Haploscleridamine, a Novel Tryptamine-Derived Alkaloid from a Sponge of the Order Haplosclerida: An Inhibitor of Cathepsin K

Ashok D. Patil,[†] Alan J. Freyer,*[,‡] Brad Carte,[§] Paul B. Taylor,[‡] Randall K. Johnson,[‡] and D. John Faulkner[⊥]

*Department of Drug Discovery, Millennium Pharmaceuticals Inc., 75-5 Sidney Street, Cambridge, Massachusetts 02139-1562, Departments of Biomolecular Discovery, Analytical Sciences, and Cellular Biochemistry, GlaxoSmithKline Pharmaceuticals, 709 Swedeland Road, PO Box 1539, King of Prussia, Pennsylvania 19406-0939, Drug Discovery Division, S*BIO Pte. Ltd., 1 Science Park Road, #05-09/15 The Capricorn, Singapore, 117528, and Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0212*

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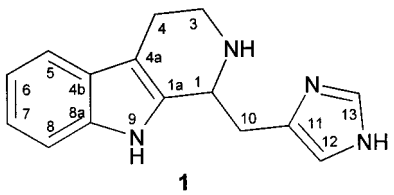
As part of a search for novel inhibitors of cathepsin K, the MeOH extract of a Micronesian sponge of the order Haplosclerida was shown to be active. Bioassay-guided fractionation of the extract yielded halitoxins, tryptamine, and a novel tryptamine-derived alkaloid, haploscleridamine (**1**). The tetrahydro-β-carboline structure of haploscleridamine (**1**) was elucidated through spectral techniques. Haploscleridamine (**1**) was found to be an inhibitor of cathepsin K with an IC_{50} of 26 μM.

Cathepsin K is a novel cysteine protease that has been implicated as playing a role in osteoporosis. It has been established that unlike serine protease inhibitors, cysteine protease inhibitors are very effective in preventing bone resorption.[1] The enzyme itself was identified from a cDNA library that was prepared from enriched osteoclasts present in osteoclastoma tissue.

In the last two decades, the search for marine-derived natural products with useful pharmacological properties has been extended to all oceans of the world; the results of this search have been reported in numerous reviews.[2] As part of our search for biologically active compounds with utility against osteoporosis, we initiated a screen to evaluate natural product extracts for the inhibition of cathepsin K. An extract of the sponge of the order Haplosclerida, collected in Palau, showed cathepsin K inhibitory activity and was therefore selected for fractionation.

The freeze-dried sponge was sequentially extracted with ethyl acetate and methanol. The bioassay-guided fractionation of the methanol extract that exhibited cathepsin K inhibitory activity was chromatographed over a column of Sephadex LH-20. Initial fractions that displayed potent activity in the cathepsin K assay were readily identified from their ^{1}H NMR spectra as mixtures of halitoxins[3] which were considered nuisance compounds and therefore discarded. Further purification of the weakly active fractions by column chromatography (RP-18) followed by Si gel preparative TLC yielded tryptamine and a novel tryptamine-derived tetrahydro-β-carboline alkaloid, haploscleridamine (**1**), as colorless gums.



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The molecular formula for haploscleridamine (**1**) was established by HR-DCMS, which provided a molecular ion at *m/z* 253.1444 $(M + H)^+$ corresponding to a $C_{15}H_{17}N_4$ molecular formula (calcd 253.1453). The mass spectrum taken with ND_3 gas produced a 4-Da increase in the molecular ion, signifying the presence of three exchangeable hydrogens. This molecular formula required 10 double-bond equivalents. The ^{1}H NMR spectrum of haploscleridamine (**1**) contained six downfield multiplets between δ 7.65 and 6.87 and seven aliphatic protons between δ 4.48 and 2.78. The COSY data for haploscleridamine (**1**) revealed the presence of three discrete spin systems, one of which was a tightly coupled aromatic ABCD spin system. One of the two remaining spin systems consisted solely of two pairs of adjacent methylene protons, H-3 and H-4 (between δ 3.36 and 2.78), while the other spin system included an H-1 methine multiplet (δ 4.48), two H-10 aliphatic methylene multiplets (δ 3.32 and 3.01), and two imidazole signals corresponding to H-12 (δ 6.87) and H-13 (δ 7.65).

The ^{13}C GASPE spectrum of haploscleridamine (**1**) indicated the presence of 15 carbon atoms including seven methine, three methylene, and five quaternary carbons. The presence of a tryptamine moiety in **1** was evidenced by HMBC correlations between the ABCD aromatic protons (H-5 to H-8) and the ring carbons (C-4b to C-8a) as well as correlations between H-5 (δ 7.39) and C-4a (δ 108.7) and between H-4 and C-1a (δ 134.2), C-4a, C-4b (δ 128.2), and C-3 (δ 43.0). Consistent with these assignments, H-5 shared an NOE with methylene H-4.

The number of double-bond equivalents suggested that the tryptamine moiety in haploscleridamine (**1**) had cyclized into a tetrahydro-β-carboline unit. This proposal was supported by HMBC correlations observed between H-1 (δ 4.48) and C-1a. In addition, the H-10 methylene hydrogen atoms that connect the pendant imidazole moiety to the β-carboline moiety correlated to C-1a and C-1 in the tryptamine portion of the molecule as well as to C-11 and C-12 in the imidazole portion. The remaining correlation data confirmed that haploscleridamine (**1**) was a tetrahydro-β-carboline entity with a pendant methylimidazole substituent. The 7-bromo analogue of **1**, named lissoclin C, was previously isolated from the tropical ascidian *Lissoclinum* sp.[4]

The H-12 imidazole proton shared NOEs with H-1 and H-10, while the H-13 doublet showed no NOEs at all owing to its isolated position. The specific rotation of haplo-

* To whom correspondence should be addressed. Tel: 610-270-6315. Fax: 610-270-6608. E-mail: Alan_J_Freyer@gsk.com.
[†] Millennium Pharmaceuticals Inc.
[‡] GlaxoSmithKline Pharmaceuticals.
[§] S*BIO Pte. Ltd.
[⊥] Scripps Institution of Oceanography.

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scleridamine (**1**) was nearly zero, suggesting a racemic mixture. A previous study has shown that C-1-substituted tetrahydro-β-carbolines readily racemize in acidic solution.[5] Therefore, haploscleridamine (**1**) may initially have been chiral and subsequently racemized during isolation.

Haploscleridamine (**1**) and tryptamine had moderate potency as inhibitors of cathepsin K with IC_{50}'s of 26 and 15 μM, respectively.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet Model 20 DXB-FTIR spectrometer. All homo- and heteronuclear one- and two-dimensional NMR data were recorded on a Bruker AMX-400 spectrometer in CD_3OD. Low-resolution mass spectra were obtained using a Finnigan Model 4610 quadrupole mass spectrometer with CH_4, NH_3, and ND_3 gases. The HR-DCMS was acquired on a VG-70SE with CH_4 and NH_3 gases. Analytical and preparative TLC were carried out on precoated Si gel G (Kisel gel G254) and reverse-phase (Whatman KC18F) plates. The UV spectrum was recorded on a Beckman-DU-7 spectrophotometer. Optical rotation was recorded on a Perkin-Elmer 241 MC polarimeter. Reagent grade chemicals (Fisher and Baker) were used throughout.

Biological Assay. The experimental procedure employed was modeled after a previously described method for the proteolytic cleavage of small peptide fluorogenic substrates.[6] All screening was performed using an Orca robotic arm that facilitated the addition of components to a total volume of 100 μL per well. The final buffer composition in the assay was 100 mM sodium acetate, 5 mM EDTA, and 20 mM L-cysteine, pH 5.5. The L-cysteine was added to the buffer on the same day that the assay was performed. The substrate used was the cleavable fluorogenic peptide conjugate ZFR-AMC (phenylalanine-arginine-aminomethylcoumarin, Bachem). With the exception of the inhibitor source plates, all assay components were kept at 4 °C until they were added to the test plates that were left at room temperature for 1 h. It was unnecessary to quench the cleavage reaction because the assay duration could be accurately constrained by robot scheduling. The final assay composition was 160 ng/mL (6 nM) cathepsin K, 20 μM ZFR-AMC, and 100 μg/mL (or lower if necessary) inhibitor. The final assay mix contained 2% DMSO, which was the summation of the substrate and inhibitors each contributing 1%. After substrate cleavage by cathepsin K, the AMC fluorescence signal was recorded by a Fluostar fluorimeter (Tecan) which had custom filters installed (Omega Optical) possessing excitation and emission wavelengths of 360 nm (15 nm bandwidth) and 440 nm (20 nm bandwidth), respectively.

Biological Material. The sponge (PAL93-055) is a firm brown lobate mass and was collected by hand using scuba at a depth of 170 feet near "Blue Hole" in the Republic of Palau on January 24, 1993. The specimen was frozen immediately and kept at −20 °C until extraction. The sponge skeleton is an irregular complex reticulation of oxeas (150−170 μm) with a surface of spicule tuffs echinated by smaller oxeas (50−90 μm). Within a deeper choanosome spicules form irregular palisades around cavities. The sponge produces a copious slime and turns reddish brown in alcohol preservative. The sponge probably represents a new genus that shares the spiculation and structural skeletal features of *Pellina* and *Biminia* (order Haplosclerida, family Oceanoplidae) and *Petrosia* (order Haplosclerida, family Petrosiidae). The sample was identified by Michelle Kelly-Borges, and a voucher sample has been deposited in the National History Museum, London, U.K. (BMNH 1996.9.17.1).

Extraction and Isolation. The freeze-dried sponge (120 g) was extracted with EtOAc (500 mL × 2) and MeOH (500 mL × 2), yielding 1.4 and 21.6 g extracts, respectively. Part of the dark red MeOH extract (3.5 g), which showed cathepsin K inhibitory activity, was applied to a Sephadex LH-20 column and eluted with MeOH. Several fractions (15 mL each) were collected and monitored by TLC. Like fractions were combined to yield nine (A−I) individual fractions. Cathepsin K active fraction A (0.437 g) was determined to be a mixture of halitoxins on the basis of its ^{1}H NMR spectrum and was not pursued further. Weakly active fractions E and F were combined (0.274 g) and chromatographed over a column of RP-18 Si gel using H_2O/MeOH (15:85) as a solvent system. Fatty acids and other impurities were eluted first, and finally the strongly retained material was eluted with 1% NH_4OH in MeOH, yielding the cathepsin K active residue (43 mg). The Si gel PTLC of this residue using MeOH/CH_2Cl_2/H_2O/HCOOH (25:75:2.5:3.5) afforded tryptamine (14 mg) and haploscleridamine (**1**) (19 mg).

Haploscleridamine (1): colorless gum, $[\alpha]_D$ −3.4° (*c* 0.78, MeOH); IR (KBr) ν_{max} 3370−3380, 2800−3100, 1623, 1589, 1570, 1453, 1318, and 745 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 235 (3.72), 270 (3.41), 278 (3.55), and 288 (3.06) nm; DCMS *m*/*z* 253 $(M + H)^+$; HR-DCMS *m*/*z* 253.1444 $(M + H)^+$ calcd for $C_{15}H_{17}N_4$, 253.1453; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.65 (1H, d, J = 1.2 Hz, H-13), 7.39 (1H, ddd, J = 1.0, 1.0, and 7.7 Hz, H-5), 7.31 (1H, ddd, J = 1.0, 1.0, and 8.0 Hz, H-8), 7.07 (1H, ddd, J = 1.0, 8.0, and 8.0 Hz, H-7), 6.98 (1H, ddd, J = 1.0, 7.7, and 8.0 Hz, H-6), 6.87 (1H, dt, J = 1.2 and 1.0 Hz, H-12), 4.48 (1H, m, H-1), 3.36−3.32 (2H, m, H-3, 10), 3.04−3.01 (2H, m, H-3, 10), 2.82−2.78 (2H, m, H-4, 4); ^{13}C NMR (CD_3OD, 100 MHz) δ 137.9 (s, C-8a), 136.6 (d, C-13), 135.4 (s, C-11), 134.2 (s, C-1a), 128.2 (s, C-4b), 122.6 (d, C-7), 120.0 (d, C-6), 118.8 (d, C-5), 117.8 (d, C-12), 112.1 (d, C-8), 108.7 (s, C-4a), 54.3 (d, C-1), 43.0 (t, C-3), 32.2 (t, C-10), 21.9 (t, C-4).

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