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Antibacterial Strategy against *H. pylori*: Inhibition of the Radical SAM Enzyme MqnE in Menaquinone Biosynthesis

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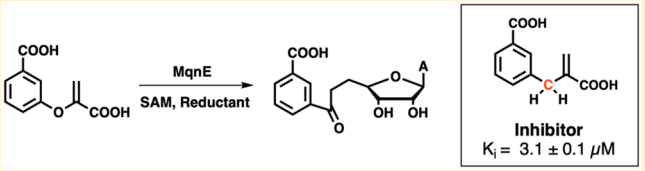
Supporting Information

ABSTRACT: Aminofutalosine synthase (MqnE) catalyzes an important rearrangement reaction in menaquinone biosynthesis by the futalosine pathway. In this Letter, we report the identification of previously unreported inhibitors of MqnE using a mechanism-guided approach. The best inhibitor shows efficient inhibitory activity against *H. pylori* (IC_{50} = 1.8 ± 0.4 μM) and identifies MqnE as a promising target for antibiotic development.



KEYWORDS: *Radical SAM enzyme, MqnE, bisubstrate inhibitor, Helicobacter pylori, antibiotic*

Menaquinone is a lipid-soluble, redox-active cofactor involved in the transmembrane electron transport chain of the majority of microbes.[1] Humans use menaquinone (vitamin K) as an essential blood clotting vitamin[2−4] and acquire it from dietary sources and from its biosynthesis in the gut microbiome.[5] Menaquinone biosynthesis is therefore an attractive target for antibiotic development,[6] and inhibitors against Gram-positive organisms such as *Mycobacterium tuberculosis* and *Staphylococcus aureus* have been identified.[7] The recent discovery of a new, futalosine-dependent, menaquinone biosynthesis pathway has presented new opportunities for antibacterial development[8,9] because important human pathogens including *Helicobacter pylori* (causes gastric ulcers and cancer), *Campylobacter jejuni* (causes diarrhea), *Chlamydia* strains (cause urethritis and respiratory tract infections), and *Spirochetes* (cause syphilis and Lyme disease) utilize this pathway.[10] The absence of this pathway in humans and in most of the human gut bacteria potentially provides the required selectivity for targeting this pathway without affecting the commensal bacteria. Potent, transition-state analog inhibitors against the 5′-methylthioadenosine nucleosidase (MTAN) from *H. pylori*[11−13] and *C. jejuni*[14] have been developed, and long chain fatty acids and macrolides targeting the later steps of the pathway have been reported.[15−19] The antibiotic potential of the other enzymes on the futalosine pathway, including the two radical SAM enzymes MqnE and MqnC, has not been explored. In this Letter, we report the identification of a mechanism-based inhibitor of MqnE and demonstrate its antibacterial activity against *H. pylori* and *C. jejuni*.

MqnE is a radical SAM enzyme[20,21] in the futalosine-dependent menaquinone biosynthesis pathway that catalyzes a key C−C bond formation.[22] We have previously reported mechanistic studies on this enzyme with successful trapping of the captodative radical **3** and the aryl radical anion **7** (Figure 1).[23,24]

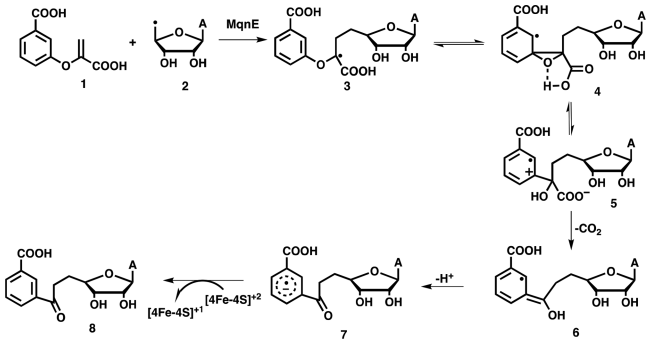


Figure 1. Mechanistic proposal for the MqnE-catalyzed conversion of **1** to **8**.

High throughput screening for inhibitors of radical SAM enzymes is technically demanding because these enzymes are extremely oxygen sensitive and have low turnover. We therefore undertook a mechanism-guided approach for the development of an inhibitor of MqnE. The captodative radical intermediate **3** is expected to be the most stable radical intermediate in the conversion of **1** to **8**. We therefore anticipated that a structural analog of this intermediate might act as a substrate or transition state mimic and form a bi-substrate inhibitor of MqnE. A bi-substrate inhibitor is a molecule that is chemically synthesized or enzymatically

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generated by covalent linking of two substrates of a bi-substrate enzyme reaction and mimics the ternary enzyme substrate complex.[25] This inhibitor design strategy has been demonstrated to be effective in achieving enhanced potency and selectivity and has led to the development of FDA approved therapeutics such as finasteride, mupirocin, and isoniazid.[25]

We hypothesized that replacing the bridging oxygen of the native substrate **1** with a methylene group (compound **9**) would block the conversion of **11** to **13**/**14** due to the instability of a primary carbanion (or radical). This would allow the accumulation of **10**, which, after hydrogen atom abstraction (or electron/proton transfer), would result in the formation of the shunt product **12**, a potential bi-substrate inhibitor (Figure 2).

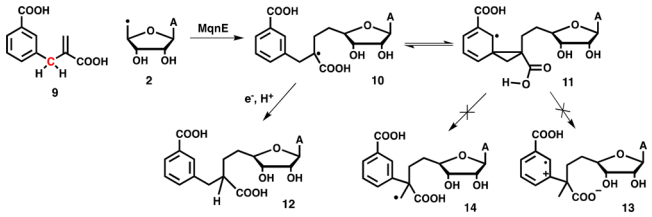

Figure 2. Mechanistic proposal for the MqnE reaction with **9**.

The methylene analog **9** was synthesized as shown in Figure S1[26,27] and tested with the *Thermus thermophilus* ortholog of MqnE. HPLC analysis of the reaction mixture indicated the formation of one major product that was absent in the controls (Figure S2). This product had a molecular ion m/z of 456 Da consistent with the mass of the shunt product **12** (Figure S3). This structure was confirmed using MS fragmentation and NMR analysis (Figures S4–S9). On running the reaction in 95% D_2O buffer, this peak showed one deuterium incorporation implying that the added proton in **12** originated from solvent or a solvent exchangeable protein residue (Figure S3).

The *T. thermophilus* MqnE enzyme catalyzed >25 turnovers under our *in vitro* conditions with the native substrate (Figure S10). The MqnE reaction was slow with the methylene analog **9**, providing a single turnover (Figure S10). Encouraged by this result, we used competitive inhibition experiments in which MqnE-$[4Fe\text{-}4S]^{2+}$ was preincubated with variable concentrations of the methylene analog **9** in the presence of excess SAM and substrate **1**. Reactions were then initiated by reducing the enzyme with Ti(III) citrate, and the rate of aminofutalosine **8** formation was followed by a discontinuous HPLC analysis. The normalized relative initial reaction rates were plotted as a function of inhibitor concentration to generate a dose–response curve, and an IC_{50} value of 38.7 ± 3.4 μM was obtained (Figure S11). Since this IC_{50} value was within 5-fold of the enzyme concentration used, the dose–response curve data was fitted to the Morrison equation for tight-binding inhibition,[28] which gave an inhibition constant K_i of 3.1 ± 0.1 μM (Figure 3). Irreversible inhibition was eliminated by demonstrating full restoration of enzyme activity after the enzyme was preincubated with **9** for 1 h, followed by removal of the inhibitor by gel filtration (Figure S12).

The bi-substrate analog **12** was enzymatically synthesized and also tested as a competitive inhibitor. This compound was a weaker inhibitor of MqnE with an IC_{50} value of 839 ± 187 μM (Figure S13). This suggests that the enzyme undergoes a major conformational change after the formation of **10** resulting in reduced affinity of the enzyme for **12** and avoiding product inhibition by **8**.

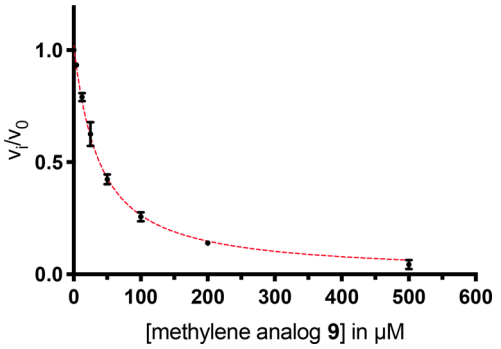

Figure 3. Inhibition kinetics with the methylene analog **9**.

The human pathogens *H. pylori* and *C. jejuni* were selected to test the antibiotic activity of the methylene analog **9** and the bi-substrate analog **12**. The effect of these inhibitors on *C. jejuni* and *H. pylori* growth was measured using the 96-well plate liquid culture method.[29−31] As shown in Table 1, the IC_{50} values for the methylene analog **9** and the bi-substrate analog **12** on *C. jejuni* were 13.6 ± 1.5 and 83.3 ± 3.4 μM, respectively. Gentamicin was used as a control and had an IC_{50} value of 1.9 ± 0.2 μM (Table 1). The measured IC_{50} values for methylene analog **9** and bi-substrate analog **12** on *H. pylori* were 1.8 ± 0.4 and 16.1 ± 3.9 μM, respectively. BTDIA, a transition state analog of the *H. pylori* MTAN (Figure S14),[12] was tested as a control and displayed IC_{50} values of 0.012 ± 0.001 and 1.4 ± 0.3 μM for *H. pylori* and *C. jejuni*,[14] respectively (Table 1).

Table 1. IC_{50} Values for the Inhibitors Tested against *H. pylori* and *C. jejuni*

	IC_{50} (μM)			
	12	**9**	gentamicin	BTDIA
C. jejuni	83.3 ± 3.4	13.6 ± 1.5	1.9 ± 0.2	1.4 ± 0.3
H. pylori	16.1 ± 3.9	1.8 ± 0.4	0.26[a]	0.012 ± 0.0001

[a]Literature reported value.[32]

Radical SAM enzymes are widespread in cofactor biosynthesis pathways.[21] While these enzymes are reasonable targets for antibiotic development, technical difficulties working with highly oxygen sensitive low turnover enzymes have retarded the development of inhibitors against this family of enzymes. The methylene analog **9** is a potential lead compound as an antibiotic against *H. pylori*. It has comparable antibacterial activity to amoxicillin and clarithromycin, currently approved antibiotics in the treatment of *H. pylori* infections.[33] In addition, this compound is resistant to acid hydrolysis, making it a suitable lead compound for the development of an orally available antibiotic against an acidophile like *H. pylori*.

In summary, we have identified methylene analog **9** as an inhibitor of MqnE and have demonstrated its antibacterial activity against *H. pylori* (IC_{50} = 1.8 ± 0.4 μM). These studies set the stage for the future development of antibiotics against *H. pylori* with MqnE as the target.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00649.

Procedures for the overexpression and purification of MqnE, protocols for *in vitro* and *in vivo* inhibition studies, synthetic procedures for compound **9**, NMR and MS characterization of compound **12** (PDF)

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Notes

The authors declare no competing financial interest.

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