

Novel Bromotyrosine Alkaloids: Inhibitors of Mycothiol S-Conjugate Amidase

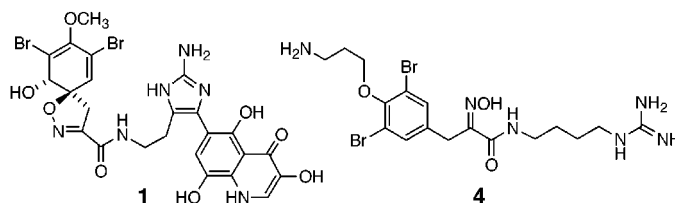
Gillian M. Nicholas,[†] Gerald L. Newton,[‡] Robert C. Fahey,[‡] and
Carole A. Bewley^{*,†}

Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health,
Bethesda, Maryland 20892-0820, and Department of Chemistry and
Biochemistry, University of California, San Diego, La Jolla, California 92093

bewley@speck.nidk.nih.gov

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ABSTRACT



The novel alkaloids 1 and 4 were isolated from an Australian non-verongid sponge, *Oceanapia* sp. Compound 1 contains an unprecedented imidazolyl-quinolinone substructure attached to a bromotyrosine-derived spiro-isoxazoline. Three other known alkaloids were isolated in addition to 1 and 4 and together represent the first examples of inhibitors of a new mycobacterial enzyme mycothiol S-conjugate amidase (MCA).

Mycobacterium tuberculosis, the causative agent of tuberculosis, is a leading pathogenic cause of death worldwide.¹ The rise of mycobacterial resistance to common antituberculars such as isoniazid and rifampin, along with the high prevalence of tuberculosis and *Mycobacterium avium* complex in AIDS patients, has led to a renewed interest in the discovery of antimycobacterial agents with new modes of action. In an effort to identify new classes of antimycobacterials, we have screened a variety of marine extracts for their ability to inhibit a novel mycobacterial enzyme, mycothiol S-conjugate amidase (MCA).² Herein we report the isolation and structure elucidation of new alkaloids that inhibit MCA.

Mycothiol (MSH)³ is a low molecular weight thiol that replaces glutathione in actinomycetes.⁴ In conjunction with MCA, MSH plays a central role in protecting actinomycetes

against alkylating agents and other toxins.^{4,5} Recently a second highly homologous amidase from *M. tuberculosis* that is involved in the biosynthesis of MSH has been described.⁶ Because these mycothiol-dependent pathways are not found in eukaryotes, the enzymes involved represent potentially useful new antimycobacterial targets since inhibition of both enzymes would permit blocking of MSH-dependent detoxification at two distinct levels, namely, biosynthesis and detoxification.

A preliminary screen of the extract of a specimen of *Oceanapia* sp.⁷ showed strong activity against MCA,⁸ as

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(6) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *J. Bacteriol.* **2000**, *24*, 6958.

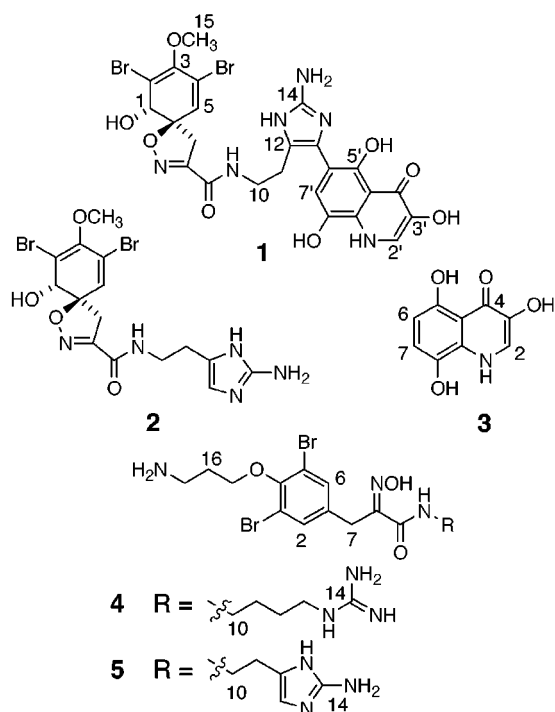
[†] National Institutes of Health.

[‡] The University of California.

(1) Zumla, A.; Grange, J. *Clinical Review: Tuberculosis. Br. Med. J.* **1998**, *316*, 1962.

(2) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, *35*, 10739.

determined by the absence of the MCA/MSH cleavage product AcCys-bimane in a fluorescence-detected HPLC assay.² Bioassay-guided purification of the MeOH/10% H₂O soluble material by reverse-phase (C18) chromatography, followed by chromatography on LH20 (eluting with MeOH), yielded known compounds pseudoceratine⁹ (**2**, 24.7 mg, 1.4%) and uranidine¹⁰ (**3**, 17.7 mg, 1.0%) and a small amount of compound **1** (2 mg, 0.11%). Compound **1** exhibited the strongest inhibition of MCA,¹¹ but initial efforts to establish the structure were hampered by extensive decomposition upon reverse-phase HPLC with CH₃CN/H₂O (0.05% TFA). Purification of a second MeOH/10% H₂O soluble fraction of the crude extract by LH20 chromatography (MeOH) alone provided increased amounts of compound **1** (6.0 mg 0.24%). Repeated reverse-phase (C18) HPLC of other fractions from this LH20 column gave compounds **4** (2.0 mg, 0.11%) and **5** (1.5 mg, 0.08%).



Compound **1** showed an isotopic cluster of MH⁺ ions in the ratio of 1:2:1 in the FABMS at *m/z* 681, 683, and 685. A molecular formula of C₂₄H₂₂O₈N₆Br₂ was determined by HRFABMS.¹² An initial comparison of the ¹H and ¹³C NMR spectra of compound **1** showed obvious similarities with the spectra for compounds **2** and **3** and indicated that the dibrominated cyclohexadienol–spiro-isoxazoline system was intact. This was confirmed by HSQC and HMBC 2D NMR

(7) Extract obtained from the NCI open repository.

(8) Mycothiol *S*-conjugate amidase and mycothiol-bimane were isolated from a culture of *Mycobacterium smegmatis* mc²155 and purified as described in ref 2.

(9) Benharref, A.; Pais, M. *J. Nat. Prod.* **1996**, *59*, 177.

(10) Cimino, G.; De Rosa, S.; De Stefano, S.; Spinella, A.; Sodano, G. *Tetrahedron Lett.* **1984**, *25*, 2925.

(11) Compounds **1**, **2**, **4**, and **5** inhibited mycothiol *S*-conjugate amidase by 50% at 2, 100, 3, and 37 mM, respectively. A full report of biological data will be reported elsewhere.

(12) HRFABMS MH⁺ *m/z* 680.9940, calcd 680.9944.

experiments (see Supporting Information). The spin system from C9 to C11 was assigned by HMBC correlations from the methylene protons at δ_{H} 3.40 (H10) and the exchangeable proton at δ_{H} 8.62 (C9–NH) to the amide carbon at C9 (δ_{C} 158.9), along with TOCSY correlations between these protons and the second methylene at δ_{H} 2.75 (H11). In elucidating the complete structure of **1**, HMBC correlations from H11 (δ_{H} 2.75) to two aromatic quaternary carbons at δ_{C} 120.4 (C12) and 118.7 (C13) and from an exchangeable proton at δ_{H} 11.95 (12–NH) to the carbons at δ_{C} 120.4 (C12) and 146.2 (C14) were critical (Figure 1).

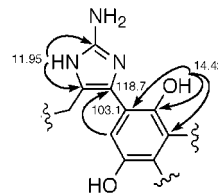


Figure 1. HMBC correlations that establish the connection between the 2-amino imidazolyl and quinolinone fragments.

For the imidazolyl-quinolinone portion of **1**, all but two of the proton and carbon resonances were consistent with those reported for uranidine (**3**) and were supported by HMBC correlations.¹⁰ Notable differences included the absence of the resonances corresponding to H6 and H7 in uranidine and the appearance of a singlet at δ_{H} 6.96. This new singlet was assigned as H7' in compound **1** on the basis of observed HMBC correlations to carbons at δ_{C} 128.9 (8a'), 137.0 (C8'), and 149.3 (C5'), confirming substitution at the C6' carbon. Analysis of HMBC spectra recorded with varying values of Δt (set to observe long-range ¹H–¹³C couplings of 6, 8, 13, and 20 Hz) allowed the detection of correlations from the hydrogen-bonded hydroxyl proton at δ_{H} 14.42 (5'-OH) to three aromatic carbons at δ_{C} 112.2, 149.3, and 103.1, which were assigned as C4a', C5', and C6', respectively. An HMBC correlation from H7' (δ_{H} 6.96) to a quaternary carbon at δ_{C} 118.7 (C13) gave the last carbon–carbon connection and linked together the quinolinone and imidazole subunits (Figure 1). Full NMR data are presented in Table 1.

The absolute stereochemistry of compound **1** was determined by comparing the spectral data with those of compound **2** and its enantiomer.^{9,14} Both enantiomers of compound **2** have been described, and the absolute stereochemistry has been determined by comparing CD spectra with previously published data.^{9,14} On the basis of these studies, the specific rotation and the negative Cotton effect observed in the CD spectrum for compound **2**¹⁵ confirmed it to be the

(13) 1-D NMR spectra were recorded on a Varian Mercury300 and 2-D NMR spectra at 27 °C on a Bruker DMX500 and referenced to residual solvent (DMSO-*d*₆, δ_{C} 39.5, δ_{H} 2.50). Protons were assigned from ¹H NMR or HSQC spectra.

(14) Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull.* **1995**, *43*, 403.

(15) [α]_D²⁰ –150° (*c* 0.19, MeOH); CD (MeOH) λ 260 nm $\Delta\epsilon$ –2.1, 292 nm $\Delta\epsilon$ –2.8 (see Supporting Information for spectrum).

Table 1. NMR Data for **1** in DMSO-*d*₆¹³

atom	δ_C	δ_H	mult ^a	<i>J</i> (Hz)
1	73.5	3.89	d	0.6
2	113.1			
3	147.1			
4	120.8			
5	131.2	6.56	d	0.6
6	90.1			
7a	39.4	3.12	d	18.0
7b		3.60	*	
8	154.4			
9	158.9			
10	38.1	3.40	*	
11	24.6	2.75	m	
12	120.4			
13	118.7			
14	146.2			
15	59.6	3.69	s	
2'	124.2	7.70	s	
3'	137.7			
4'	173.2			
4a'	112.2			
5'	149.3			
6'	103.1			
7'	113.8	6.96	s	
8'	137.0			
8a'	128.9			
1-OH		6.42	br s	
9-NH		8.62	br t	5.7
12-NH		11.95	br s	
14-NH ₂		7.27	br s	
1'-NH		9.00	br s	
3'-OH		11.81	br s	
5'-OH		14.42	br s	
8'-OH		10.36	br s	

^a s = singlet, d = doublet, m = multiplet, br = broad, (*) = overlapped.

levorotatory enantiomer, pseudoceratine,⁹ with absolute configuration 1-(*S*), 6-(*R*). Comparison of the ¹H NMR chemical shifts for H1, H7a, and H7b of compounds **1** and **2** indicated that they have the same relative stereochemistry. Thus the negative Cotton effect in the CD spectrum for compound **1** indicated that it also has the absolute configuration 1-(*S*), 6-(*R*).^{9,14,16}

The ¹H and ¹³C NMR data for compound **5** were consistent with those reported previously.¹⁷ A molecular formula of C₁₇H₂₆N₆O₃Br₂ for compound **4** was determined by HR-

(16) [α]_D²⁰ -59° (*c* 0.25, MeOH); UV (MeOH) λ_{\max} 238 nm (ϵ 32000), 280 nm (ϵ 20000), 340 nm (ϵ 2800); CD (MeOH) λ 290 nm $\Delta\epsilon$ -2.7, 260 nm $\Delta\epsilon$ -1.0 (see Supporting Information for spectrum).

(17) Litaudon, M.; Guyot, M. *Tetrahedron Lett.* **1986**, 27, 4455.

FABMS.¹⁸ The obvious similarities in the ¹H NMR spectra of compounds **4**¹⁹ and **5** were the presence of the aromatic singlet at δ_H 7.49 (H2 and H6), the methylene signals corresponding to H15–H17, and the singlet at δ_H 3.82 (H7). In contrast, the ¹H NMR spectrum of compound **4** lacked the methylenes corresponding to C10 and C11 of **5** and the aromatic signal at δ_H 6.51 (H13). From the HMBC and COSY spectra of compound **4** a chain of four methylenes, H10–H13 (δ_H 3.27, 1.56 (2CH₂) and 3.17), ending with a guanidine group (C14, δ_C 156.8) was evident. These carbon and proton resonances are consistent with those reported for other compounds that contain this fragment.¹⁴

In conclusion, we have isolated and elucidated the structure of the novel alkaloids **1** and **4**. Compound **1** contains a rare example of an amino-imidazole coupled to another aromatic substituent; a second example occurs in the histidino-tyrosine bridge of the bicyclic glycopeptidolipids aciculitins A–C.²⁰ We note that, with the exception of this example, bromo-tyrosine-derived metabolites have been limited exclusively to sponges of the order Verongida and arguably exemplify the most solid chemotaxonomic grouping among the Porifera. While a voucher specimen corresponding to the sponge from which compounds **1–5** were obtained was reidentified as an *Oceanapia* sp., it remains possible that a sample of verongid sponge was present in the actual collection. Compounds **1**, **2**, **4**, and **5** are the first examples of natural products that inhibit an enzyme central to a mycothiol-dependent detoxification pathway found in mycobacteria.

Acknowledgment. We thank Noel Whittaker and Tom Spande for mass spectrometry, John Hooper for reexamining voucher specimen Q66C-1919a, and Mary Kay Harper for helpful discussions on sponge taxonomy. G.M.N. acknowledges a Visiting Fellow Award, NIH. This work was supported in part by NSF Grant MCB-998150 (R.C.F.) and the Intramural AIDS Targeted Antiviral Program of the Office of the Director, National Institutes of Health (C.A.B.).

Supporting Information Available: ¹H and ¹³C NMR spectra and a complete table of COSY, HMBC, and ROESY correlations for compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(18) HRFABMS MH⁺ *m/z* 521.0501, calcd 521.0511; FABMS MH⁺ *m/z* 521, 523, 525 in a ratio of 1:2:1.

(19) ¹H NMR (CD₃OD) δ_H 7.49 (s, 2H, H2, 6), 4.09 (t, *J* = 5.5 Hz, H15), 3.82 (s, 1H, H7), 3.27 (m, 2H, H10), 3.25 (m, 2H, H17), 3.17 (m, 2H, H13), 1.56 (m, 4H, H11, 12); ¹³C NMR (CD₃OD) δ_C 165.7 (C9), 156.8 (C14), 151.0 (C8), 150.5 (C4), 136.5 (C1), 132.9 (C2, 6), 117.2 (C3, 5), 70.7 (C15), 41.8 (C13), 39.5 (C17), 38.9 (C10), 33.6 (C16), 28.1 (C7), 27.2 (C12), 27.1 (C11); IR (ZnSe, film) 3400, 1678, 1420 cm⁻¹.

(20) Bewley, C. A.; He, H.; Williams, D. H.; Faulkner, D. J. *J. Am. Chem. Soc.* **1996**, 118, 4314.