Pure Appl. Chem., Vol. 81, No. 6, pp. 1009–1018, 2009.
doi:10.1351/PAC-CON-08-08-22

Chemistry and biology of Okinawan marine natural products*

Jun'ichi Kobayashi

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060-0812, Japan

Abstract: Marine macro- and micro-organisms collected in Okinawa are good sources of compounds with intriguing structures and interesting biological activities. Synthetic hybrid molecules of caffeine and eudistomin D from tunicates *Eudistoma* sp. were found to show better potency as adenosine receptor ligands than caffeine, and one of them exhibits potent activity for adenosine receptors tested, especially for A_3 subtype. Potent cytotoxic polyene macrolides from a tunicate *Cystodytes* sp. were found to be potent osteoclast inhibitors and to inhibit vacuolar type H^+-ATPase (V-ATPase) of both mammalian and yeast cells. Amphidinolactones A and B are new macrolides from a dinoflagellate *Amphidinium* sp., and a potent cytotoxic macrolide from another strain was found to target actin cytoskeleton. Theonezolide A, a long-chain polyketide from a sponge *Theonella* sp., induces a drastic shape change in platelets by reorganization of microtubules. The stereochemistry of many chiral centers in theonezolide A was elucidated by spectral data and chemical means. Metachromins L–Q are new sesquiterpenoid quinones with an amino acid residue, while nakijiquinones E and F were the first dimeric sesquiterpenoid quinones possessing a 3-aminobenzoate moiety. Halichonadin E is the first hetero-dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment isolated from a sponge *Halichondria* sp. Pyrinadine A and nakinadine A are novel bis-pyridine alkaloids from sponges, while nagelamides are new bromopyrrole alkaloids from a sponge *Agelas* sp. Here, the structures and bioactivities of these interesting marine natural products will be described.

Keywords: marine natural products; tunicates; dinoflagellates; sponges; alkaloids; polyketides; terpenoids.

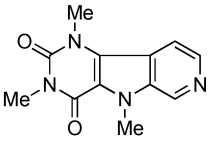
BIOACTIVE MARINE NATURAL PRODUCTS FROM TUNICATES

Synthetic hybrid molecules of caffeine and eudistomin D

Four hybrid molecules (**1** and **4–6**) of caffeine and eudistomin D, a β-carboline alkaloid from tunicates *Eudistoma* sp., were synthesized, and their affinity and selectivity for adenosine receptors A_1, A_{2A}, and A_3 were examined. It was found that all the compounds showed better potency as adenosine receptor ligands as compared with caffeine. Among them, a compound (**5**) possessing a nitrogen at the δ-position of the pyridine ring (δ-N type) showed the most potent affinity for adenosine receptor A_3 subtype, while N-methylation (**6**) of a pyrrole ring in **5** significantly lowered the potency as adenosine receptor

*Paper based on a presentation at the 26th International Symposium on Chemistry of Natural Products (ISCNP-26) and 6th International Conference on Biodiversity (ICOB-6), 13–18 July 2008, Charlottetown, Prince Edward Island, Canada. Other presentations are published in this issue, pp. 1001–1129.

ligands. Compounds (**1** and **4**) having a nitrogen at the β-position of the pyridine ring (β-N type) showed lower affinity than the corresponding δ-N type compounds (**5** and **6**), while compounds (**2**, **3**, and **7**) lacking a pyrrole ring between the pyridine and pyrimidine rings exhibited almost no affinity to the adenosine receptor subtypes examined [1].



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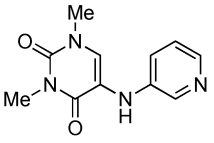

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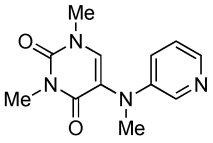

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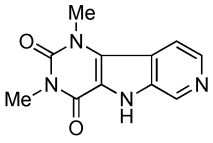

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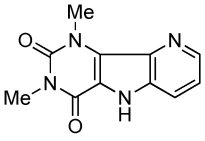

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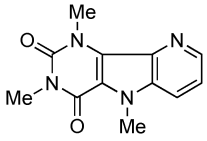

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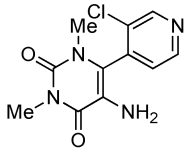

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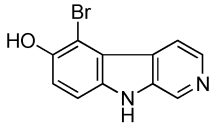
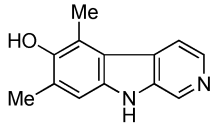
Furthermore, six analogs (**8–13**) of eudistomin D, a β-carboline alkaloid from a marine tunicate *Eudistoma olivaceum*, were synthesized, and their affinity and selectivity for adenosine receptors A_1, A_{2A}, and A_3 were examined. All the synthetic compounds **8–13** did not show affinity to the adenosine A1 receptor. δ-Carboline **10** exhibited the most potent affinity to the adenosine receptor A_3 among compounds **8–13**. δ-Carbolines **10** and **11** showed better affinity than the corresponding β-carbolines **8** and **9**, respectively, while N-methylation (**9**, **11**, and **13**, respectively) of the pyrrole ring in **8**, **10**, and **12** resulted in the reduced affinity to the adenosin A_3 receptor. On the other hand, a eudistomin D derivative, BED, exhibited modest affinity to all the receptors A_1, A_{2A}, and A_3, but no selectivity [2].



eudistomin D



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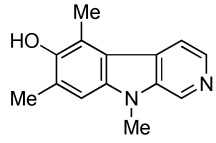

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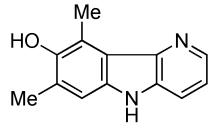

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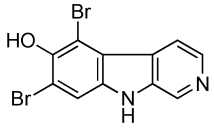
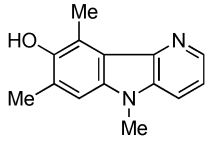


BED



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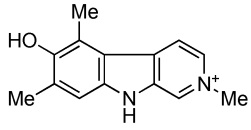

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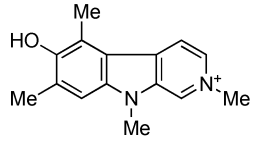

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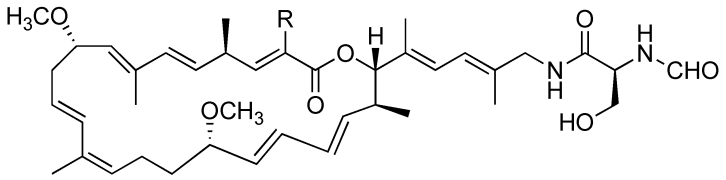
Iejimalides, potent cytotoxic polyene macrolides from a tunicate, as a new type of V-ATPase inhibitors

Iejimalides are unique 24-membered macrolides having two methoxy groups, four diene units, and an *N*-formyl-L-serine terminus isolated from marine tunicates *Eudistoma* cf. *rigida* and *Cystodytes* sp. Although these macrolides exhibit potent cytotoxic activity in vitro and antitumor activity in vivo, their target molecule remained to be revealed. Recently, it was found that iejimalides show antiosteoporotic activity in vitro, and irreversibly inhibited the V-ATPase activity of mammalian and yeast cells. Furthermore, *Saccharomyce cerevieae vma3-T32I* mutant that is resistant to bafilomycin A1, the most potent inhibitor of V-ATPase, also showed resistance for iejimalides, suggesting that iejimalides might interact with the enzyme in a manner similar to the bafilomycins. These results suggest that iejimalides show antitumor and antiosteoporotic activity via V-ATPase inhibition [3].



iejimalide A (R = H)
iejimalide B (R = CH_3)

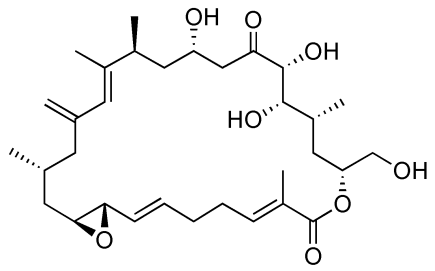
BIOACTIVE MARINE NATURAL PRODUCTS FROM DINOFLAGELLATES

Amphidinolide H, a potent cytotoxic macrolide from dinoflagellate *Amphidinium* sp., targets actin cytoskeleton

Marine dinoflagellates have proven to be one of the most important sources of bioactive natural products, which have been investigated worldwide. We have continuously studied structurally intriguing and biologically interesting macrolides and polyketides from dinoflagellates *Amphidinium* sp., which are symbionts of Okinawan marine flatworms *Amphiscolops* sp. Cytotoxic macrolides, designated amphidinolides, have been isolated from *Amphidinium* sp. by our group [4,5].

The molecular target of amphidinolide H has been investigated as follows. The analysis of phenotypes of amphidinolide H-treated cells suggested that amphidinolide H disrupted the actin organization in the cells, and the polymerization/depolymerization assay using purified actin indicated that amphidinolide H stimulated actin polymerization and stabilized F-actin. Matrix-assisted laser desorption/ionization with time-of-flight (MALDI-TOF) mass analysis and the halo assay using the yeast harboring site-directed mutagenized actin revealed that the covalent binding of amphidinolide H to actin and the binding site was Tyr200 of actin subdomain 4. Time-lapse analyses showed that amphidinolide H stimulated the formation of small actin patches, followed by F-actin rearrangement into aggregates via the retraction actin fibers. These results indicated that amphidinolide H is a novel F-actin stabilizer that covalently binds on actin .

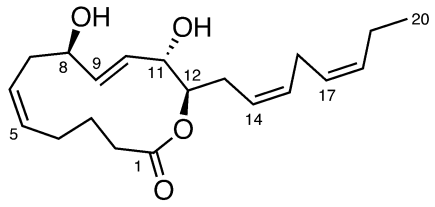
To evaluate whether amphidinolide H competes at the same binding site with phalloidin, the effect of amphidinolide H on phalloidin–actin binding was measured using fluorescein isothiocyanate conjugated phalloidin. Amphidinolide H does not compete with, instead enhances, the binding of phalloidin to F-actin, indicating that amphidinolide H increased susceptibility of F-actin to phalloidin [6].



amphidinolide H

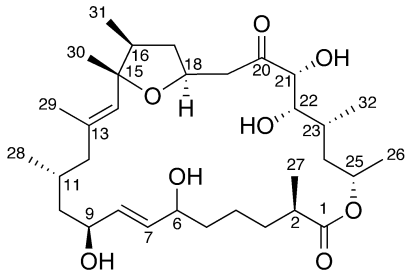
Amphidinolactones A and B, new macrolides from a dinoflagellate *Amphidinium* sp.

A new 13-membered macrolide, amphidinolactone A, $C_{20}H_{30}O_4$, was isolated from a strain (Y-25) of a dinoflagellate *Amphidinium* sp. Detailed analyses of the ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum of amphidinolactone A revealed connectivities of a long carbon chain from C-2 to C-20. ^{1}H and ^{13}C chemical shifts of CH_2-2 and CH-12 suggested that C-12 was involved in an ester linkage with C-1. ^{1}H-^{1}H couplings of the two disubstituted double bonds at C-5 and C-9 indicated the *Z* and *E* geometries, respectively. Geometries of two disubstituted double bonds at C-14 and C-17 were assigned as both *Z* by nuclear Overhauser enhancement spectroscopy (NOESY) correlations and the carbon chemical shift of C-16, which was a typical value for a methylene carbon between two *Z* double bonds. The relative configurations at C-8, C-11, and C-12 in amphidinolactone A were deduced from NOESY correlations [7].



amphidinolactone A

A new 26-membered macrolide, amphidinolactone B, $C_{32}H_{54}O_8$, has been isolated from the same dinoflagellate *Amphidinium* sp. (strain Y-25) as described above. Detailed analyses of the ^{1}H-^{1}H COSY and total correlation spectroscopy (TOCSY) spectra as well as heteronuclear multiple-bond correlation (HMBC) correlations indicated connectivities of a long carbon chain from C-2 to C-26. ^{1}H and ^{13}C chemical shifts of C-25 indicated that C-25 was involved in an ester linkage with C-1. The NOESY correlation for H-2/H-25 also supported the connectivity of C-25 to C-2. The connectivity of C-19 to C-21 through a remaining keto carbonyl at C-20 was deduced from the molecular formula of amphidinolactone B and the NOESY correlation. The presence of a tetrahydrofuran ring was deduced from deuterium-induced shift of oxymethine carbons in the heteronuclear single-quantum coherence (HSQC) spectra of amphidinolactone B. The ^{1}H-^{1}H coupling of the disubstituted double bond at C-7 indicated the *E* geometry. The *E* geometry of the double bond at C-13 was suggested from the NOESY correlation and the ^{13}C chemical shift of C-29. The relative stereochemistry of the tetrahydrofuran ring was deduced from NOESY correlations, while that of C-2, C-22, C-23, and C-25 was assigned from ^{1}H-^{1}H couplings and NOESY correlations. Furthermore, considering conformation of the macrocyclic ring, the relative stereochemistries of the C-21~C-25 and C-1~C-2 moieties were elucidated [8].

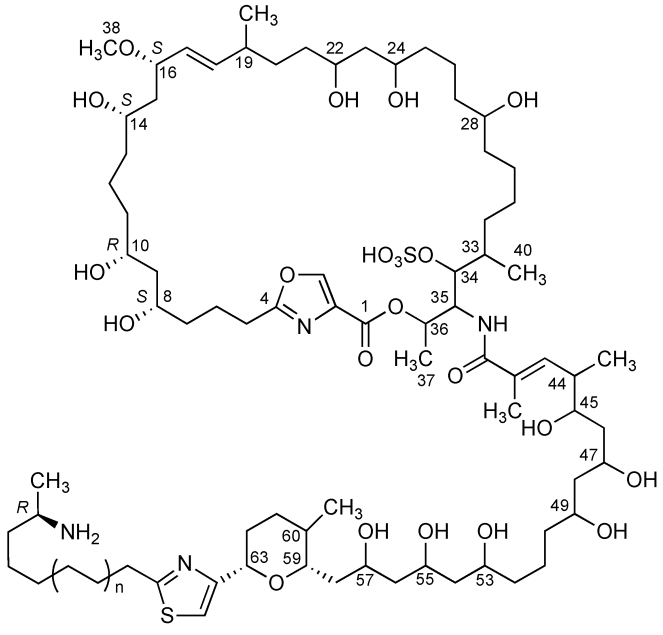


amphidinolactone B

BIOACTIVE MARINE NATURAL PRODUCTS FROM SPONGES

Stereochemistry of theonezolides, unique long-chain polyketides from a sponge *Theonella* sp.

Theonezolides A~C are unique cytotoxic 37-membered macrolides isolated from an Okinawan marine sponge *Theonella* sp., having unique bioactivity to induce a drastic shape change in rabbit platelets by reorganization of microtubules. Theonezolides A~C contain 23 chiral centers, and synthesis of their ozonolysis products disclosed absolute configurations at 5 chiral centers so far. The absolute configurations in the C-4~C-17 moiety were established as 8*S*, 10*R*, 14*S*, and 16*S*, while one chiral center at the terminal position (C-75, C-73, and C-77 of theonezolides A~C, respectively) of theonezolides A~C were determined as all *R*. Though the comparison of spectral and optical data of resulting products obtained from theonezolides A~C by ozonolysis suggested that the absolute configurations at the remaining 18 chiral centers in each macrolide were identical to each other, their absolute configurations are

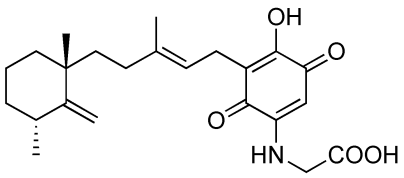


theonezolide A : n=2
theonezolide B : n=1
theonezolide C : n=3

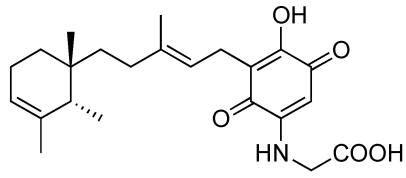
unknown. Recently, we have elucidated the absolute configurations at the remaining chiral centers in theonezolides A~C on the basis of the NMR analysis and a modified Mosher's method [10–14].

Metachromins L–Q and nakijiquinones E and F, new sesquiterpenoid quinones from marine sponges

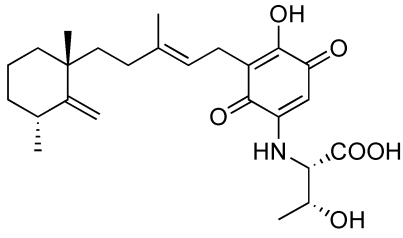
In our continuing search for bioactive compounds from marine sponges, we previously isolated new sesquiterpenoid quinones, metachromins J and K, from an Okinawan sponge *Spongia* sp. (SS-1037). Futher investigation of extracts of this sponge resulted in the isolation of six new sesquiterpenoid quinones with an amino acid residue, metachromins L–Q. Metachromins L and M showed modest cytotoxicity [15].



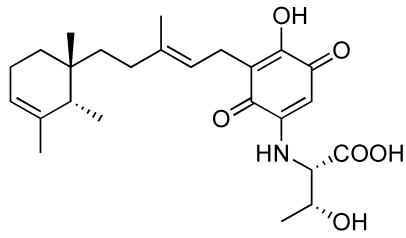
metachromin L



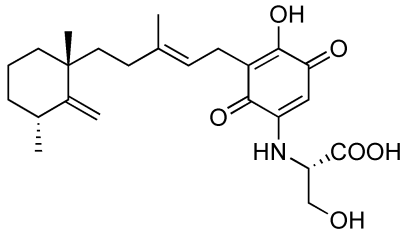
metachromin M



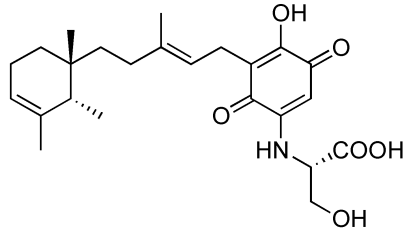
metachromin N



metachromin O



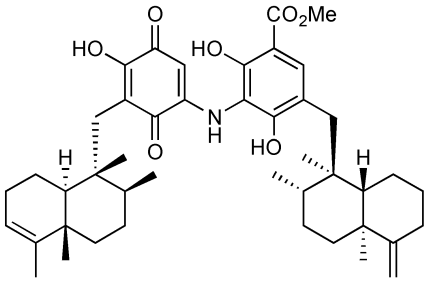
metachromin P



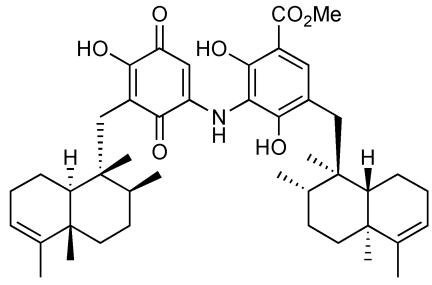
metachromin Q

Investigation of extracts of another lot of the sponge resulted in the isolation of five new sesquiterpenoid quinones, nakijiquinones E–I. Nakijiquinones E and F were the first dimeric sesquiterpenoid quinones possessing a 3-aminobenzoate moiety, though some dimeric sesquiterpenoid quinones from sponges *Dysidea* sp. have been reported so far. The bioactivity of nakijiquinones E and F are currently investigated. Nakijiquinones G–I are new sesquiterpenoid quinones having an amine residue such as histamine, agmatine, and 3-(methylsulfinyl)propan-1-amino group, respectively, although some sesquiterpenoid quinones containing an amino acid residue have been isolated from a marine sponge. Nakijiquinones G–I showed modest cytotoxicity against P388 murine leukemia (IC_{50}, 3.2, 2.4, and

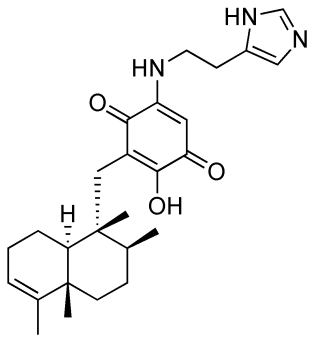
2.9 μg/ml, respectively), L1210 murine leukemia (IC_{50}, 2.9, 8.5, and 2.4 μg/ml, respectively), and KB human epidermoid carcinoma cells (IC_{50}, 4.8, >10, and 5.6 μg/ml, respectively) in vitro. Nakijiquinones G–I exhibited inhibitory activity against HER2 kinase. Nakijiquinone H showed antibacterial activity against *Micrococcus luteus* (MIC, 16.7 μg/ml), and antifungal activities against *Cryptococcus neoformans* (MIC, 8.35 μg/ml), *Candida albicans* (MIC, 8.35 μg/ml), and *Aspergillus niger* (MIC, 16.7 μg/ml) [16, 17].



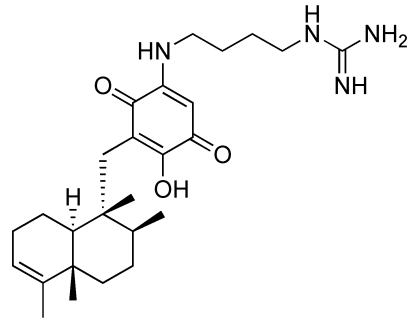
nakijiquinone E



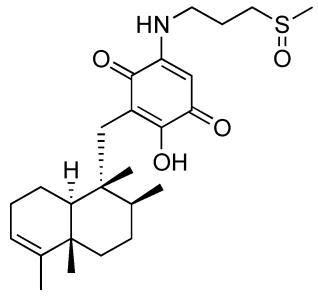
nakijiquinone F



nakijiquinone G



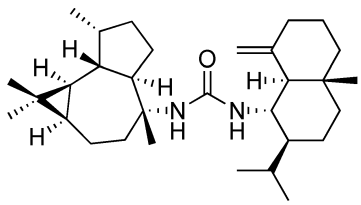
nakijiquinone H



nakijiquinone I

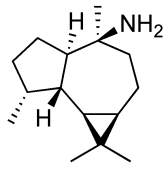
Halichonadin E, a dimeric sesquiterpenoid from sponge *Halichondria* sp.

A new dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment, halichonadin E, was isolated from a marine sponge *Halichondria* sp., and the gross structure and relative configuration of halichonadin E were elucidated on the basis of spectroscopic data. Halichonadin E is the first hetero-dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment isolated from a sponge *Halichondria* sp., although some homo-dimeric sesquiterpenoids such as halichonadin A5 and *N,N*'- bis[(1*Z*,4*Z*)-7R*H*-germacra-1(10),4-dienyl]urea have been isolated from sponges of the genera *Halichondria* and *Axinyssa*, respectively. Halichonadin E showed cytotoxicity against L1210 murine leukemia (IC_{50}, 3.0 μg/ml) and KB human epidermoid carcinoma cells (IC_{50}, 2.6 μg/ml) in vitro [18].

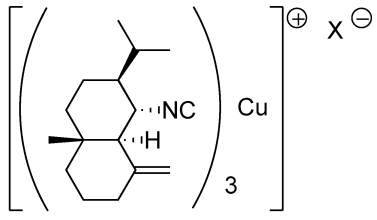


halichonadin E

Further investigation of extracts of this sponge resulted in the isolation of a new sesquiterpenoid with an aromadendrane skeleton, halichonadin F, and the Cu(I) complex of halichonadin C. Halichonadin F is a new aromadendrane sesquiterpenoid having an amino group, although aromadendrane sesquiterpenoids with a thioisocyanate group, an isonitrile group, and an *N*-formylamide group, and a dimeric sesquiterpenoid with eudesmane and aromadendrane skeletons linked through a urea fragment have been isolated from marine sponges *Epipolasis kushimotoensis*, *Axinella cannabina*, or *Halichondria* sp. Halichonadin F and the Cu(I) complex of halichonadin C showed antimicrobial activity against *Micrococcus luteus* (MIC, 4 μg/ml, both), *Trichophyton mentagrophytes* (MIC, 16 and 8 μg/ml, respectively), and *Cryptococcus neoformans* (MIC, 16 μg/ml, both) [19].



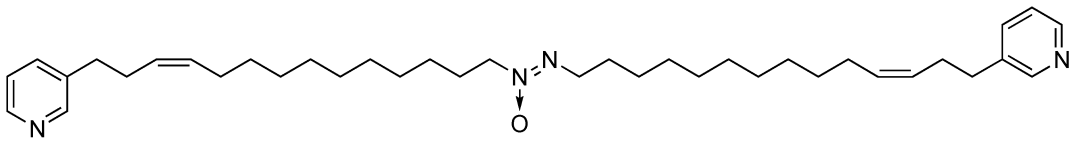
halichonadin F



Cu(I) complex of haliconadin C

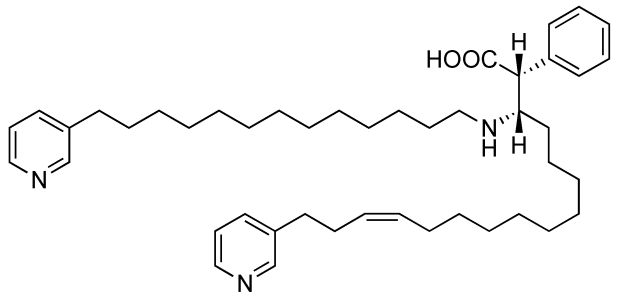
Pyrinadine A and nakinadine A, novel bis-pyridine alkaloids from marine sponges

A novel cytotoxic bis-3-alkylpyridine alkaloid with an azoxy moiety, pyrinadine A, has been isolated from an Okinawan marine sponge *Cribrochalina* sp., and the structure was elucidated by spectroscopic data and chemical means. Pyrinadine A is the first pyridine alkaloid with an azoxy moiety from natural origins. Pyrinadine A showed cytotoxicity against L1210 murine leukemia (IC_{50}, 10 μg/ml) and KB human epidermoid carcinoma cells (IC_{50}, 7 μg/ml) in vitro [20].



pyrinadine A

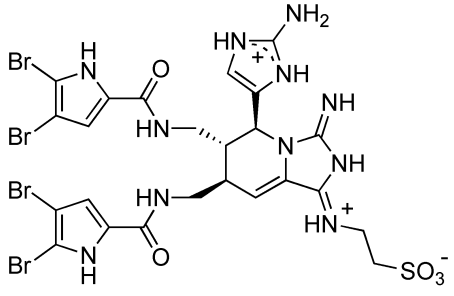
A novel cytotoxic bis-3-alkylpyridine alkaloid with a β-amino acid moiety, nakinadine A, has been isolated from an Okinawan marine sponge *Amphimedon* sp., and the structure and stereochemistry were elucidated by spectroscopic data. Nakinadine A is the first bis-3-alkylpyridine alkaloid with a β-amino acid moiety. Nakinadine A showed cytotoxicity against L1210 murine leukemia (IC_{50}, 1.3 μg/ml) and KB human epidermoid carcinoma cells (IC_{50}, 2.5 μg/ml) in vitro [21].



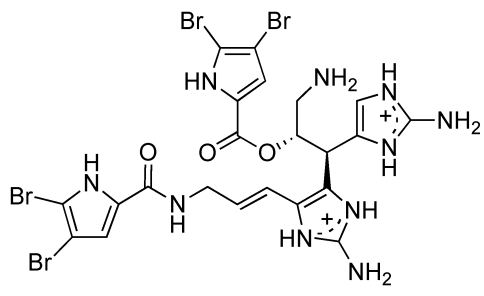
nakinadine A

Nagelamides K and L, dimeric bromopyrrole alkaloids from sponge *Agelas* sp.

Bromopyrrole alkaloids are known to be one of common metabolites from marine sponges of several genera. During our search for bioactive substances from marine sponges, we have isolated some bromopyrrole alkaloids with unique cyclic skeletons from sponges of the genus *Agelas* or *Hymeniacidon*. Recently, we have investigated extracts of two collections of Okinawan marine sponges *Agelas* sp. (SS-1134 and SS-1077) and isolated two new dimeric bromopyrrole alkaloids, nagelamides K and L, respectively. Nagelamide K is a new dimeric bromopyrrole alkaloid possessing a piperidinoiminoimidazolone ring with an aminoimidazole ring and a taurine unit, while nagelamide L is a unique dimeric bromopyrrole alkaloid containing an ester linkage. Nagelamides K and L exhibited antimicrobial activity against *Micrococcus luteus* (MIC, both 16.7 μg/ml) [22].



nagelamide K



nagelamide L

ACKNOWLEDGMENTS

These works were supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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