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Repurposing Cryptosporidium Inosine 5′-Monophosphate Dehydrogenase Inhibitors as Potential Antibacterial Agents

Kavitha Mandapati,[†] Suresh Kumar Gorla,[†] Amanda L. House,[§] Elizabeth S. McKenney,[§] Minjia Zhang,[†] Suraj Nagendra Rao,[†] Deviprasad R. Gollapalli,[†] Barbara J. Mann,[§,¥] Joanna B. Goldberg,[§,⊥] Gregory D. Cuny,[∥] Ian J. Glomski,[§] and Lizbeth Hedstrom*[,†,‡]

[†]Departments of Biology and [‡]Chemistry, Brandeis University, 415 South Street, Waltham, Massachusetts 02454, United States

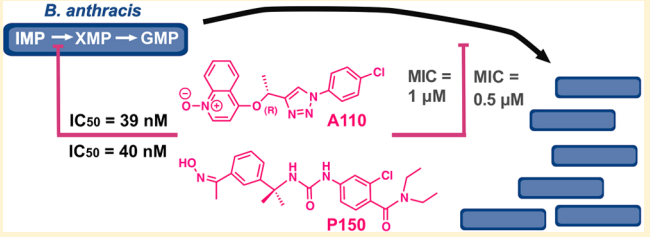
[§]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, Virginia 22908, United States

[∥]Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, 549A Science and Research Building 2, Houston, Texas 77204, United States

[¥]Department of Medicine, Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia 22908, United States

Supporting Information

ABSTRACT: Inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the pivotal step in guanine nucleotide biosynthesis. IMPDH is a target for immunosuppressive, antiviral, and anticancer drugs, but, as of yet, has not been exploited for antimicrobial therapy. We have previously reported potent inhibitors of IMPDH from the protozoan parasite *Cryptosporidium parvum* (*Cp*IMPDH). Many pathogenic bacteria, including *Bacillus anthracis*, *Staphylococcus aureus*, and *Listeria monocytogenes*, contain IMPDHs that should also be inhibited by these compounds. Herein, we present the structure−activity relationships for the inhibition of *B. anthracis* IMPDH (*Ba*IMPDH) and antibacterial activity of 140 compounds from five structurally distinct compound series. Many potent inhibitors of *Ba*IMPDH were identified (78% with $IC_{50} \leq 1$ μM). Four compounds had minimum inhibitory concentrations (MIC) of less than 2 μM against *B. anthracis* Sterne 770. These compounds also displayed antibacterial activity against *S. aureus* and *L. monocytogenes*.



KEYWORDS: *IMP dehydrogenase, antibiotic, antibacterial, Gram-positive, inhibitor*

The emergence of drug-resistant bacteria has severely compromised the arsenal of antibiotic drugs.[1] New compounds and targets are needed to meet the growing threat from drug-resistant strains of *Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium tuberculosis, Neisseria gonorrheae, Enterobacteriaceae, Enterococcus, Salmonella, Shigella*, and *Campylobacter*.[2] New drugs are also needed to treat infections of naturally drug resistant bacteria such as *Acinetobacter baumanni* and *Pseudomonas aeruginosa*. The possibility that *Bacillus anthracis* and other bacteria have been perniciously engineered as biowarfare agents creates another demand for new treatment options.

Inosine 5′-monophosphate dehydrogenase (IMPDH) is an attractive target for the development of new antibiotics.[3−6] This enzyme catalyzes the rate determining penultimate step in guanine nucleotide biosynthesis. We continue to develop inhibitors against IMPDH from the protozoan parasite *Cryptosporidium* (*Cp*IMPDH)[7−14] and recently reported compounds that are efficacious in a mouse model of infection.[14] Curiously, the *Cp*IMPDH gene was obtained from an epsilon proteobacteria by lateral gene transfer.[15] Consequently, many bacterial IMPDHs are structurally similar to *Cp*IMPDH and likely to be inhibited by the same compounds.[16] X-ray crystal structures of *Cp*IMPDH inhibitors with parasite and bacterial IMPDHs have defined the structural determinants of inhibition (Figure 1A).[10,14] The key residues Ala165 and Tyr358 (*Cp*IMPDH numbering) are conserved in many bacterial IMPDHs, including those found in both Gram-negative and Gram-positive pathogens.[16] Here, we report the structure−activity relationships (SARs) for enzyme inhibition and antibacterial activity of five structurally distinct inhibitor series against the representative Gram-positive bacteria *B. anthracis*. The best compounds also displayed antibacterial activity against two other Gram-positive organisms, *S. aureus* and *Listeria moncyotogenes*.

The *Cp*IMPDH inhibitors typically consist of two aromatic groups separated by a linker (Figure 1B). The structures of two *Cp*IMPDH·IMP·inhibitor complexes suggest that one aromatic group forms a π-stacking interaction with the purine base of IMP, while the other interacts with Tyr358′ from the adjacent subunit (*Cp*IMPDH numbering, ′ denotes residue from neighboring subunit) and the linker bends around Ala165 (Figure 1A).[10,14] These interactions are likely to be major determinants of inhibitor

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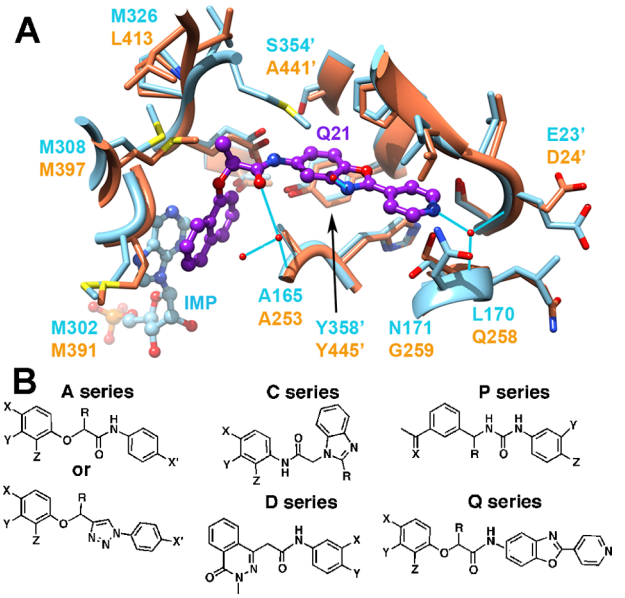


Figure 1. Structures of *Cp*IMPDH and *Ba*IMPDH and their inhibitors. (A) E·IMP·**Q21** complex of *Cp*IMPDH (PDB 4IXH, chains C and D), blue. **Q21**, purple. E·P_i complex of *Ba*IMPDH (PDB 3TSB, A chain), orange. Residues within 5 Å of **Q21** are displayed. Residues from the neighboring subunit are denoted with′. The key determinants of selectivity are Tyr358′/445′ and Ala165/253 (*Cp*IMPDH/*Ba*IMPDH numbering). (B) Representative general structures of *Cp*IMPDH inhibitors.

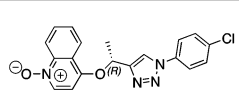
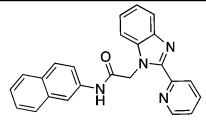
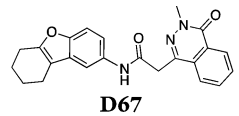
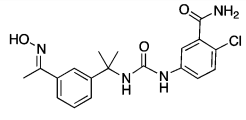
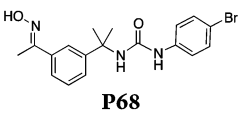
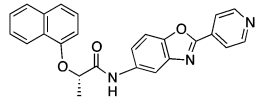
Table 1. Selected Inhibitors of *Ba*IMPDH[a]

	IC_{50} (nM)	
compd	*Cp*IMPDH	*Ba*IMPDH
A50	1100 ± 100[b]	150 ± 30
A52	>5000[b]	760 ± 60
A66	>5000[b]	2400 ± 40
A69	700 ± 100[b]	120 ± 20
A72	>5000[b]	160 ± 60
P47	190 ± 10[c]	95 ± 30
P68	9 ± 0.4[c]	4 ± 1
P77	>5000[c]	1200 ± 300
P82	1.0 ± 0.1	2.0 ± 0.5
P94	330 ± 70[c]	100 ± 20
P106	4 ± 1[c]	8 ± 2
Q36	1.2 ± 0.2[d]	10 ± 1
Q43	>5000[d]	300 ± 100
Q48	>5000[d]	900 ± 200
Q52	49 ± 9[d]	14 ± 2
Q59	0.6 ± 0.5[d]	10 ± 3
Q67	0.5 ± 0.1[d]	5 ± 1

[a]The values of IC_{50} for potent (IC_{50} ≤ 10 nM) or selective (*Ba*IMPDH/*Cp*IMPDH ≤ 0.5) inhibitors are shown. Molecular structures are depicted in Figure 2. Table S1, Supporting Information includes the values of IC_{50} for all 140 compounds. [b]Data from ref 8. [c]Data from ref 12. [d]Data from ref 14.

binding. In the **Q21** complex, the methyl group interacts with Met308 and Met326, while the amide carbonyl oxygen and the pyridine nitrogen form water-mediated hydrogen bonds with the main chain (Figure 1). *B. anthracis* IMPDH (*Ba*IMPDH) contains the Ala/Tyr′ motif, suggesting that it could be sensitive to *Cp*IMPDH inhibitors. However, several substitutions are found in the inhibitor binding site, including Leu413 for Met326, Gly259 for Asn171, and Ala441 for Ser354 (Figure 1A; note that unlike

Table 2. Mechanism of *Ba*IMPDH Inhibition[a]

Cmpd	S	*Ba*IMPDH		*Cp*IMPDH	
		Mech	K_i (nM)	Mech	Ki (nM)
A110	IMP	NC	48 ± 8	UC	12 ± 4
	NAD^+	NC	58 ± 4	C[b]	5 ± 1
C91	IMP	NC	38 ± 3	UC[b]	5 ± 3
	NAD^+	NC	44 ± 5	NC	14 ± 2
D67	IMP	NC	820 ± 80	UC	13 ± 2
	NAD^+	NC	500 ± 60	NC	49 ± 3
P32	IMP	NC[b]	12 ± 2	UC[b]	5 ± 1
	NAD^+	NC[b]	8 ± 2	NC[b]	6 ± 1
P68	IMP	NC[b]	1.5 ± 0.3	UC[b]	2.0 ± 0.6
	NAD^+	NC[b]	2.3 ± 0.3	NC	15 ± 2
Q21	IMP	NC	24 ± 3	UC	64 ± 12
	NAD^+	NC	17 ± 2	C	13 ± 4
 **A110**				 **C91**	
 **D67**				 **P32**	
 **P68**				 **Q21**	

[a]C, competitive inhibition; UC, uncompetitive inhibition; NC, non-competitive inhibition. [b]Data analyzed using the Morrison tight binding equations.[18]

most IMPDHs, *Cp*IMPDH lacks the cystathionine beta synthetase (CBS) subdomain, which accounts for the large difference in sequence numbering). In addition, Met302, Met308, and Met326 are in mobile structural elements, so the affinities of *Cp*IMPDH inhibitors for *Ba*IMPDH are difficult to predict.

We examined 140 compounds from five structurally distinct classes of *Cp*IMPDH inhibitors for inhibition of *Ba*IMPDH and antibacterial activity against *B. anthracis*, including 22 amides and triazoles from the **A** series,[8,9] 6 benzimidazoles from the **C** series,[10,11] 13 phthalazinones from the **D** series,[13] 55 ureas from the **P** series,[14] and 44 benzoxazoles from the **Q** series[14] (Figure 2 and Table S1, see Supporting Information). The **P** and **Q** series are the most successful in our *Cp*IMPDH program, accounting for the greater number of representative derivatives.[14] Ten compounds have not been reported previously, including 1 in the **A** series, 3 in the **D** series, 4 in the **P** series, and 2 in the **Q** series. Not surprisingly given that the compounds were optimized for potency against *Cp*IMPDH, most compounds were significantly poorer inhibitors of *Ba*IMPDH. Nonetheless, many compounds (78%) displayed values of IC_{50} less than 1 μM, and approximately 47% displayed values of IC_{50} less than 100 nM. Seven compounds had values of IC_{50} less than or equal to 10 nM (Figure 2).

The SARs for enzyme inhibition displayed distinct differences from that of *Cp*IMPDH in all five series (Figure 2). Twelve compounds were more potent inhibitors of *Ba*IMPDH

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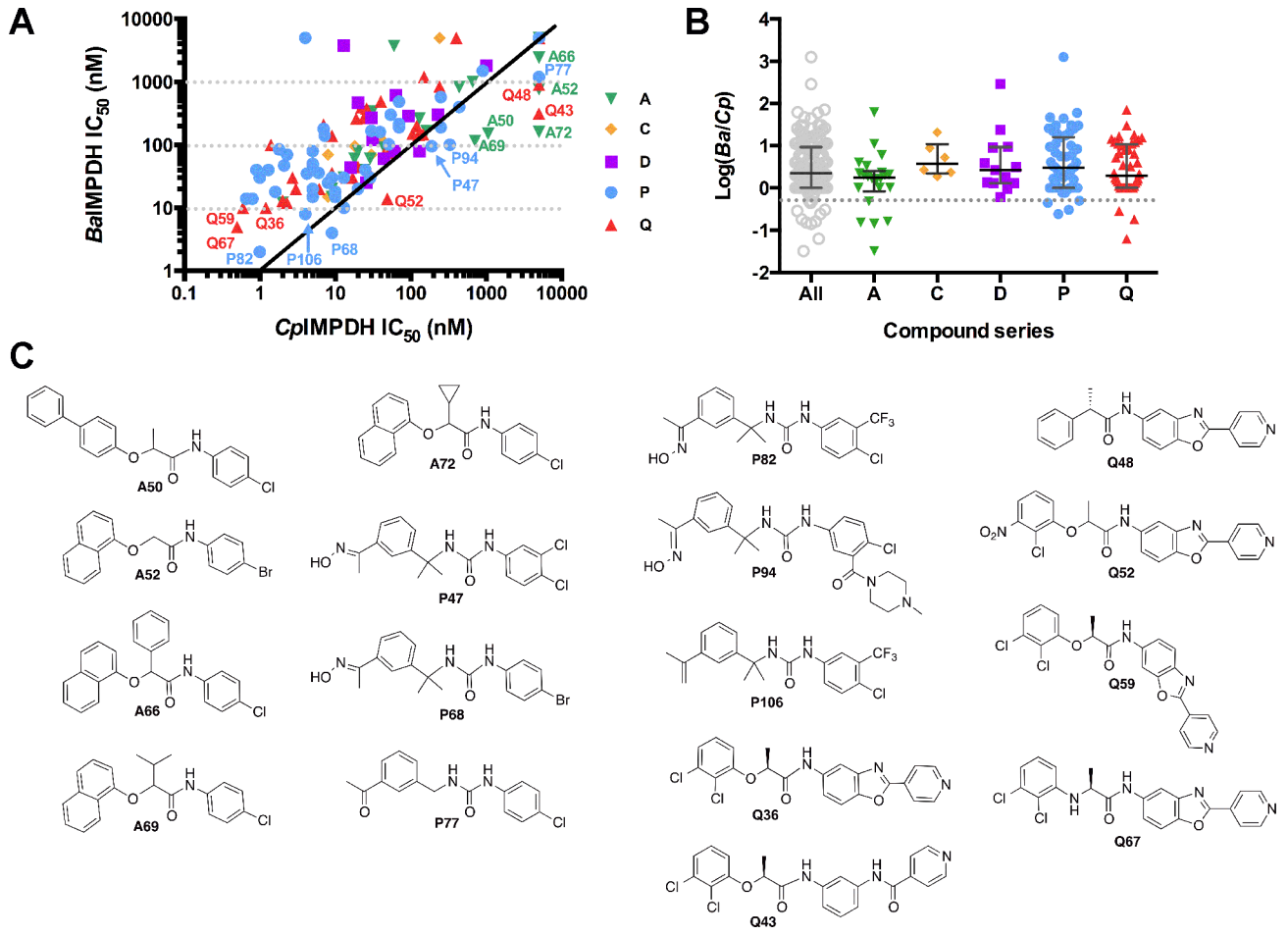

Figure 2. Effects of *Cp*IMPDH inhibitors on *Ba*IMPDH. (A) Comparison of the values of IC_{50} for *Cp*IMPDH and *Ba*IMPDH. **A** series, green triangles (pointed down); **C** series, orange diamonds; **D** series, purple squares; **P** series, blue circles; **Q** series, red triangles (pointed up). The black line denotes equivalent values of IC_{50} for both enzymes. The gray lines demarcate IC_{50} = 10 nM, 100 nM and 1 μM for *Ba*IMPDH. Compounds that display $IC_{50} \leq 10$ nM or a preference for *Ba*IMPDH are labeled. Tables S2−S14, Supporting Information, contain all structures and IC_{50} values. (B) Variation in the selectivity of *Cp*IMPDH inhibitors. The log of the ratio of the values of IC_{50} for *Ba*IMPDH and *Cp*IMPDH are shown. The median and quartiles are marked. The dotted gray line denotes the ratio = 0.5, i.e., a 2-fold preference for *Ba*IMPDH. (C) Structures of the compounds that display $IC_{50} \leq 10$ nM or a preference for *Ba*IMPDH.

than *Cp*IMPDH (Figure 2C and Table 1), including five from the **A** series, four from the **P** series, and three from the **Q** series. No compounds from the **C** and **D** series were found to be more potent for *Ba*IMPDH, though this likely reflects the small number of these compounds tested. In contrast, the **A** series appears to be over-represented in the set of compounds with a preference for *Ba*IMPDH, possibly suggesting that this framework is more amenable to the development of broad spectrum prokaryotic IMPDH inhibitors. Six of these compounds contain substitutions in the linker region that are not well tolerated in *Cp*IMPDH, including phenyl (**A66**), isopropyl (**A69**), cyclopropyl (**A72**), epimeric stereochemistry (**Q48**, compared to **Q36**, **Q43**, **Q59**, and **Q67**), and non-α-substituted amides and ureas (**A52** and **P77**). This SAR may reflect the substitution of Leu413 for Met326 in this region of *Ba*IMPDH (Figure 1A). Several compounds, e.g., **A50**, **P68**, **P94**, and **Q43**, contain larger substitutions on one aromatic ring that may reflect the additional space created by the substitution of Gly259 for Asn171 (Figure 2C).

The *Cp*IMPDH inhibitors bind in the NAD^+ site and can have uncompetitive or noncompetitive mechanisms with respect to IMP depending on their relative affinities for the E, E·IMP, and E-XMP* complexes. Similarly, the inhibitors can have competitive, uncompetitive, or noncompetitive mechanisms with respect to NAD^+. We determined the mechanism of representative compounds in each series, **A110**, **C91**, **D67**, **P32**, **P68**, and **Q21**, for both *Ba*IMPDH and *Cp*IMPDH (Table 2). With respect to IMP, all of the compounds are noncompetitive inhibitors of *Ba*IMPDH but uncompetitive inhibitors of

Table 3. Antibacterial Activity of *Cp*IMPDH Inhibitors[a]

		MIC (μM)						
		Ba		*Sa #1*		*Sa #2*		*Lm*
compd	*Ba* IC_{50} (nM)	−	+	−	+	−	+	−
A98	15 ± 4	1	8	1.8	>30	2	16	7.5
A110	30 ± 3	2	>30	7.5	>30	8	>30	3.8
P146	170 ± 10	1	8	3.8	7.5	2	>30	>30
P150	40 ± 20	0.5	>30	0.9	>30	4	>30	15

[a]Compounds with values of MIC ≤ 2 μM versus *B. anthracis* Sterne 7702 are shown. Structures can be found in Figure 3 and in the Supporting Information. *Ba*, *B. anthracis* Sterne 7702; *Sa #1*, *S. aureus* NCTC 8325; *Sa #2*, *S. aureus* ATCC 13709 (Smith); *Lm*, *L. monocytogenes* 10403S. *B. anthracis* and *S. aureus* were cultured in RPMI1640 medium in the presence (+) and absence (−) of 0.01% guanine. *L. monocytogenes* were cultured in Mueller−Hinton medium.

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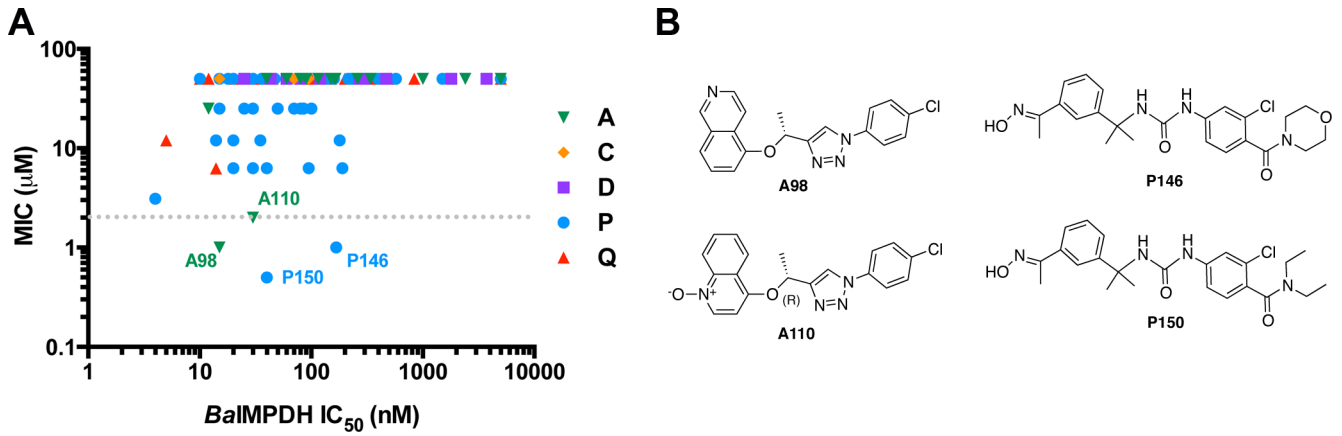

Figure 3. Antibacterial activity of *Cp*IMPDH inhibitors. (A) Plot of minimum inhibitory concentration for *B. anthracis* growth versus the value of IC_{50} for *Ba*IMPDH for 106 *Cp*IMPDH inhibitors. *B. anthracis* Sterne 7702 was cultured in RPMI 1640, which does not contain purines. **A** series, green triangles (pointed down); **C** series, orange diamonds; **D** series, purple squares; **P** series, blue circles; **Q** series, red triangles (pointed up). The gray dotted line denotes MIC = 2 μM. Compounds with values of MIC $\leq$ 2 μM are labeled. Table S15, Supporting Information, contains all MIC values. (B) Structures of compounds with values of MIC $\leq$ 2 μM.

*Cp*IMPDH. With respect to NAD^+, all of compounds are noncompetitive inhibitors of both *Ba*IMPDH and *Cp*IMPDH, with the exception of **A110** and **Q21**, which were competitive inhibitors of *Cp*IMPDH. These differences in inhibitory mechanism were unanticipated and suggest that significant differences exist in the catalytic cycle of *Ba*IMPDH and *Cp*IMPDH.

The antibacterial activity of 106 *Cp*IMPDH inhibitors was assessed by monitoring the growth of *B. anthracis* Sterne 7702 in RPMI 1640, a defined medium that lacks purines. No antibacterial activity was observed for compounds with IC_{50} > 300 nM (Figure 3). Sixteen compounds displayed MICs less than or equal to 12 μM. The active compounds are significantly less hydrophobic (average cLogP = 3.5 $\pm$ 0.5) and have larger topological polar surface areas (average tPSA = 83 $\pm$ 20 $Å^2$) than the set of *Cp*IMPDH inhibitors as a whole (average cLogP = 4.5 $\pm$ 1.1 and average tPSA = 66 $\pm$ 19 $Å^2$). The tPSA values of the active compounds are similar to those of fluoroquinolone antibiotics (average tPSA = 82 Å), though the cLogP values are higher (average cLogP = 1.3).[17] Averaged over all antibacterials, the values of tPSA are 243 and 165 $Å^2$ for Gram-positive and Gram-negative bacteria, respectively, while the values of cLogP are 2.1 and −0.1, respectively.[17] These observations suggest that the antibacterial activity can be improved with further optimization of hydrophobicity.

Four compounds, **A98**, **A110**, **P146**, and **P150**, had values of MIC less than or equal to 2 μM (Figure 3 and Table 3). The values of MIC increased at least 8-fold in the presence of guanine, suggesting that these antibacterial activities resulted from the on-target inhibition of IMPDH. These four compounds also displayed antibacterial activity against *S. aureus*. However, only **A98** and **A110** displayed potent antibacterial activity against *L. monocytogenes*, further suggesting that the **A** scaffold may be more amenable for the development of IMPDH inhibitors ith broad spectrum antibacterial activity.

In conclusion, we have initiated a program to repurpose *Cp*IMPDH inhibitors for antibiotic discovery. Many of these compounds were potent inhibitors of *Ba*IMPDH, though few displayed antibacterial activity, as expected given the very different uptake processes that operate in *C. parvum* and bacteria. Nonetheless, three compounds were identified that displayed antibacterial activity against three Gram-positive pathogens, *B. anthracis*, *S. aureus*, and *L. monocytogenes*. These findings demonstrate the promise of IMPDH as an antibiotic target. Further optimization of these compounds to increase potency and uptake into bacteria is ongoing.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, compound data for **A119**, **D85**, **D87**, **D89**, **P67**, **P68**, **P146**, **P150**, **Q81**, and **Q82**, compound structures, and tables of IC_{50} and MIC values. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(L.H.) E-mail: hedstrom@brandeis.edu. Tel: 781-736-2333. Fax: 781-736-2349.

Present Address

⊥(J.B.G.) Department of Pediatrics, Emory University School of Medicine, 1510 Clifton Rd, NE, Suite 3009, Atlanta, GA 30322

Author Contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

Biography

Lizbeth Hedstrom received her Ph.D. in Biochemistry from Brandeis University, MA. After postdoctoral training at UCSF, she returned to Brandeis as an assistant professor and is currently Professor of Biology and Chemistry. The Hedstrom laboratory studies structure/function relationships in proteases and enzymes involved in nucleotide metabolism. Current projects address the development of IMPDH-targeted antibiotic and the structural basis of reaction specificity in the IMPDH/GMPR family. Her laboratory is also developing small molecules strategies to induce selective protein degradation. She is a Searle Scholar (1993), Beckman Young Investigator (1995), and AAAS Fellow (2010).

■ ABBREVIATIONS

IMP, inosine 5′-monophosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; IMPDH, inosine monophosphate dehydrogenase; *Cp*IMPDH, IMPDH from *Cryptosporidium parvum*; *Ba*IMPDH, IMPDH from *Bacillus anthracis*

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