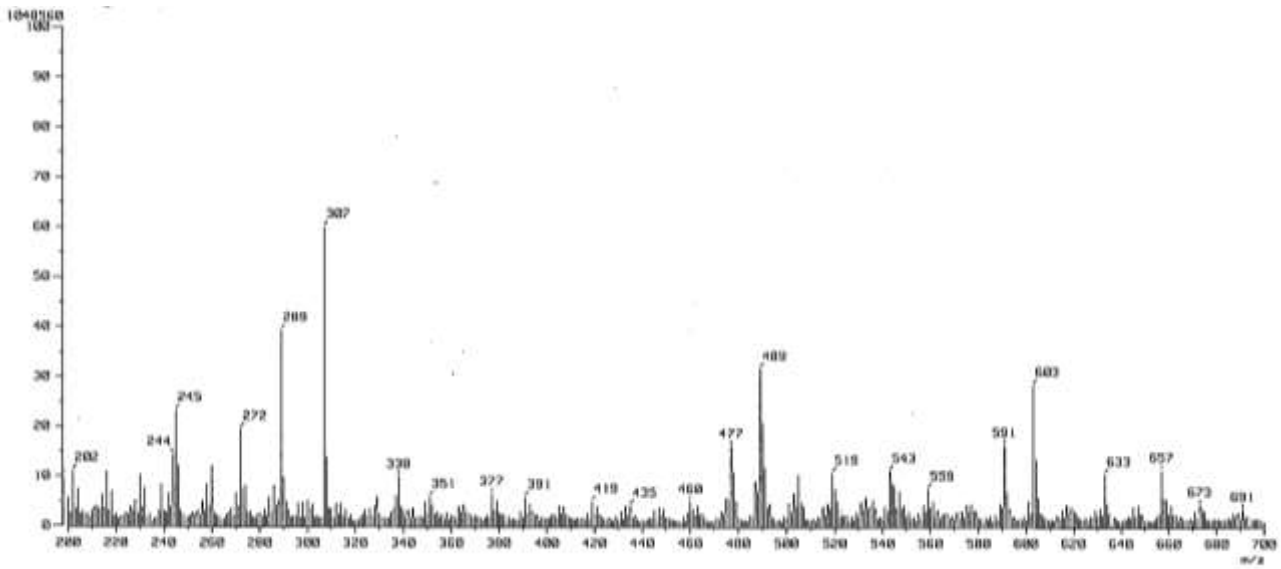


**Figure 3:** Positive FAB-MS of compound **1**

The positive FABMS spectrum exhibited ion peaks at m/z 489 (two monomer units-H$^+$), and 603 (two monomers + $CF_3COO^-$), which showed that **1** is a cyclic dimer with two positively charged nitrogen. The formation of the [M-H]+ ion can be attributed to Hoffman-type elimination during the FAB ionization process. [15] The FAB-MS spectra showed an ion peak at 245 m/z, confirming the symmetrical structure of 1, namely two $C_{12}$ linear alkyl chains connecting the N-1 and C-3 between two pyridiniums. The location of the double bond was clarified by $^1$H-$^1$H-COSY and HMBC experiments. HMBC correlations of **1** were (H-7 to C-2/C-8, H-8 to C-9, H-10 to C-12, H-11 to C-12), as shown in Figure **2**. Meanwhile, its $^1$H-$^1$H-COSY correlations include (H-4/H-5, H-7/H-8, H-10/H-11, H-16/H-17), indicating the presence of the double bond at C-9 and C-10. The coupling constants H-9 and H-10 for 1 (5.32 t, J = 4.8 Hz), as well as the $^{13}$C chemical shift values of its allylic methylenes (32.1, 30.3) were used to determine the *E* geometry of the double bond [16]. Compounds **1 - 9** were assessed for their antibacterial efficacy against *M. smegmatis* using the disc diffusion method. At 10 g/disc, they were active against the test bacteria, as shown in Table 2. Furthermore, compound **2**, namely haliclocyclamine A, has a 17 mm inhibitory zone at this level, making it the most effective. Alkyl Pyridine Alkaloids (3-APAs) are generally produced by sponges of the genus Haliclona,[17-21] Amphimedon,[22-24] Topsentia,[25] Neopetrosia,[26] Xestospongia,[27] and Cribrochalina.[28] They have also been reported to have pharmacological potentials, such as cytotoxic,[17] antifungal,[29,30] anticancer,[31] and antimicrobial activities[32].

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**Table 1:** NMR Data of compound **1** in $CD_3OD$

| No | □δc | □δH, mult, (*J* in Hz) | HMBC | COSY |
|---|---|---|---|---|
| 2/2’ | 145.2 | 8.90 brs | 7, 7’ | |
| 3/3’ | 145.6 | | | |
| 4/4’ | 146.8 | 8.45 (d, *J* = 7.6) | 18, 18’ | 5, 5, |
| 5/5’ | 129.1 | 8.02 (t, *J* = 5.6) | | 4, 4’ |
| 6/6’ | 143.3 | 8.82 (d, *J* = 5.6) | 4, 4’ 5, 5’ | 5, 5’ |
| 7/7' | 62.9 | 4.61 (t, *J* = 4.0) | 2, 2’ 8, 8’ | 8, 8’ |
| 8/8’ | 32.1 | 2.01, m | 9 | 9 |
| 9 | 130.6 | 5.32 (t, *J* = 5.6) | | |
| 9’ | 26.8 | 1.23-1.33, m | | |
| 10 | 130.6 | 5.32 (t, *J* = 5.6) | 12 | 11 |
| 10’ | 29-31, br | 1.23-1.33, m | | |
| 11 | 30.3 | 2.01, m | 12 | |
| 11’ | 29-31, br | 1.23-1.33, m | | |
| 12 | 26.7 | 1.23-1.33, m | | |
| 12’ | 29-31, br | 1.23-1.33, m | | |
| 13/13’ | 29-31, br | 1.23-1.33, m | | |
| 14/14’ | 29-31, br | 1.23-1.33, m | | |
| 15/15’ | 29-31, br | 1.23-1.33, m | | |
| 16/16’ | 29-31, br | 1.23-1.33, m | | 17, 17’ |
| 17/17’ | 29-31, br | 1.72, m | 3, 3’, 18, 18’ | 16, 16’, 18, 18’ |
| 18/18’ | 33.3 | 2.88, m | 3, 3’, 17, 17’ | 17, 17’ |

**Table 2:** Biological activity of compounds **1**–**9** against *M. smegmatis*

| Compound | *M. smegmatis* (inhibition zone, mm)10 g/disc |
|---|---|
| 1 | 10 |
| 2 | 17 |
| 3 | 10 |
| 4 | 13 |
| 5 | 14 |
| 6 | 8 |
| 7 | 8 |
| 8 | 12 |
| 9 | 12 |
| streptomycin sulfate (1 □μg/*disc*) | 20 |

## Conclusion

From the Indonesian marine sponge *Haliclona* sp., a new cyclostelletamine alkaloid **1**, and eight previously identified compounds, including haliclocyclamines A (2), B (3), C (4), as well as cyclostettetamines A (5), B (6), C (7), E (8), and F (9) were isolated. At 10 μg/disc, they exhibited inhibitory effects against *M. smegmatis*. Compounds 2, 4, 5, 8, and 9 are more effective than 1, 3, 6, and 7 in inhibiting mycobacterial growth.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors’ Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. World Health Organization (WHO). *Global Tuberculosis Report*. 2020.
2. World Health Organization (WHO). *Global Tuberculosis Report*. 2021.
3. Ryan NJ, and Han J. Delamanid : First Global Approval. Drugs. 2014; 74(9):1041–1045.
4. Xavier AS, and Lakshmanan M. Molecules of the Millennium Delamanid : A New Armor in Combating Drug - Resistant Tuberculosis. J. Pharmacol. Pharmacother. 2014; *5*(3): 222–225.
5. Carrol AR, Cop BR, Davis RA, Keyzers RA, Prinsep MR. Marine Natural Products. Nat. Prod. Rep. 2021; 38, 362-413
6. Carrol AR, Cop BR, Davis RA, Keyzers RA, Prinsep MR. Marine Natural Products. Nat. Prod. Rep. 2020; 37:175-223
7. Carrol AR, Cop BR, Davis RA, Keyzers RA, Prinsep MR. Marine Natural Products. Nat. Prod. Rep. 2019; 36:122-173
8. Maarisit W, Yamazaki H, Abdjul B, Takahashi O, Kirikoshi R, Namikoshi M. A New Pyranonaphtoquinone Derivative, 4-Oxo-Rhinacanthin A, from Roots of Indonesian *Rhinacanthus nasutus*. Chem. Pharm. Bull. 2017; 65(6):586–588.
9. Maarisit W, Abdjul DB, Yamazaki H, Kato H, Rotinsulu H, Wewengkang DS, Sumilat DA, Kapojos MM, Ukai K, Namikoshi M. Anti-Mycobacterial Alkaloids, Cyclic 3-Alkyl Pyridinium Dimers, from the Indonesian Marine Sponge Haliclona Sp. Bioorg. Med. Chem. Lett. 2017; 27(15):3503–3506.
10. Yamazaki H, Nakayama W, Takahashi O, Kirikoshi R, Izumikawa Y, Sumilat DA, Mangindaan REP, Namikoshi M. Verruculides A and B, Two New Protein Tyrosine Phosphatase 1B Inhibitors from an Indonesian Ascidian-Derived Penicillium Verruculosum. Bioorg. Med. Chem. Lett. 2015; *25*(16):3087–3090.
11. Yamazaki H, and Sumilat DA. A Polybromodiphenyl Ether from an Indonesian Marine Sponge Lamellodysidea Herbacea and Its Chemical Derivatives Inhibit Protein Tyrosine Phosphatase 1B, an Important Target for Diabetes Treatment. Nat. Med. 2012; 67(4):730–735.
12. Abdjul DB, Yaki A, Yamazaki H, Kirikoshi R, Takahashi O, Namikoshi M, Uchida R. Anti-mycobacterial haliclonadiamine alkaloids from the Okinawan marine sponge *Haliclona sp*. collected at Iriomote Island. Phytochem. Lett. 2018; 26:130–133.

13. Bu YY, Yamazaki H, Ukai K, Namikoshi M. Anti-Mycobacterial Nucleosides Antibiotics from a Marine Derived Streptomyces sp. TPU 1236A. Mar. Drug. 2014; 12:6102-6112.
14. Fusetan N, Asai N, and Matsunaga S. Cyclostellettamines A-F, Pyridine Alkaloids Which Inhibit Binding of Methyl Quinuclidinyl Benzilate (QNB) to Muscarinic Acetylcholine Receptors, from the Marine Sponge, *Stefletta maxitnal*. Tetrahedron Lett. 1994; *35*(23):3967–3970.
15. Davies-coleman MT, Faulkner DJ, Dubowchik GM, Roth GP, Polson C, Fairchildt C. New EGF-Active Polymeric Pyridinium Alkaloid from the Sponge Callyspongia Fibrosa. J. Org. Chem. 1993; 58(22):5925–5930.
16. Wang GYS, Kuramoto M, Uemura D, Akihiro Y, Yamaguchi K, Yazawa K. Three Novel Anti-Microfouling Nitroalkyl Pyridine Alkaloids from the Okinawan Marine Sponge *Callyspongia sp*. Tetrahedron Lett. 1996; *37*(11):1813–1816.
17. Zhang H, Loveridge ST, Tenney, K, and Crews, P. A new 3-alkylpyridine alkaloid from the marine sponge *Haliclona* sp. and its cytotoxic activity. Nat. Prod. Res. 2016; 30(11):1262-1265.
18. Schmidt G, Timm C, Grube A, Volk CA, Ko¨ck M. Viscosalines B1,2 and E1,2: challenging new 3-alkyl pyridinium alkaloids from the marine sponge *Haliclona viscosa*. Chem Eur J. 2012; 18:8180–8189.
19. Köck M, Muñoz J, Cychon C, Timm C, and Schmidt G. The Arctic sponge *Haliclona viscosa* as a source of a wide array of 3-alkyl pyridine alkaloids. Phytochem Rev. 2013; 12(3):391–406.
20. Einarsdottir E, Magnusdottir M, Astarita G, Köck M, Ögmundsdottir H, Thorsteinsdottir M, Rap HT, Omarsdottir S, Paglia G. Metabolic Profiling as a Screening Tool for Cytotoxic Compounds: Identification of 3-Alkyl Pyridine Alkaloids from Sponges Collected at a Shallow Water Hydrothermal Vent Site North of Iceland. Mar.Drugs, 2017; 15(2):3-14.
21. Timm C, Mordhorst T, and Köck M. Synthesis of 3-alkyl pyridinium alkaloids from the Arctic sponge Haliclona viscosa. Mar.Drugs. 2010; 8:483–49
22. Kubota T, Kura K, Fromont J, and Kobayashi J. Pyrinodemins G–I, new bis-3-alkylpyridine alkaloids from a marine sponge Amphimedon sp. Tetrahedron. 2013; 69(1):96–100.
23. Xu NJ, Sun X, and Yan XJ. (2007). A new cyclostellettamine from sponge *Amphimedon compressa*. Chin. Chem. Lett. 2007; 18(8):947–950.
24. Hirano K, Kubota T, Tsuda M, Mikami Y, Kobayashi, J. Pyrinodemins B-D, Potent Cytotoxic Bis-Pyridine Alkaloids from Marine Sponge *Amphimedon sp*. Chem. Pharm. Bull. 2000; 48(7): 974–977.
25. Sun JZ, Jiang CS, Chen XQ, Chen KS, Zhen XC, van Soest RWM, and Guo YW. Topsendines A–F, new 3-alkylpyridine alkaloids from a Hainan sponge Topsentia sp. Tetrahedron. 2014; 70(19):3166–3171.
26. Hitora, Y, Maeda R, Honda K, Sadahiro Y, Ise Y, Angkouw ED, Mangindaan REP, Tsukamoto S. Neopetrosidines A–D, pyridine alkaloids isolated from the marine sponge *Neopetrosia chaliniformis* and their cell cycle elongation activity. Bioorg. Med. Chem. 2021; 50:116461
27. Arai M, Kamiya K, Shin D, Matsumoto H, Hisa T, Setiawan A, Kotoku N, Kobayashi M. N-Methylniphatyne A, a New 3-Alkylpyridine Alkaloid as an Inhibitor of the Cancer Cells Adapted to Nutrient Starvation, from an Indonesian Marine Sponge of Xestospongia sp. Chem. Pharm. Bull. 2016; 64;766–771
28. Elissawy AM, Dehkordi ES, Mehdinezhad N, Ashour ML, and Pour PM. Cytotoxic Alkaloids Derived from Marine Sponges: A Comprehensive Review. Biomolecules. 2021, 11;258.
29. Damodaran V, Ryan JL, and Keyzers RA. Cyclic 3-alkyl pyridinium alkaloid monomers from a new Zealand Haliclona sp. Marine sponge. J. Nat. Prod.. 2013;76(10):1997–2001.
30. Andrade JT, Lima WG, Barbosa CS, Goncalves AMMN, Silva MKP, Morais FB, Palumbo JMC, Viana GHR, and Ferreira JMS. Antifungal Activity of a Novel 3-Alkylpyridine analog derived from Marine Sponge Alkaloid. An Acad Bras Cienc. 2021; 93(supll.4):e20200944.
31. Barbosa MCS, Barbosa CSB, Oliveira JT, Moreira NCS, Martins NRM, Gomes GKA, Caldeira CA, Costa MLA, Uimaraes DSL, Guimaraes L, Nascimento CS, Varotti FP, Viana GHR, Santos FV. Synthesis and evaluation of the mutagenicity of 3-alkylpyridine marine alkaloid analogues with anticancer potential. Mutat Res Toxicol Environ Mutagen. 2018; 825:31-39.
32. Kaplan AR, Schrank, CL, and Wuest WM. An Efficient Synthesis of 3-Alkylpyridine Alkaloids Enables Their Biological Evaluation. Chem. Med. Chem. 2021; 16(16):2487–2490.