Bioorganic & Medicinal Chemistry Letters 26 (2016) 4960–4965

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of anabaenopeptin 679 from freshwater algal bloom material: Insights into the structure–activity relationship of anabaenopeptin protease inhibitors

Henrik Harms [a], Kenji L. Kurita [d], Li Pan [b], Paul G. Wahome [c], Haiyin He [c], A. Douglas Kinghorn [b], Guy T. Carter [c], Roger G. Linington [a,d,*]

[a] Department of Chemistry and Biochemistry, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA
[b] Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210, USA
[c] Biosortia Pharmaceuticals, Hollings Marine Lab, 331 Ft. Johnson Road, Charleston, SC 29412 USA
[d] Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

ARTICLE INFO

Article history:
Received 1 June 2016
Revised 2 September 2016
Accepted 3 September 2016
Available online 6 September 2016

Keywords:
Anabaenopeptin
Carboxypeptidase inhibitor
Cyanobacteria
Algal bloom

ABSTRACT

Cyanobacteria possess a unique capacity for the production of structurally novel secondary metabolites compared to the biosynthetic abilities of other environmental prokaryotes such as bacteria of the genus Streptomyces. Two different strategies to explore cyanobacteria-derived natural products have been explored previously: (1) cultivation of single cyanobacterial strains, in bioreactors for example; (2) bulk collections from the environment of so called 'algal blooms' that are dominated by cyanobacteria. In this study a new environmentally friendly collection technique for obtaining large quantities of algal bloom biomass was utilized. Algal biomass derived from eight million liters of lake water was concentrated using a novel continuous countercurrent filtration system. Analysis of this freshwater algal bloom from Grand Lake-Saint Marys, Ohio led to the discovery of anabaenopeptin 679 (**1**), as well as the known anabaenopeptins B, F, H and 908. Anabaenopeptin 679 is unusual in that it possesses the classical anabaenopeptin-like cyclic pentapeptide core, but lacks the typical sidechain attached to the constitutive ureido group. Screening of all anabaenopeptin derivatives in an enzymatic assay for inhibitory activity toward carboxypeptidase A identified anabaenopeptin 679 as a strong inhibitor of carboxypeptidase A with an IC_{50} value of 4.6 μg/mL. This result defines a new minimal core structure for carboxypeptidase activity among the anabaenopeptin class, and provides further insight into the structure–activity relationship of anabaenopeptin-like carboxypeptidase A inhibitors.

Freshwater algal blooms constitute a rapid growth and accumulation of a complex assemblage of unicellular organisms including phytoplankton, cyanobacteria and other prokaryotes that can lead to discoloration and chemical contamination of affected waters.[1] Freshwater cyanobacteria have been shown to produce a variety of structurally diverse and biologically valuable natural products including cytotoxic macrocyclic depsipeptides like cryptophycin 1,[2] antimicrobial alkaloids like ambiguine H[2] and cyclic peptides with protease inhibitory activity such as the anabaenopeptins.[2] Anabaenopeptins are cyclic pentapeptides produced by several cyanobacterial species, containing a ureido moiety as part of the amino acid sidechain adjacent to a D-lysine residue, which in turn cyclizes the main ring system.[2] Several anabaenopeptins have been identified as potent inhibitors of exopeptidases like carboxypeptidase A (CPA).[2]

Carboxypeptidases are exopeptidase enzymes that catalyze the cleavage of certain carboxyl-terminal peptide bonds in peptides and proteins.[3,4] Carboxypeptidase A (CPA) is considered a model enzyme for physiologically relevant exopeptidases such as angiotensin-converting enzyme.[4] Additionally several inhibitors of CPA are also inhibitors of carboxypeptidase U (CPU),[5] which plays an important role in the regulation of fibrinolysis.[6] Therefore carboxypeptidase inhibitors are considered a potential treatment strategy for certain cardiovascular diseases.[7–9] Furthermore carboxypeptidase inhibitors have been identified as antagonists of human epithelial growth factor and are therefore involved in the inhibition of tumor cell growth for certain cancers.[10]

The constitution of the side chain connected to the ureido moiety of anabaenopeptin-like CPA inhibitors has previously been postulated as crucial for the potency of protease inhibitory effects.[11,12]

* Corresponding author. Tel.: +1 778 782 3517; fax: +1 778 782 3765.
E-mail address: rliningt@sfu.ca (R.G. Linington).

http://dx.doi.org/10.1016/j.bmcl.2016.09.008
0960-894X/

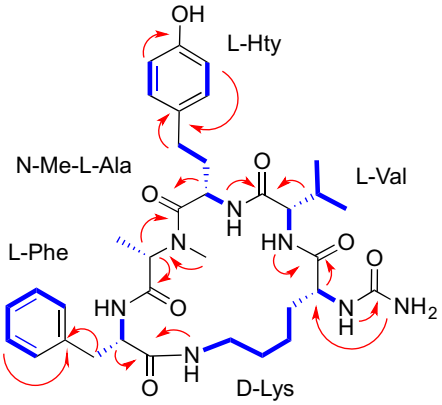
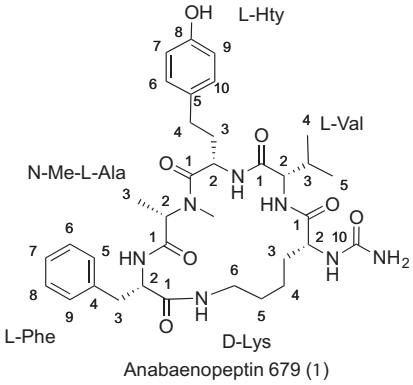


Figure 1. Structure determination for anabaenopeptin 679 (**1**). Key $^{1}H–^{1}H$ COSY (blue lines) and HMBC (red arrows) correlations for anabaenopeptin 679 in DMSO-d_6.

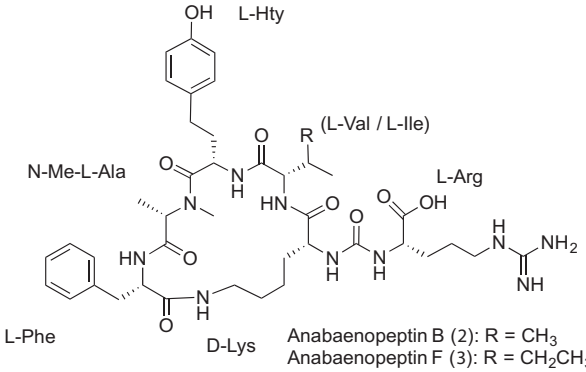
Previous work has suggested that the amino acid residue attached to the ureido group is essential to interact with CPA. Furthermore it has been postulated that the cyclic pentapeptide moiety of anabaenopeptins might also be an important pharmacophoric element for CPA inhibitory activity.[12] Here we describe the isolation of a new truncated anabaenopeptin, anabaenopeptin 679 (**1**), which lacks the side chain residue usually found in this class of compounds. This new addition to the anabaenopeptin family affords further insight into the structure–activity relationship for this compound class.

In order to capture novel micro-algal metabolites present at sub-ppm concentrations, we have developed a unique continuous counter-current filtration system. For this work, processing of eight million liters of water from Grand Lake-Saint Marys, Ohio in May 2012 provided 3495 kg of concentrated algal biomass as a dark green paste. Prior to solvent extraction, batches of the paste were carefully freeze-dried yielding a friable solid. In this study a 16.5 kg aliquot of this dry material was extracted and used to create a fraction library. In brief, freeze-dried material was extracted exhaustively with $MeOH/CH_2Cl_2$ to give a dark green gum. This crude material was sequentially fractionated under normal- and reversed-phase conditions to give nine pre-fractions whose fractionation provided 230 fractions. Aliquots of these fractions were resuspended in DMSO at a standard *w*/*v* ratio for biological evaluation.

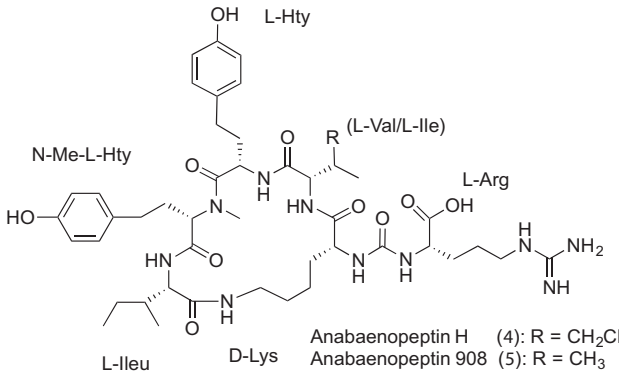
As part of an ongoing program to use integrated biological and chemical profiling to discover novel cytotoxic agents from natural product libraries, this fraction library was evaluated in an image-based cytological profiling assay against HeLa cells.[13–15] Simultaneously, these fractions were subjected to metabolomics profiling by UPLC-ESI-TOF mass spectrometry. In the course of analyzing these two complex datasets an exact mass feature at 678.3618 *m*/*z* was identified as having no match to any known cyanobacterial metabolite in our in-house database. Initial inspection of ^{1}H and ^{13}C NMR spectra for this new metabolite suggested that it was closely related to anabaenopeptin-like cyclic peptides, although the molecular weight was significantly lower than other representatives of this compound class. Given this potential structural novelty, this compound was selected for further investigation. HRMS analysis suggested a molecular formula of $C_{35}H_{48}N_7O_7$ ($[M-H]^-$ = 678.3618, Δ0.44 ppm), indicating 15 degrees of unsaturation. Extensive examination of 2D NMR data identified the presence of three proteinogenic (lysine, valine, phenylalanine) as well as two nonproteinogenic (homo-tyrosine and *N*-methyl-alanine) amino acids. NOESY experiments as well as MS^n fragmentation experiments allowed us to establish the amino acid sequence of the cyclic peptide as Lys-Val-Hty-*N*Me-Ala-Phe. Finally $^{1}H–^{13}C$ HMBC correlations between the proton at the ε-NH_{Lys} position and the carbon at position C1 of the phenylalanine residue implied that the phenylalanine residue was attached to the adjacent lysine through the lys ε-NH, completing the planar assignment of the cyclic portion of the structure and accounting for all but one of the degrees of unsaturation (Fig. 1).



Anabaenopeptin 679 (1)



Anabaenopeptin B (2): R = CH_3
Anabaenopeptin F (3): R = CH_2CH_3



Anabaenopeptin H (4): R = CH_2CH_3
Anabaenopeptin 908 (5): R = CH_3

The remaining portion of the molecule possessed a formula of CNH_2O. Examination of the proton spectrum revealed two protons, displayed as a broad singlet at 5.68 ppm, that were not directly linked to a carbon atom. When combined with the one additional signal in the carbon spectrum at 158.2 ppm and the one remaining degree of unsaturation, these data suggested the presence of a terminal primary amide motif. HMBC correlations between the unassigned carbon at 158.2 ppm and both the unassigned broad singlet at 5.68 ppm and the α-NH atom of the lysine amino acid indicated the presence of a mono-substituted ureido moiety attached at the α-CH position of the lysine amino acid (Fig. 1), thus completing the planar structure assignment for this new metabolite (Table 1).