Bioorganic & Medicinal Chemistry Letters 18 (2008) 5963–5966

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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ARTICLE INFO

Article history:
Received 26 June 2008
Revised 28 July 2008
Accepted 29 July 2008
Available online 12 August 2008

Keywords:
Acyl-CoA synthetase
Mycobacterium tuberculosis
Staphylococcus aureus
Mechanism-based inhibitor
Antibiotic

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

☆ Dedicated to Professor Benjamin F. Cravatt, in honor of his outstanding contributions to chemical biology and his receipt of the 2008 Tetrahedron Young Investigator Award.

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doi:10.1016/j.bmcl.2008.07.130

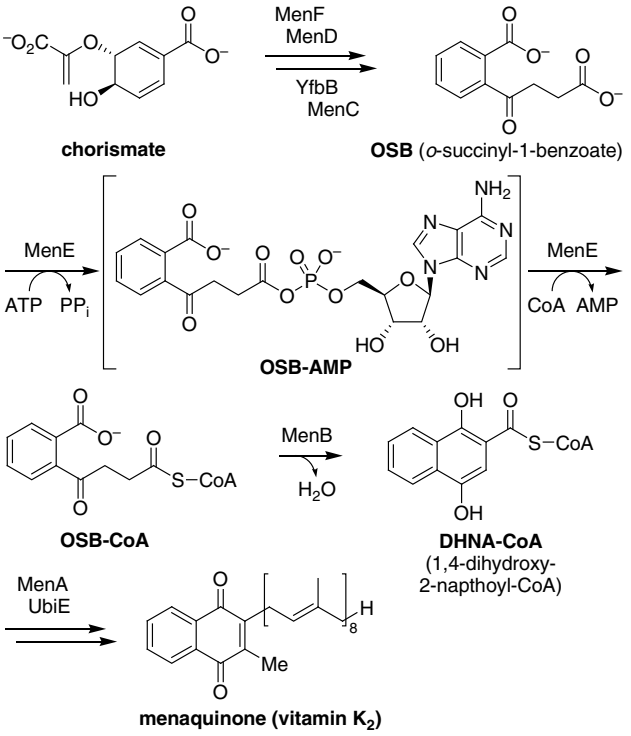

Figure 1. Bacterial biosynthesis of menaquinone from chorismate. The acyl-CoA synthetase MenE catalyzes initial adenylation of OSB (*o*-succinyl-1-benzoate) to form an OSB-AMP intermediate, followed by transthioesterification with CoA to form an OSB-CoA thioester adduct. MenB then catalyzes Dieckmann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

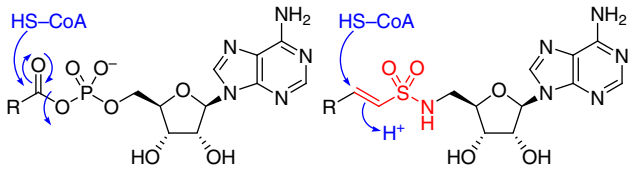

Figure 3. Mechanism of covalent inhibition. *Left*: The CoA thiol nucleophile attacks the carbonyl group in the acyl-AMP intermediate during the second half-reaction catalyzed by acyl-CoA synthetases. *Right*: A vinyl sulfonamide Michael acceptor is appropriately positioned to trap the incoming nucleophile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

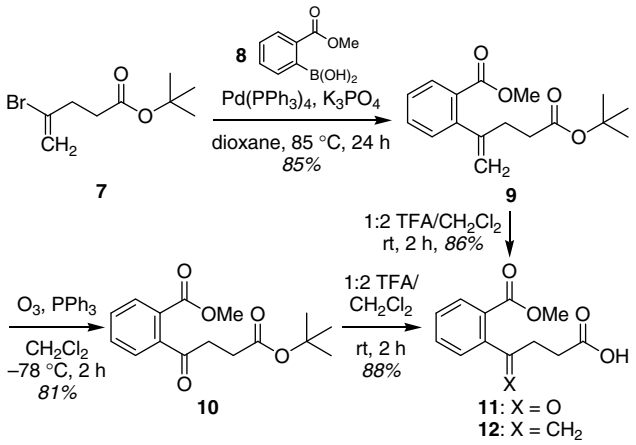

Figure 4. Synthesis of MeOSB (**11**) and the corresponding *exo*-methylene analog **12**.

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.

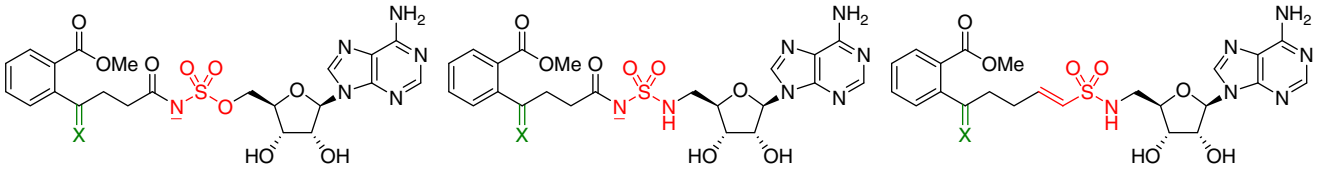

Figure 2. Structures of designed inhibitors of MenE. The sulfamate (**1**, **4**) and sulfamide (**2**, **5**) functionalities (red) are designed to mimic the phosphate group in the cognate OSB-AMP reaction intermediate. The vinyl sulfonamide moiety (**3**, **6**) is designed to trap the incoming CoA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

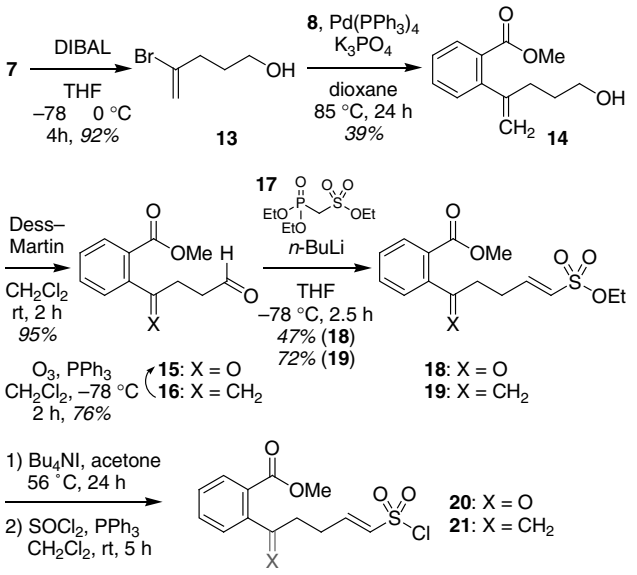

Figure 5. Synthesis of vinyl sulfonyl chloride reagents **20** and **21**.

With these OSB analogs in hand, MeOSB-AMS (**1**) and its *exo*-methylene analog **4** were synthesized by analogy to our established procedures,[14h] via *N*-acylation of a protected 5′-*O*-sulfamoyladenosine derivative with **11** and **12**, respectively, followed by deprotection.[23] Sulfamide analogs **2** and **5** were synthesized similarly from a protected 5′-*N*-sulfamoylaminodeoxyadenosine.[23] The vinyl sulfonamide analogs **3** and **6** were prepared by acylation of a protected 5′-aminodeoxyadenosine with **20** and **21**, respectively.[23]

To test these compounds for inhibition of MenE, we used a coupled assay with MenE and MenB, the DHNA-CoA synthetase that follows MenE in the biosynthetic pathway.[4,23] *E. coli* MenE and *M. tuberculosis* MenB were separately cloned and expressed with *N*-terminal His_6-tags in *E. coli* (BL21) cells, then purified to homogeneity using affinity chromatography. Reactions were initiated by adding MenE (final concentration 20 nM) to a solution containing MenB (7.2 μM), ATP (240 μM), CoA (240 μM), OSB (240 μM) and inhibitor (0–200 μM). Formation of DHNA-CoA was monitored at 392 nm, and IC_{50} values were determined.

We were gratified to find that both the sulfamate MeOSB-AMS (**1**) and sulfamide MeOSB-AMSN (**2**) were effective inhibitors of MenE (Table 1). Moreover, the vinyl sulfonamide analog MeOSB-AVSN (**3**) proved to be the most potent inhibitor, with an IC_{50} of 5.7 ± 0.7 μM; kinetic analysis indicated that this compound is a slow-binding inhibitor, suggesting a conformational change during binding. In contrast, none of the corresponding *exo*-methylene analogs (**4**–**6**) inhibited the enzyme at up to 200 μM concentration. No inhibition was observed when assays were performed using a limiting concentration of MenB (100 nM) in the presence of excess MenE (5 μM), indicating that the compounds do not inhibit MenB directly. In a preliminary experiment, **1–6** (up to 300 μM) did not inhibit *M. smegmatis* growth, suggesting that additional pharmacological issues may need to be addressed. Further investigations of cellular activity are ongoing.

Table 1
Inhibition of MenE by designed inhibitors **1**–**6**

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)
1	38.0 ± 3.0	**4**	>200
2	34.1 ± 2.8	**5**	>200
3	5.7 ± 0.7	**6**	>200

[a] Values are means of three experiments with standard deviation indicated.

It is interesting to note that the vinyl sulfonamide analog MeOSB-AVSN (**3**) is the most potent inhibitor of MenE. In contrast to the sulfamate and sulfamide analogs **1** and **2**, this compound lacks the carbonyl and adjacent heteroatom of the acyl phosphate group in OSB-AMP, which may be involved in hydrogen bonding interactions, based on the cocrystal structure of a related fatty acyl-CoA synthetase with myristoyl-AMP.[8d] These results also contrast with the relative potencies of related inhibitors of the NRPS salicylate adenylation enzyme MbtA.[18b] This may be due to a variety of factors, including possible structural differences between these enzymes,[24] different binding requirements for the inhibitors or resulting covalent adducts, and/or the different thiol nucleophiles involved: CoA in the case of MenE and a protein (MbtB) phosphopantetheine group in the case of MbtA. Our results also suggest that the OSB ketone group is required for inhibition, as shown by the complete lack of activity in *exo*-methylene analogs **4**–**6**.

In conclusion, we have designed, synthesized, and evaluated a series of mechanism-based inhibitors of the OSB-CoA synthetase MenE, which is used in bacterial menaquinone biosynthesis. This work expands the scope of sulfonyladenosine-based inhibitors to the acyl-CoA synthetase class of the adenylate-forming enzyme superfamily and sets the stage for future assessment of these inhibitors and additional analogs in cellular and animal models of infection to evaluate the potential of targeting MenE in antibacterial drug discovery.

Acknowledgments

We thank Dr. George Sukenick, Hui Liu, Hui Fang, and Sylvi Rusli (MSKCC Analytical Core Facility) for expert mass spectral analyses. D.S.T. is an Alfred P. Sloan Research Fellow. Financial support from the NIH (R01 AI068038 to D.S.T.; R01 AI044639 and R21 AI058785 to P.J.T.), NYSTAR Watson Investigator Program (D.S.T.), William H. Goodwin and Alice Goodwin and the Commonwealth Foundation for Cancer Research, and MSKCC Experimental Therapeutics Center is gratefully acknowledged.

Supplementary data

Experimental procedures and analytical data for all new compounds are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.130.

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23. See Supplementary data for full details.
24. We have previously noted a key structural difference in the *C*-terminal region of certain adenylate-forming enzymes.[14g].