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Mechanism-based inhibitors of MenE, an acyl-CoA synthetase involved in bacterial menaquinone biosynthesis ☆

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ABSTRACT

Menaquinone (vitamin K_2) is an essential component of the electron transfer chain in many pathogens, including *Mycobacterium tuberculosis* and *Staphylococcus aureus*, and menaquinone biosynthesis is a potential target for antibiotic drug discovery. We report herein a series of mechanism-based inhibitors of MenE, an acyl-CoA synthetase that catalyzes adenylation and thioesterification of *o*-succinylbenzoic acid (OSB) during menaquinone biosynthesis. The most potent compound inhibits MenE with an IC_{50} value of 5.7 μM.

The growing incidence of drug-resistant strains of pathogens such as *Mycobacterium tuberculosis* and *Staphylococcus aureus* poses a serious threat to human health and necessitates the development of novel antibiotics.[1] While humans and some bacteria use ubiquinone as the lipid-soluble electron carrier in the electron transport chain, this function is fulfilled solely by menaquinone (vitamin K_2) in *M. tuberculosis*, most Gram positive bacteria, including *S. aureus*, and some Gram negative organisms.[2] Although menaquinone plays an important role in the mammalian blood clotting cascade,[3] humans lack the biosynthetic pathway for generating this compound and instead obtain it from the diet or intestinal bacteria. Thus, bacterial menaquinone biosynthesis is an attractive target for drug discovery.[4] Toward this end, we report herein a series of mechanism-based inhibitors of MenE, an acyl-CoA synthetase used in menaquinone biosynthesis.

Menaquinone is biosynthesized from chorismate by the action of at least eight enzymes (Fig. 1).[5] The first studies on menaquinone biosynthesis focused on *Escherichia coli*, *Mycobacterium phlei*, and *Bacillus subtilis*, and the pathway is best understood in *E. coli*, where the first six enzymes are present in an operon. These and other genetic experiments delineated many of the components of the pathway and also demonstrated the essential role menaquinone plays in bacterial viability.[5b,6]

Our initial efforts to target this pathway have focused on MenE,[7] an acyl-CoA synthetase (ligase) that is essential in *M. tuberculosis*.[6b] MenE converts *o*-succinyl-1-benzoate (OSB) to OSB-CoA via a two-step process involving initial ATP-dependent adenylation of OSB to form a reactive OSB-AMP intermediate, followed by thioesterification with CoA to form OSB-CoA.

Acyl-CoA synthetases[8] belong to a superfamily of structurally and mechanistically related adenylate-forming enzymes that also includes non-ribosomal peptide synthetase (NRPS) adenylation domains[9] and firefly luciferase.[10] Analogous adenylation reactions are also catalyzed by structurally unrelated aminoacyl-tRNA synthetases.[11] We and others have used 5′-*O*-(*N*-acylsulfamoyl)adenosines (acyl-AMS) and related compounds to inhibit such adenylate-forming enzymes by mimicking the cognate, tightly bound acyl-AMP intermediates.[10,12–14] These molecules were inspired by a class of sulfamoyladenosine natural products that includes nucleocidin and ascamycin.[15] To avoid potential liabilities of the aromatic carboxylate moiety with respect to cell permeability or chemical instability via spirodilactone formation (observed for OSB-CoA), we posited that it might be replaced with a neutral methyl ester, since this carboxylate is not directly involved in the

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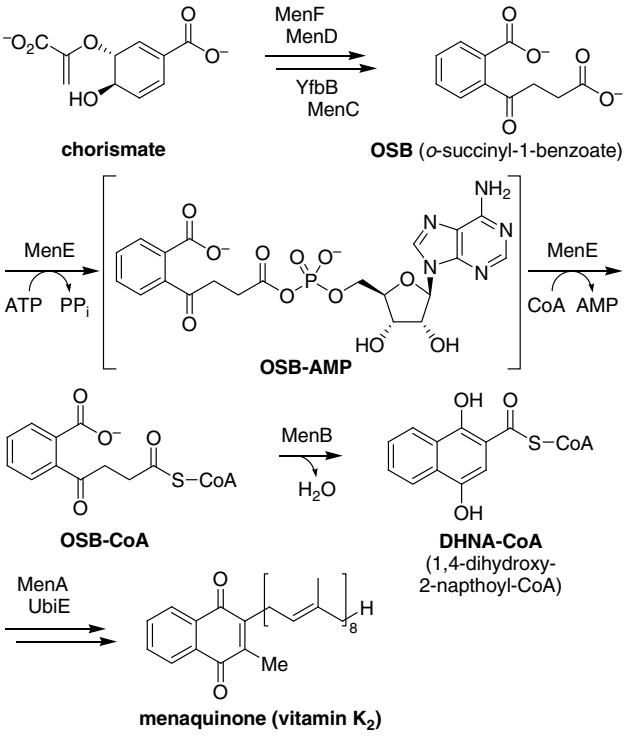

Figure 1. Bacterial biosynthesis of menaquinone from chorismate. The acyl-CoA synthetase MenE catalyzes initial adenylation of OSB (*o*-succinyl-1-benzoate) to form an OSB-AMP intermediate, followed by transthioesterification with CoA to form an OSB-CoA thioester adduct. MenB then catalyzes Dieckmann condensation to form DHNA-CoA, which is ultimately converted to menaquinone.

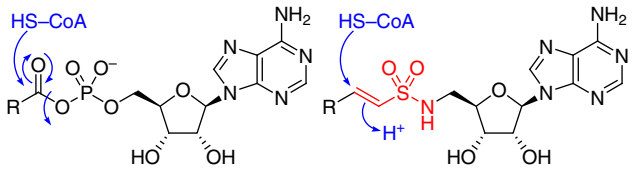

Figure 3. Mechanism of covalent inhibition. *Left*: The CoA thiol nucleophile attacks the carbonyl group in the acyl-AMP intermediate during the second half-reaction catalyzed by acyl-CoA synthetases. *Right*: A vinyl sulfonamide Michael acceptor is appropriately positioned to trap the incoming nucleophile and form a covalent adduct.

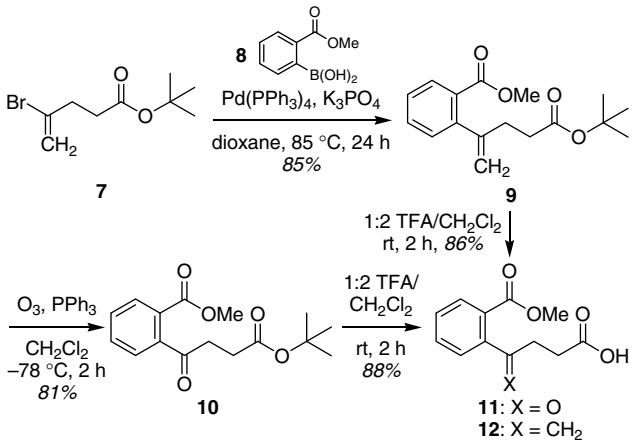

Figure 4. Synthesis of MeOSB (**11**) and the corresponding *exo*-methylene analog **12**.

reaction mechanism.[16] Thus, we envisioned that MeOSB-AMS (**1**) or its sulfamide analog MeOSB-AMSN (**2**) might be effective inhibitors of MenE and menaquinone biosynthesis (Fig. 2).

We also considered that the corresponding vinyl sulfonamide MeOSB-AVSN (**3**) might inhibit MenE through covalent binding to the incoming CoA thiol nucleophile during the second half-reaction (Fig. 3), forming a mimic of the tetrahedral intermediate. Michael acceptors have been used extensively to inhibit cysteine proteases,[17] and also to target protein thiol nucleophiles in polyketide and non-ribosomal peptide synthetases.[18] Based on studies of Roush and coworkers on the inherent reactivities of various sulfonyl-based Michael acceptors,[19] we selected the vinyl sulfonamide moiety to provide the requisite balance of reactivity and selectivity to bind CoA in the MenE active site without reacting promiscuously with other nucleophiles.

Synthesis of these inhibitors began with the preparation of MeOSB (**11**, Fig. 4). OSB was first synthesized by Roser in 1884 from phthalic anhydride and succinic acid.[20] MeOSB has also been synthesized by selective monohydrolysis of the corresponding CDI-derived bis(acylimidazole), followed by methanolysis.[16] To provide more efficient and flexible access to OSB and analogs thereof, we developed a new synthesis from the known vinyl bromide **7**, prepared by alkylation of *tert*-butyl acetate with 2,3-dibromopropene (Fig. 4).[21] Suzuki cross-coupling with aryl boronate **8** provided styrene **9**. Ozonolysis of the vinyl group afforded the orthogonally protected OSB diester **10**. Acid deprotection of the *tert*-butyl ester then yielded the desired aromatic monoester MeOSB (**11**). This modular approach should provide access to a wide range of OSB analogs. Indeed, the *exo*-methylene intermediate **9** provided immediate access to the corresponding OSB analog **12**, which we envisioned would allow us to remove the potentially enolizable ketone functionality in OSB-AMP analogs **4**–**6** (Fig. 2) and to assess its importance in binding.

The corresponding vinyl sulfonyl chlorides **20** and **21** were also prepared by a similar route (Fig. 5), featuring selective Horner–Wadsworth–Emmons coupling of ketoaldehyde **15** with sulfonyl phosphonate **17**[22] to afford the vinyl sulfonate **18**. The *exo*-methylene analog **19** was similarly prepared from **16**. The esters were purified and converted to vinyl sulfonyl chlorides **20** and **21**, which were used without further purification.

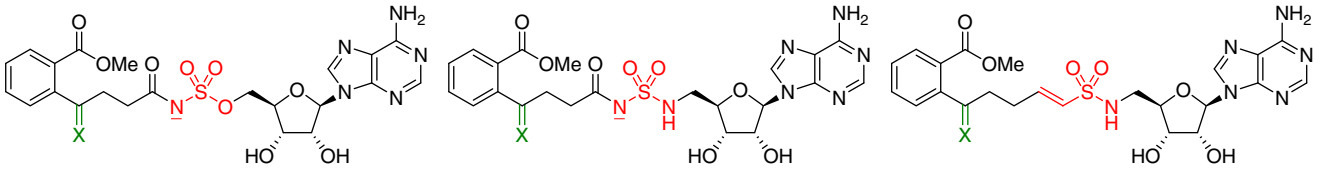

Figure 2. Structures of designed inhibitors of MenE. The sulfamate (**1**, **4**) and sulfamide (**2**, **5**) functionalities (red) are designed to mimic the phosphate group in the cognate OSB-AMP reaction intermediate. The vinyl sulfonamide moiety (**3**, **6**) is designed to trap the incoming CoA nucleophile covalently. The corresponding *exo*-methylene analogs (**4**–**6**) are designed to probe the importance of the aromatic ketone functionality (green) for binding.