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Trichothiazole A, a dichlorinated polyketide containing an embedded thiazole isolated from *Trichodesmium* blooms

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Abstract

Mass spectrometry-guided isolation of the lipophilic extract of *Trichodesmium* bloom material led to the isolation and structure characterization of a new thiazole-containing di-chlorinated polyketide (**1**). The structure of **1** was deduced using 1D and 2D NMR analysis, high-resolution mass spectrometry analysis and complementary spectroscopic procedures. Trichothiazole A possesses interesting structural features, such as a terminal alkyne, two vinyl chlorides and a 2,4-disubstituted thiazole. Trichothiazole A showed moderate cytotoxicity to Neuro-2A cells (EC₅₀: 13.3 ± 1.1 μM).

Graphical Abstract

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Supplementary Data

Supplementary data: Additional experimental detail. Figures S1–S7. ¹H NMR, ¹³C NMR, HSQC, HMBC, COSY, TOCSY and NOESY of **1**. Figure S8. HRESIMS spectrum of **1**. Figure S9. Dose-response of **1** tested against Neuro-2A cells. Figure S10. HRESIMS spectrum of putative trichothiazole analog. Figure S11. Possible biosynthetic pathway of **1**. The supplementary material associated with this article can be found in the online version.

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Keywords

Trichodesmium; thiazole; heterocycles

Heterocyclic moieties contained within small natural molecules and synthetics are desired functional groups in lead molecules in drug discovery.¹ Heterocyclic groups such as thiazoles, thiazolines, oxazoles and oxazolines are often reported in cyanobacteria natural products as modified amino acids in peptides, and hybrid polyketide-peptides.² Thiazoles and thiazolines are contained in structurally diverse, biologically active linear hybrid polyketide-peptides from cyanobacteria such as kalkitoxin,³ curacin,⁴ and barbamide.⁵ These functionalities are additionally found in cyclic compounds such as hectochlorin⁶ and wewakazole.⁷ Cytotoxicity is a desired property for chemotherapeutic lead molecules. Kalkitoxin showed potent cytotoxicity against the HCT-116 human colon cancer cell line,⁸ while curacin has been shown to act as a cytotoxin by tubulin inhibition.⁴

Our laboratory has been intensively chemically profiling blooms of the cyanobacterium *Trichodesmium* collected from the Gulf of Mexico. Thus far we have characterized the chlorinated polyketides trichophycin A and trichotoxins A and B,^{9,10} as well as the macrocyclic hybrid polyketide-peptides tricholides A and B and unnarmicin D.¹¹ We have employed bioassays and NMR examination to guide our isolations. However, having uncovered several chlorinated metabolites from bloom chemical fractions,^{9,10} mass spectrometry-guided isolation offers means to ultimately purify metabolites based on molecular features identified from mass spectrometric isotopic data.

Herein, we have utilized mass spectrometry-guided isolation to characterize a new highly functionalized dichlorinated polyketide, trichothiazole A (**1**) (Figure 1). In addition to multiple chlorinations, **1** features a terminal alkyne and 2,4-disubstituted thiazole embedded in the linear polyketide chain.

Results and Discussion

In our search for bioactive molecules from *Trichodesmium* sp. blooms, chemical fractionation and isolation guided by mass spectrometric molecular features such as the ratio of the M+, M+2 and M+4 peaks led to the isolation of a colorless oil (**1**). HRESIMS analysis of **1** gave a protonated molecule [M+H]⁺ of *m/z* 342.0857, suggesting a molecular formula of C₁₇H₂₁Cl₂NS and a requirement of 7 degrees of unsaturation. An isotopic ratio of 9:6:1 for the M+, M+2, and M+4 peaks provided additional support for the presence of two chlorine atoms.

Examination of ¹³C NMR and 2D NMR spectra of **1** identified 8 methylenes, 4 methines, 4 quaternary carbon atoms and signals consistent with a terminal alkyne (δ_C 68.2, δ_H 2.19). A partial structure was deduced from C-1 to C-6 in which two methylene groups were correlated by COSY (H₂-5, δ_H 2.59; H₂-6, δ_H 2.86) (cf. Table 1 and Figure 2). A second methylene group (H₂-3, δ_H 2.82) was correlated by COSY to an olefin (H-2, δ_H 5.86; H-1, δ_H 6.17). Methylenes from these two spin systems (H₂-3 and H₂-5) were correlated by HMBC to a quaternary carbon (C-4, δ_C 139.3). A singlet methine proton (H-17, δ_H 5.99) showed an HMBC correlation to C-4. The polarization of the C-4–C-17 olefin (δ_C , 139.3 and 114.7) and the C-1–C-2 olefin (δ_C 130.1 and 119.2) supported the assignment of two vinyl chloride groups. The C-1–C-2 olefin in **1** was determined to be *E* by virtue of a large vicinal ¹H–¹H coupling constant (13.2 Hz). The 13.2 Hz coupling constant was very close to that determined for the terminal vinyl chloride group in kimbeamide A (13.4 Hz).¹² An NOE correlation between H-17 and H-2 supported the C-4–C-17 vinyl chloride group configuration as *E*. A second partial structure was constructed from C-10 to C-16. A deshielded methylene group (H₂-10, δ_H 3.00) was correlated by COSY to H₂-11 (δ_H 1.79). COSY correlations extended the aliphatic chain to position 14 with three additional methylene groups. A moderately deshielded methylene group (H₂-14, δ_H 2.17) was correlated by HMBC to a quaternary carbon (C-15, δ_C 83.4). Examination of the HMBC spectrum additionally correlated the H-16 proton (δ_H 2.19) to C-15. H-16 itself was correlated to C-16 (δ_C 68.2) by HSQC and strongly supported the assignment of a terminal alkyne functionality. Four of the seven degrees of unsaturation were accounted for by the assigned double and triple bonds. Three carbons remained unassigned. Two were quaternary carbons δ_C 171.2 and 155.0. The latter of was part of an extremely polarized olefin with a second carbon (C-8, δ_C 113.0) as indicated by an HMBC correlation from H-8 (δ_H 7.01) to C-7 (δ_C 155.0). Examination of the molecular formula and the remaining carbon chemical shifts supported assignment of a thiazole functionality. HMBC correlations from H₂-10 to C-9 and H₂-6 to C-7 supported a 2,4-disubstituted thiazole moiety and the two double bonds in the thiazole and the ring satisfied the remaining three degrees of unsaturation and completed the structure of **1** (Figure 2).

Trichothiazole A (**1**) showed moderate cytotoxicity against the murine neuroblastoma cell line Neuro-2A (EC₅₀: 13.3 ± 1.1 μM). The small quantity of **1** isolated (0.2 mg) precluded further testing against more cell lines and further biological evaluation at the present time.

Both natural and synthetic 2,4-disubstituted thiazole containing compounds have shown intriguing biological activities other than cytotoxicity such as anti-inflammatory, anti-viral,

anti-bacterial, anti-fungal, and anti-plasmodial activity.¹³ Continued access to diverse structural elements surrounding the thiazole functionality are important for continued understanding of structure-activity relationships for natural and synthetic derivatives containing an embedded thiazole. Trichothiazole A (**1**) represents a new addition to the diverse group of polyketides with embedded thiazoles isolated from the marine realm. In addition to the thiazole-containing polyketide compounds isolated from cyanobacteria referenced above, the antihelminthic mycothiazole was isolated from the marine sponge *Spongia mycofijiensis*,¹⁴ and WS75624 A and B were isolated from the fermentation broth of *Saccharothrix* sp. No. 75624.¹⁵ These compounds showed potent endothelin converting enzyme inhibition.¹⁵ Trichothiazole A (**1**) shows unprecedented structural features for a natural molecule in the acyclic portions surrounding the thiazole, a terminal alkyne and two vinyl chloride moieties. The terminal alkyne has been observed in the previously reported trichotoxin B¹⁰ (Figure 1) and vinyl chloride functionalities have been observed in trichophycin A and trichotoxins A and B.⁹ However, these molecules are constructed as linear polyketides without any modified amino acid portions.

We predict that the biosynthetic pathway for the construction of trichothiazole contains elements previously observed in cyanobacterial metabolites such as jamaicamide A and curacin A.^{16,17} The terminal alkyne may be formed by a fatty acid desaturase following loading of an octanoic acid starter unit as predicted in jamaicamide biosynthesis.¹⁶ Next a cysteine amino acid would be incorporated by an NRPS module with a cyclization domain to generate the thiazole. PKS modules would add three acetate units to the linear molecule and the cysteine carbonyl would be fully reduced by domains contained in the first PKS module. We predict the first acetate unit would be modified by an HMG CoA synthase to an exomethylene vinyl group¹⁸ and the second and third would be modified by a decarboxylating thioesterase as observed in curacin¹⁷ to create a terminal alkene. The two alkenes would be halogenated to generate the vinyl chlorides (Figure S11).

A second putative trichothiazole analog with an $[M+H]^+$ of m/z 344.1005 suggesting a molecular formula of $C_{17}H_{23}Cl_2NS$ (calcd for $C_{17}H_{24}Cl_2NS$, 344.1007) was detected in our HPLC fractionation and HRESIMS analysis. The molecule showed greater retention time than trichothiazole and the ion had the same isotopic pattern of **1** indicating dichlorination. However, this likely analog was not isolable in pure quantities necessary for NMR analysis. Overall, *Trichodesmium* blooms appear to be an intriguing source of highly functionalized natural molecules and our future work will focus on metabolic profiling of *Trichodesmium* blooms spatially and temporally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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19. *Trichothiazole A* (1): colorless oil; $\alpha_{25D} -6.3$ (MeOH, c 0.04); UV (MeOH) $\lambda_{\max}$ (log ϵ) 201 (3.7), 242 (3.2) nm; (^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (175 MHz, CD_3OD), see Table 1; HRESIMS m/z 342.0857 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{22}\text{Cl}_2\text{NS}$, 342.0850).

Figure 1.
Structure of trichothiazole A (**1**) and the previously reported trichotoxin B.⁹

Figure 2.
Key 2D NMR correlations of **1**.

Table 1.NMR data for trichothiazole A (**1**) (500 MHz for ^1H NMR and 175 MHz for ^{13}C NMR, CD_3OD)

position	δ_{C} , type	δ_{H} (J in Hz)	HMBC	COSY
1	119.2, CH	6.17, dt (13.2, 1.5)		H-2
2	130.1, CH	5.86, dt (13.2, 7.5)	C-17	H-1, H-3
3	35.4, CH_2	2.82, dt (7.5, 1.4)	C-1, C-2, C-4, C-5, C-17	H-2
4	139.3, qC			
5	29.4, CH_2	2.59, m	C-3, C-4, C-6, C-7, C-17	H-6
6	27.9, CH_2	2.86, m	C-4, C-5, C-7, C-8	H-5
7	155.0, qC			
8	113.0, CH	7.01, s	C-7, C-9	
9	171.2, qC			
10	32.4, CH_2	3.00, m	C-9, C-11, C-12	H-11
11	29.5, CH_2	1.79, m	C-10, C-12	H-10, H-12
12	27.6, CH_2	1.51, m	C-11, C-13	H-11
13	27.8, CH_2	1.56, m	C-12, C-15	H-14
14	17.5, CH_2	2.17, m	C-13, C-15	H-13
15	83.4, qC			
16	68.2, CH	2.19, t (2.6)	C-15	
17	114.7, CH	5.99, s	C-4	