Stereoselective Synthesis, Docking, and Biological Evaluation of Difluoroindanediol-Based MenE Inhibitors as Antibiotics

Christopher E. Evans,* Joe S. Matarlo,§⊥ Peter J. Tonge,*§∥ and Derek S. Tan*†‡

1Pharmacology Program, Weill Cornell Graduate School of Medical Sciences, and 2Chemical Biology Program and Tri-Institutional Research Program, Memorial Sloan Kettering Cancer Center, New York, New York 10065, United States
2Institute of Chemical Biology and Drug Discovery, 3Department of Chemistry, and 4Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York 11794, United States

Supporting Information

ABSTRACT: A stereoselective synthesis has been developed to provide all four side-chain stereoisomers of difluoroindanediol 2, the mixture of which was previously identified as an inhibitor of the o-succinylbenzoyl-CoA synthetase MenE in bacterial menaquinone biosynthesis, having promising in vitro activity against methicillin-resistant Staphylococcus aureus and Mycobacterium tuberculosis. Only the (1R,3S)-diastereomer inhibited the biochemical activity of MenE, consistent with computational docking studies, and this diastereomer also exhibited in vitro antibacterial activity comparable to that of the mixture. However, mechanism-of-action studies suggest that this inhibitor and its diastereomers may act via other mechanisms beyond inhibition of menaquinone biosynthesis.

Novel antibiotics with new mechanisms of action are urgently needed to counter the growing threat of antibiotic-resistant bacterial infections.† Bacterial menaquinone biosynthesis is an attractive new antibacterial target.‡ Menaquinone (vitamin K₂) is a lipid-soluble electron carrier used in the electron-transport chain of cellular respiration in many bacterial species. It is the sole electron carrier in Gram-positive bacteria, mycobacteria, and all anaerobically growing bacteria.‡ In contrast, humans use ubiquinone for electron transport, and although menaquinone is an important clotting factor, humans lack the de novo biosynthetic pathway for menaquinone and acquire it from diet and gut flora.‡ Menaquinone is biosynthesized in bacteria through at least nine distinct enzymes (Figure 1),§ and inhibitors have been reported for MenD,§ MenC,8 MenE,9,10 MenB,11 and MenA.12 The antimicrobial activity of these inhibitors corroborates genetic evidence indicating that menaquinone is essential for proliferation and survival of bacteria in which it is the sole electron carrier.† In particular, our laboratories have previously reported inhibitors of MenE9, an acyl-CoA (coenzyme A) synthetase (ligase) in the ANL (acyl-CoA synthetase/non-ribosomal peptide synthetase adenylation domain/luciferase) family of adenylate-forming enzymes.13 MenE catalyzes a two-step process involving initial adenylation of o-succinylbenzoyl (OSB) to form the tightly bound OSB-AMP (adenosine 5′-monophosphate) intermediate, followed by thioesterification with CoA to form OSB-CoA.13 We9 and others10 have used 5′-O-(N-acetylaminomethyl)adenosine (acyl-AMS) analogues of the tightly bound OSB-AMP reaction intermediate to target MenE. Based upon an initial inhibitor OSB-AMS (1), we recently discovered a difluoroindanediol analogue 2 with improved in vitro antibacterial activity against methicillin-resistant Staphylococcus aureus and Mycobacterium tuberculosis (Figure 2). The difluoroindanediol side chain was originally prepared as a mixture of four stereoisomers. Thus, to assess the activity of the individual stereoisomers, we report herein the development of a stereoselective synthesis leveraging enzymatic kinetic resolution. The individual stereoisomers of 2 were then evaluated in biochemical, computational, and cell culture studies to assess selectivity and mechanisms of action.

In our original synthesis of 2, a racemic difluoroindanol side chain bearing a ketone at the C3 position was coupled to the AMS scaffold, with the ketone undergoing non-stereoselective reduction during a subsequent hydrogenation step.9c Initial efforts to resolve this racemic keto acid side chain by recrystallization with a chiral amine or chromatographic separation of corresponding chiral amine-derived diastereomeric Schiff bases were unsuccessful. Thus, to access the individual diastereomers of 2 in a stereoselective fashion, we envisioned an alternative retrosynthetic approach in which both the C1 and C3 stereocenters of the side chains 4 would be set prior to coupling to the AMS scaffold 3 (Figure 2). C1 stereochemistry would be set via diastereoselective alkyne addition to protected keto alcohol 5, with absolute stereochemistry at C3 established in 3-hydroxy-1-indanone 6. Notably, initial efforts to achieve stereinduction by asymmetric reduction or alkyne addition to 2,2-difluoroindan-1,3-dione...
IC_{50} (E. coli[MenE]) = 0.025 µM and 1.5 µM
MIC (MRSAs) = 31.25 µg/mL and 15.6 µg/mL
MIC (M. tuberculosis) = 125 µg/mL and 15.6 µg/mL

Figure 1. (a) Menaquinone biosynthetic pathway. m = 4–13; n = 9 in M. tuberculosis; n = 8 in S. aureus and E. coli. (b) MenE inhibitors that mimic the tightly bound OSB-AMP reaction intermediate. AMP = adenosine 5′-monophosphate; ATP = adenosine 5′-triphosphate; CoA = coenzyme A; IC_{50} = 50% inhibitory concentration; MIC = minimum inhibitory concentration; MRSAs = methicillin-resistant S. aureus; PP = inorganic pyrophosphate.

Figure 2. Stereoselective retrosynthesis of difluoroundanediol-based inhibitor 2. PG = protecting group. Red circles indicate key stereocenters.

(not shown) yielded no enantiocontrol, perhaps due to the high reactivity of this diketone.

To access both enantiomers of 3-hydroxy-1-indanone (6), we carried out an enzymatic kinetic resolution with vinyl acetate and Amano Lipase PS (Burkholderia cepacia, formerly Pseudomonas cepacia), reported previously by Nair and co-workers. At 50% conversion, the reaction provided the starting alcohol (3S)-6 in 46% yield and >98% ee (Chiracel OB-H) and the enantiomeric acetate (3R)-7 in 43% yield and >98% ee, corresponding to an E value \(^\text{15}\) of >200 (Figure 3).

With the C3 stereochemistry established, synthesis of the syn-difluoroundanediol inhibitors (1R,3S)-2 and (1S,3R)-2 commenced with conversion of the acetate (3R)-7 to silyl ether (3S)-8. Mild fluorination of the corresponding Schiff base with Selectfluor provided α-difluoroketone (3S)-9.\(^\text{16}\) Propiolate addition under optimized conditions provided syn-diol (1R,3S)-10 (>20:1 dr). The tert-butyl ester was cleaved, and the resulting acid was coupled to protected AMS scaffold 12.\(^\text{9}\) Hydrogenation of the alkene and global deprotection provided syn-difluoroundanediol (1R,3S)-2. The other syn-diol diastereomer (1S,3R)-2 was synthesized analogously from the enantiomeric alcohol (3S)-6. Absolute and relative stereochemistry were confirmed by X-ray crystallographic analysis (CuKα radiation for determination of absolute configuration \(^\text{17}\) of the diol obtained via desilylation of silyl ether (1S,3R)-11.\(^\text{18}\)

To access the corresponding anti-difluoroundanediol inhibitor (1R,3R)-2, we used an oxidation/reduction approach starting from protected syn-diol intermediate (1R,3S)-10 to afford anti-diol acid (1R,3R)-15 (Figure 4). The intermediate anti-diol ester (1R,3R)-14 exhibited a diagnostic \(^1^H\) NMR shift of 5.41 ppm for C3-H, compared to 5.11 ppm for the epimeric syn-diol ester (1R,3S)-14 obtained after the initial desilylation of (1R,3S)-10.\(^\text{18}\) The enantiomeric excess was also confirmed for all four diastereomeric diol esters 14 by chiral HPLC analysis.
Figure 4. Synthesis of antidifluoroindanediol inhibitors (1R,3R)-2 and (1S,3S)-2. Yields in parentheses are for synthesis of (1S,3S)-2. TBAF = tetrabutylammonium fluoride.

(>98% ee), ruling out possible racemization at earlier stages of the synthesis (6 → 10, Figure 3). Coupling of anti-diol acid (1R,3R)-15 to protected AMS scaffold 12, alkyne hydrogenation, and global deprotection afforded anti-difluoroindanediol (1R,3R)-2. The other anti-diol diastereomer (1S,3S)-2 was synthesized analogously from the enantiomer protected syn-diol intermediate (1S,3R)-10.

Next, we carried out computational docking (Glide, Schrödinger) using our recently reported cocrystal structure of E. coli MenE (R195K mutant) in complex with OSB-AMS (1) (Figure 5 and Figure S1).6c,18 Docking of OSB-AMS into the protein provided a ligand pose well-aligned with that observed in the cocrystal structure (rmsd 0.2 Å).18 In docking of the four diastereomeric difluoroindanediols 2, the adenosine region of each diastereomer bound in an orientation consistent with that of OSB-AMS, retaining key interactions and filling the adenosine binding pocket. However, in the side-chain region, only the syn-difluoroindanediol (1R,3S)-2 fitted the binding OSB pocket fully, overlapping well with the OSB aromatic ring of cocrystallized OSB-AMS. The secondary alcohol of the difluoroindanediol appeared poised to engage in hydrogen bonding with a conserved water H2O-666 and the alcohol side chain of Thr-277, which both interact with the OSB carboxylate in cocrystallized OSB-AMS.6c

Notably, in earlier docking studies with unliganded S. aureus MenE,6c we identified a Ser-302 side chain (Thr-178 in M. tuberculosis) that could interact with the OSB ketone of OSB-AMS. Although this alcohol side chain is absent in E. coli MenE (Gly-268), the docking studies herein suggest that the tertiary alcohol of the difluoroindanediol in (1R,3S)-2 may be positioned to interact with this side chain in S. aureus and M. tuberculosis MenE.

We next tested the biochemical inhibitory activity of the four diastereomeric difluoroindanediols 2 against E. coli MenE (Table 1).5,18 Consistent with the results of the docking studies above, the syn-difluoroindanediol (1R,3S)-2 was the most potent inhibitor (entry 2), while none of the other three diastereomers inhibited the enzyme at up to 200 μM concentration (entries 3–5). The (1R,3S)-2 diastereomer was also approximately 4-fold more potent than the mixture of all four diastereomers 2 (entry 1), suggesting that this single diastereomer is responsible for the observed inhibitory activity of the mixture.

We then evaluated the antimicrobial activity of the difluoroindanediols 2 against Bacillus subtilis, methicillin-resistant S. aureus (MRSA), and M. tuberculosis (Table 1).18 Surprisingly, all four individual diastereomers exhibited MIC (minimum inhibitory concentration) values similar to that of the mixture of diastereomers. When the cultures were complemented with exogenous menaquinone-4, a 4-fold increase in MIC values was observed for the mixture of diastereomers (entry 1), while 2- to 4-fold increases were also observed for the MenE inhibitor (1R,3S)-2 (entry 2), consistent with a mechanism of action involving inhibition of menaquinone biosynthesis. Some rescue was also observed for the other syn-diastereomer (1S,3R)-2 in B. subtilis and M. tuberculosis (entry 3), while no rescue was observed for the anti diastereomers (entries 4 and 5). This suggests that the antimicrobial activity of the last three diastereomers results from other mechanisms of action, consistent with their lack of biochemical activity against MenE.

Finally, we evaluated the effects of the inhibitors on menaquinone biosynthesis in MRSA by LC-MS/MS.5,6c MRSA treated with OSB-AMS (1) showed a statistically significant 2.5-fold decrease in menaquinone-8, consistent with our previously published findings (Figure S2).5,6c,18 The mixture of four diastereomers 2 also elicited a smaller, but statistically significant, 31% decrease in menaquinone-8. However, none of the individual difluoroindanediol diastereomers caused a significant decrease in menaquinone-8. Taken together, these results suggest that even the MenE inhibitor (1R,3S)-2 may act via mechanisms other than inhibition of menaquinone biosynthesis.

In conclusion, we have developed a stereoselective synthesis of all four diastereomers of a difluoroindanediol-based inhibitor of MenE. Enzymatic kinetic resolution was used to establish absolute stereochemistry, and diastereoselective transformations were used to set relative stereochemistry. Biochemical and docking studies identified the syn-diastereomer (1R,3S)-2 as an effective MenE inhibitor that may engage in active-site interactions similar to those observed for the reaction intermediate analogue OSB-AMS (1). However, microbiological experiments suggest that (1R,3S)-2 may have multiple mechanisms of action beyond inhibition of bacterial menaquinone.
none biosynthesis. Efforts to optimize this inhibitor and to probe its mechanism of action are ongoing.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or-glett.6b03272.

X-ray crystallographic data for (1S,3R)-15 (CIF)

Experimental procedures and analytical data for all new compounds (PDF)

AUTHOR INFORMATION

Corresponding Authors

*E-mail: peter.tonge@stonybrook.edu.

*E-mail: tand@mskcc.org.

ORCID

Christopher E. Evans: 0000-0001-9777-4114

Notes

The authors declare the following competing financial interest(s): The authors are co-inventors of a patent application based on this work.

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(17) The Flack parameter was determined from Parsons’ quotients to be −0.03(5) using a Bruker Apex Duo diffractometer equipped with an ImuS CuKa radiation source (1.54178 Å), indicating the correct absolute configuration.

(18) See the Supporting Information for complete details.

Table 1. Biochemical and Antimicrobial Activity of Diastereomeric Difluoroindanediols 2

| entry | inhibitor | MenE IC<sub>50</sub> (μM) | B. subtilis MIC<sup>b</sup> (μg/mL) | MRSA MIC<sup>c</sup> (μg/mL) | M. tuberculosis MIC<sup>c</sup> (μg/mL) |
|-------|-----------|-----------------------------|-----------------------------------|-----------------------------|-----------------------------------|
| 1     | 2<sup>e</sup> | 18.3 ± 3.7<sup>d</sup> | 15.6 (62.5) | 15.6 (62.5) | 15.6 (62.5) |
| 2     | (1R,3S)<sup>e</sup>-2 | 5.0 ± 1.0 | 15.6 (31.2) | 15.6 (31.2) | 15.6 (31.2) |
| 3     | (1S,3R)<sup>e</sup>-2 | >200 | 31.2 (31.2) | 31.2 (31.2) | 31.2 (31.2) |
| 4     | (1R,3R)<sup>e</sup>-2 | >200 | 15.6 (15.6) | 15.6 (15.6) | 15.6 (15.6) |
| 5     | (1S,3S)<sup>e</sup>-2 | >200 | 15.6 (15.6) | 15.6 (15.6) | 31.2 (31.2) |
| 6     | AMS<sup>2</sup> | nd<sup>f</sup> | 3.9 (3.9) | 1.9 (1.9) | 0.16 (0.32) |

<sup>a</sup>E. coli MenE. <sup>b</sup>MIC values in parentheses determined with addition of exogenous menaquinone-4 (10 μg/mL). <sup>c</sup>Equimolar mixture of four diastereomers, prepared by the original synthetic route. <sup>d</sup>This IC<sub>50</sub> is higher than the 1.5 μM that we reported previously due to batch-to-batch variability of the enzyme preparation. <sup>e</sup>S-O-Sulfamoyladenosine. <sup>f</sup>nd = not determined.