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Bromotyrosine-Derived Natural and Synthetic Products as Inhibitors of Mycothiol-*S*-Conjugate Amidase

Gillian M. Nicholas,[a,†] Lisa L. Eckman,[a] Satyajit Ray,[a] Robert O. Hughes,[b] Jeffrey A. Pfefferkorn,[b,c] Sofia Barluenga,[b,c] K. C. Nicolaou[b,c] and Carole A. Bewley[a,*]

[a]*Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0820, USA*

[b]*Department of Chemistry and the Skaggs Institute of Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA*

[c]*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA*

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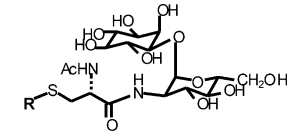
Abstract—A series of bromotyrosine-derived compounds, including marine natural products and members of a psammaplin A-inspired combinatorial synthetic library, were screened for their ability to inhibit the *Mycobacterium tuberculosis* detoxification enzyme mycothiol-*S*-conjugate amidase (MCA). Correlations between the structures and their respective IC_{50} values (which range from 3 μM to 2.7 mM) should prove valuable when optimizing more potent inhibitors of MCA.

In recent years, *Mycobacterium tuberculosis* has re-emerged as a leading cause of death by an infectious agent,[1] and the appearance of drug resistant strains continues to rise.[2] Related mycobacterial species, such as *Mycobacterium avium* and *M. avium* complex, that are otherwise non-deleterious to humans, pose serious threats to immunocompromised people.[3] Consequently, there is a continuing need for the discovery of new antituberculars with novel modes of action.

Mycothiol[4,5] (MSH, **1**, Fig. 1), the major low molecular weight thiol in actinomycetes, is important in that it appears to play an analogous role to the eukaryotic thiol glutathione in maintaining a reducing intracellular environment and facilitating detoxification from alkylating agents and other toxins.[6] Newton et al. recently characterized the MSH-dependent detoxification enzyme mycothiol-*S*-conjugate amidase (MCA).[7] With specificity for *S*-conjugates, MCA cleaves the amide bond linking cysteine and glucosamine in MSH-*S*-conjugates to form D-glu-cosamine-α(1–1)*myo*-D-inositol, which can be recycled into MSH biosynthesis,[8] and the *N*-acetyl-cysteinyl-*S*-conjugate, which can be excreted from the cell (Fig. 2).[7] Chemical *Mycobacterium smegmatis* mutants displaying decreased production of MSH have been shown to exhibit increased susceptibility to some antituberculars.[9] Since MCA is unique to actinomycetes and shares no sequence homology to other known eukaryotic enzymes, it represents a novel target for new classes of antimycobacterials or co-drugs, and we have been engaged in the identification of natural product inhibitors of MCA.

Earlier, we reported a series of novel and known bromotyrosine-derived marine natural products that inhibit MCA with IC_{50} values ranging from ~1 to 100 μM.[10] A variety of biological activities have been ascribed to bromotyrosine-derived natural products, including broad spectrum antibacterial activities,[11] moderate cytotoxicity toward several cancer cell lines,[11] and



MSH, **1** R = H

MSmB, **2** R =

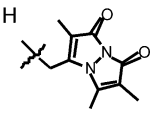


Figure 1. Mycothiol (MSH, **1**) and mycothiol bimane (MSmB, **2**).

*Corresponding author. Tel.: +1-301-594-5187; fax: +1-301-402-0008; e-mail: cb194k@nih.gov

†Current address: Hauser, CRO, Denver, CO 80221, USA.

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inhibition of many unrelated enzymes.[10,11c,12] Consequently, a number of syntheses of bromotyrosine-derived natural products have been completed.[13] Most recently, Nicolaou and co-workers constructed a combinatorial library of asymmetric disulfide-containing compounds[14] inspired by the disulfide-containing homodimer psammaplin A (**9**).[15] To further elucidate the structural requirements for inhibition of MCA, we screened members of this synthetic library alongside various related natural product inhibitors and report the efficacy of each in this paper.

Fluorescence detected enzyme inhibition assays were conducted as described previously[7] using recombinant MCA[4] (obtained from a protein G-MCA construct encoding MCA from *M. tuberculosis*) and synthetic mycothiol bimane (MSmB, **2**, Fig. 1).[4] Dose–response curves for the natural products and synthetic compounds were obtained by measuring the extent of mycothiol bimane cleavage in the presence of increasing concentrations of the natural products **3**–**8** and synthetic compounds **10**–**16** (Fig. 3). Typical of the bromotyrosine-derived natural products, these compounds feature either a spirocyclic isoxazoline core, as in compounds **3**–**6**, or the reduced, 'ring-opened' bromophenyl oximinoamide functionality, which is present in the natural products **7** and **8**. Similar to **7** and **8**, the synthetic suite of compounds preserves the oximinoamide functionality, but possesses a multi-substituted phenyl ring linked through a disulfide bond.

Non-linear least squares fitting of the inhibition curves for cleavage of MSmB by MCA yielded IC_{50} values shown in Table 1. Somewhat surprisingly, these values ranged from 3 μM for compound **3** to 2.7 mM for compound **13**. From these data, several generalizations can be made concerning inhibitor structure and corresponding activity. First, the presence of the intact spirocyclic oxazoline ring system does not appear to be essential to achieve good inhibition of MCA. Although compound **3**, the most effective inhibitor, maintains this spirocyclic system, the next most potent inhibitor, compound **8**, contains instead the reduced bromophenyl oximinoamide moiety. The natural products psammaplysins A (**5**) and B (**6**) yield similar IC_{50} values (20–30 μM) as the synthetic compounds **15** and **16** (~35 μM), which together account for the next-most effective set of MCA inhibitors. These values support the notion that the spirocyclic ring system is dispensable, but suggest that the spiro[4.5]decatriene system may be more effective than the spiro[4.6]undecatriene structure present in **5** and **6**.

Due to the large range of IC_{50} values measured for the synthetic compounds used in this study, the structure–activity relationships of these inhibitors provide valuable information. The synthetic compounds differ by the substituents of the 'left' and 'right' phenyl rings (to the

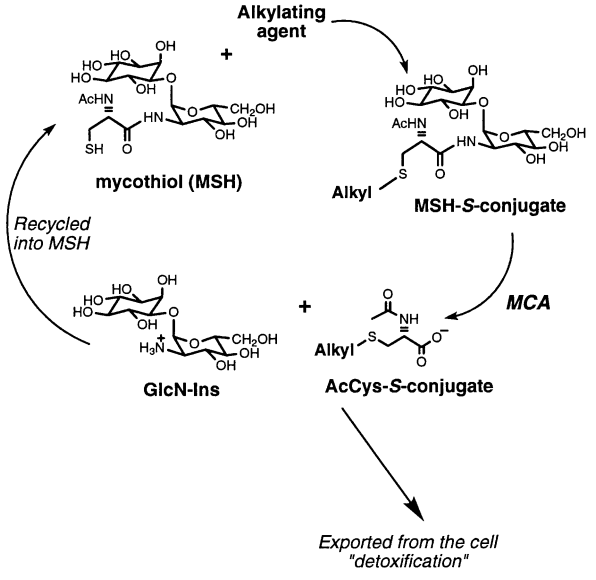
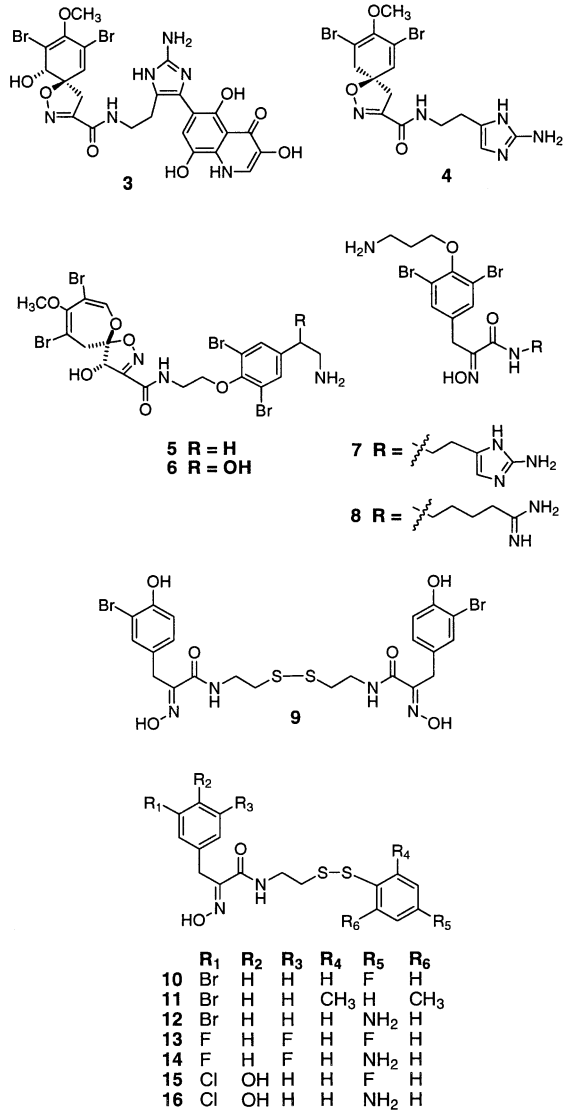


Figure 2. The role of mycothiol-*S*-conjugate amidase (MCA) in mycothiol-dependent detoxification.



	R_1	R_2	R_3	R_4	R_5	R_6
10	Br	H	H	H	F	H
11	Br	H	H	CH_3	H	CH_3
12	Br	H	H	H	NH_2	H
13	F	H	F	H	F	H
14	F	H	F	H	NH_2	H
15	Cl	OH	H	H	F	H
16	Cl	OH	H	H	NH_2	H

Figure 3. Bromotyrosine-derived natural products and members of a synthetic library. The absolute stereochemistry is known for all compounds except psammaplysins A and B[17] (**5** and **6**) for which relative stereochemistry was determined by NMR (G. M. Nicholas and C. A. Bewley, unpublished data.).

Table 1. Inhibition of mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis* by synthetic and natural bromotyrosine-derived compounds[a]

Compd	Name[b]	IC_{50}[c] (μM)
Marine natural products		
3	Nicholas et al.[10]	2.0±0.2
4	Pseudoceratine[16]	100±21
5	Psammaplysin A[17]	20±11
6	Psammaplysin B[17]	26±12
7	Litaudon and Guyot[18]	36±3
8	Nicholas et al.[10]	2.8±0.5
9	Psammaplin A[15]	2.8±0.5
Synthetic library		
10	Nicolaou et al.[14a,b]	90±4
11	Nicolaou et al.[14a,b]	65±18
12	Nicolaou et al.[14a,b]	185±62
13	Nicolaou et al.[14a,b]	2720±640
14	Nicolaou et al.[14a,b]	450±173
15	Nicolaou et al.[14a,b]	37±10
16	Nicolaou et al.[14a,b]	35±11

[a]Fluorescence-detected assays were carried out as described in ref 7 using 20 nM MCA and 30 μM synthetic mycothiol bimane[4] (**2**) at 31 °C for 20 min. The rates observed under these conditions are well within the initial linear velocity of the enzyme.
[b]Common name or first author if not named.
[c]Data were fit by non-linear least squares optimization to the equation Cleavage = $S/(1+[I]/IC_{50})$ where S is the extent of MSmB (**2**) cleavage in the absence of inhibitor.

carboxy and amino sides of the central amide, respectively): compounds **10**–**12** all possess a 3-bromophenyl ring on the left, with varying substituents on the right, and they display IC_{50} values in the range of 60–200 μM. Compounds **13** and **14** are very poor inhibitors and, unlike any of the other natural or synthetic compounds, feature a 3,5-di-fluorophenyl ring on the left. Compounds **13** and **14** differ from one another in the respective presence of a 4-fluoro- or 4-amino-phenyl ring on the right. Compounds **15** and **16** are the most potent of the synthetic inhibitors used in this study, and have in common a 3-chloro-4-hydroxy-phenyl ring on the left. As with **13** and **14**, they differ by the presence of a 4-fluoro- or 4-amino-phenyl ring on the right. Thus, in the context of the psammaplin-like compounds, several conclusions can be drawn. First, the substituents on the left phenyl ring are strong determinants of inhibitor potency. Replacement of the 3-bromo substituent with 3,5-difluoro groups nearly abolishes activity. Second, the presence of a single 3-bromo substituent is neutral. Third, the presence of the 3-chloro-4-hydroxy-phenyl ring yields the most potent inhibitors. Many of the bromophenyl oximinoamide-containing natural products possess an amino-propyl-ether on C4 of the left phenyl ring. Thus, it appears that the amino-propyl ether is not essential for activity, but an oxy-substituent on C4 lends to good inhibitory activity.

Concerning the right side of the molecules, other than our being certain that the presence of a disulfide bond is not necessary to achieve low micromolar inhibition of MCA, further structure–activity relationships are vague. Among the synthetic compounds, no trend in activity is observed among the three different patterns of substitution on the benzene ring. Similarly, for the natural products, a variety of substituents are present on the amino side of the central amide, including guanidinium and imidazole groups, a quinolinone-substituted imidazole, and a 2,5-dibromo-4-ethyl-aminobenzyl ether group. Very general commonalities include the presence of an aromatic group and/or a positively charged substituent on the right. In this set of structures, between two and five bonds separate these substituents from the amide nitrogen.

When comparing the structures of these inhibitors with that of MSH (**1**), the obvious common element is the presence of a central amide group. Given that MCA cleaves hydrophobic *S*-conjugates of mycothiol with the greatest efficiency,[7] the presence of hydrophobic, aromatic moieties to the left of the amide bond is consistent with the structure of mycothiol-*S*-conjugates. However, in the absence of a crystal or solution structure of the active site of MCA, to compare the various structures on the amino side of the central amide to the pseudodissacharide portion of MSH is difficult. Nicolaou et al. demonstrated potent antibacterial activity for compounds **10**–**16** against methicillin-resistant *Staphylococcus aureus*. In the context of *S. aureus*, these inhibitors must be affecting a different protein than MCA since *S. aureus* does not produce mycothiol. Nonetheless, the surprisingly large variations in the inhibition of MCA observed among this suite of compounds should prove valuable in the optimization of more potent inhibitors of MCA.

Acknowledgements

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