Anthranilate 4H-oxazol-5-ones: novel small molecule antibacterial acyl carrier protein synthase (AcpS) inhibitors

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Abstract—D-optimal design and Projection to Latent Structures (PLS) analysis were used to optimize screening hit 5 (B. subtilis AcpS IC50: 15 μM, B. subtilis MIC: > 200 μM) into a series of 4H-oxazol-5-one, small molecule, antibacterial, AcpS inhibitors. Specifically, 15, 16 and 18 show μM or sub-μM AcpS inhibition (IC50s: 15: 1.1 μM, 16: 1.5 μM, 18: 0.27 μM) and moderate antibacterial activity (MICs: 12.5–50 μM) against B. subtilis, E. faecalis ATCC, E. faecalis VRE and S. pneumo.

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1. Introduction

Rapidly developing resistance of known bacterial strains to currently prescribed pharmaceutical agents has stimulated a vigorous search for novel mechanisms to kill bacteria.1,2 Strains such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), penicillin-resistant Streptococcus pneumoniae (PRSP) and multiple drug-resistant Enterobacteriaceae klebsiella (MDR ESBL) do not respond to current clinical drug therapies including quinolones and β-lactams.

In order to overcome bacterial resistance to currently prescribed antibiotics, we have searched for other cellular processes that would serve as good antibacterial targets. One such process is the conversion of apo-acyl carrier protein (apo-Acp) to holo-acyl carrier protein (holo-Acp) which is catalyzed by acyl carrier protein synthase (AcpS), an enzyme that catalyzes the transfer of the 4′-phosphopantetheinyl group of coenzyme A (CoA) to a serine residue of apo-Acp. The resulting holo-Acp accepts acetyl and malonyl groups from acetyl-CoA and malonyl-CoA respectively and shuttles these acyl groups between enzymes in the fatty acid biosynthesis pathway.3 AcpS is required for the biosynthesis of important components of membrane lipids and bacterial lipopolysaccharides, and the essentiality of this enzyme was determined genetically in Streptococcus pneumoniae (S. pneumo)4 and Escherichia coli (E. coli).5

The crystal structures of S. pneumo6 and Bacillus subtilis (B. subtilis)7 AcpS have been determined but no small molecule inhibitors of AcpS have previously been reported.

In this letter, we disclose the discovery of a series of small molecule, antibacterial, anthranilate 4H-oxazol-5-one AcpS inhibitors 1. In addition, we will show how multivariate analysis (MVA) and experimental design were used to rapidly optimize the biological activity of these molecules.

2. Chemistry

Anthranilate 4H-oxazol-5-ones 1 were prepared according to literature procedures. Thus, acid chlorides 2 were converted to hippuric acids 3,8 and cyclized to
the corresponding 4H-oxazol-5-ones 4 with triethyl orthoformate and Ac₂O. The target anthranilate 4H-oxazol-5-ones 1 were prepared by heating 3 with anthranilic acids in EtOH.

3. Biology

AcpS inhibitory activity was determined using a B. subtilis GST-Acp-HTRF assay. The in vitro determination of the minimal inhibitory concentrations (MICs) against aerobic bacteria was performed by the microdilution broth method as recommended by the National Committee for Clinical Laboratory Standards.

4. Results and discussion

4-Cl Phenyl 4H-oxazol-5-one 5 was identified as an inhibitor of B. subtilis AcpS in a high-throughput screen (AcpS IC₅₀: 15 µM) (Fig. 1). Unfortunately this compound had no MIC activity against several strains of bacteria. Compound 5 showed a low PAMPA permeability (0.05×10⁻⁶ cm/s) and a low AlogD (0.43) suggesting that this compound may not enter the bacterial cell to reach its target. Molecular modeling of 5 into the active site of B. subtilis AcpS showed that variation of the R¹, R² and R³ positions could lead to optimized contacts in the active site, thereby increasing binding affinity. These modeling constraints were used to select monomers for an initial synthetic array using 4 acid chlorides and 14 anthranilic acids (chosen using a D-optimal design) of the t[1], t[2] and t[3] scores of the PCA of a 27 anthranilic acid candidate set containing variations of the R₁ and R₂ substituents (Fig. 1).

A set of 42 (Set 1) oxazolones was synthesized using the procedure outlined in Scheme 1 (Fig. 1). AcpS inhibitory and MIC data are presented for selected compounds in Table 1. MVA was used to summarize/correlate the calculated and the measured molecular physicochemical properties with in vitro AcpS potency and MIC activity for Set 1. PLS models were generated using calculated MW, hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) count, daylight clogP and cMR, polar surface area (PSA), and measured σ values and atom counts for the whole molecule as well as the R¹, R² and R³ substituents.

AcpS inhibition data (IC₅₀) and MIC data (B. subtilis, E. faecalis ATCC, E. faecalis VRE and S. pneumo) using SIMCA-P. Two PLS models were generated to understand the effects of simultaneously varying the R¹, R² and R³ substituents: a model that correlates physicochemical properties with AcpS inhibitory activity (A: 2, R²X: 0.50, R²Y: 0.71, Q²(cum): 0.63, Fig. 2), and a model that correlates physicochemical properties AcpS inhibitory activity and MIC activity A: 2, R²X: 0.50, R²Y: 0.44, Q²(cum): 0.29, Fig. 3).

Figure 2 shows AcpS inhibitory activity is primarily correlated with increasing R³clogP, R³sigma and R³MW. This effect is seen in comparing several compounds in Table 1: compare 10 (R³=Cl) to 11 (R³=CF₃) and 12 (R³=OMe) to 13 (R³=CF₃). Figure 2 also shows that AcpS inhibition can be increased by decreasing the values of the R¹cMR and R¹clogP variables. The R¹PSA, R¹HBD, R¹HBA variables are on an orthogonal axis to the R² and R³ AcpS inhibitory activity variables and thus have a minimal effect. The goodness of prediction of the model is satisfactory as the Q²(cum) term is above 0.5 (Q²(cum) = 0.63).

The PLS model in Figure 3 is even more informative as it correlates increasing AcpS inhibition and MIC activity with increasing R³clogP, R³sigma and R³MW and decreasing values of the R¹ and R² variables (primarily the w*c[1] loading). AcpS inhibitory activity and MIC activity appear to be correlated—the correlation is strongest for the B. subtilis MIC activity, and there is correlation between increasing AcpS inhibition/MIC activity and increasing molecular clogP and MW. Importantly, analysis of these two PLS models indicates that more potent antibacterial AcpS inhibitors can be synthesized via adjustment of the R³ substituent noted.

Figure 1. Retrosynthetic analysis of compound 5, monomer selection and synthesis of 4H-oxazol-5-one Set 1.
Table 1. In vitro AcpS inhibitory data and minimum inhibitory concentration (MIC) data for selected Set 1 oxazolones

<table>
<thead>
<tr>
<th>Compd</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>IC50 (µM)a</th>
<th>B. subtilis</th>
<th>E. faecalis ATCC</th>
<th>E. faecalis VRE</th>
<th>S. pneumo+</th>
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aIC50 values were determined once over a range of 7 concentrations. Standard error for each run is ±10%.
bCompounds were tested 1× against each bacterial strain. Standard error for each run is ±1 dilution.

Figure 2. PLS loadings (w*^c[1] vs w*^c[2]) of Set 1: physicochemical properties (X matrix), AcpS inhibitory data (Y matrix) (A: 2, R2X: 0.50, R2Y: 0.71, Q2(cum): 0.63). Model shows that AcpS inhibitory activity primarily increases with increasing R3clogP, R3sigma and R3MW values and decreasing R2clogP and R2cMR values. R1HBD, R1HBA and R1PSA have little effect.

Figure 3. PLS loadings (w*^c[1] vs w*^c[2]) of Set 1: physicochemical properties (X matrix), AcpS inhibitory data and bacterial MIC data (Y matrix) (A: 2, R2X: 0.50, R2Y: 0.44, Q2(cum): 0.29). Model shows that AcpS inhibitory activity and MIC activity primarily increases with increasing R3clogP, R3sigma and R3MW values and with decreasing values for the R1 and R2 variables. AcpS inhibitory activity and MIC activity also increase with increasing molecular clogP and MW.
above and by increasing overall molecular lipophilicity/MW. This conclusion was then used as the new starting point for a new library synthesis.

A second set of oxazolones (Set 2) was then synthesized as shown in Figure 4. Acid chlorides were used where the R5 substituent (R3 substituent in Set 1) was set to F, Cl, OCF3 and CF3, and anthranilic acids were used that gave active compounds in Set 1. AcpS inhibitory and MIC data are presented for selected Set 2 compounds in Table 2. Potent antibacterial AcpS inhibitors were obtained where R4=F and R5=CF3 as seen in 15, 16 and 18. Compound 18, having an AcpS IC50 = 0.27 μM and a B. subtilis MIC = 25 μM, is the most potent compound in terms of both AcpS inhibition and B. subtilis MIC activity. Compound 17 is the most potent AcpS inhibitor obtained, but it fails to show any MIC activity. A second series of antibacterial AcpS inhibitors emerged with the substitution pattern of R4=OCF3 and R5=H (19–21). These compounds show similar MIC activity as 15, 16 and 18 but have less potent enzymatic activity. These two groups of compounds do show the same trend in biological activity-AcpS IC50: R1=Cl, R1=Br > R1=Me; Overall MIC activity: R1=Me > R1=Cl, R1=Br.

Thus we have shown how MVA and experimental design can be used to rapidly optimize both in vitro AcpS inhibitory/antibacterial activity to produce a series of small molecule AcpS inhibitors represented by compound 18. MVA is a particularly powerful as it effectively summarizes variables into simpler linear combinations that are readily interpretable. It also allows one to interpret the effect of simultaneously varying multiple positions within a molecule. Additional studies on other small molecule AcpS inhibitors will be forthcoming.

Acknowledgements

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References and notes


![Figure 4. Retrosynthetic analysis of compound 14, monomer selection and synthesis of 4H-oxazol-5-one Set 2.](image)
11. Enzyme activity was measured using a homogeneous time-resolved fluorescence resonance energy transfer (HTRF\textsuperscript{TM}) assay. Assays were run in 384 well plates and consisted of compound, 15 ng/mL AcpS, 1.9 μM biotin-CoA (prepared by reacting equimolar amounts of CoA and biocytin maleimide (Molecular Probes, Eugene, OR)), and 1.25 μg/mL GST-ACP in 50 mM Tris-HCl, pH 8.0, 7.5 mM MgCl\textsubscript{2}, 3.75 mM DTT, 0.0375% Tween-20, and 37.5 μg/mL BSA in a total volume 20 μL. After 3 h at room temperature, the reaction was terminated by adding 60 μL of the stop/detection mix (82 ng/mL europium cryptate conjugate of GST monoclonal antibody (Packard, Meriden, CT), 28.7 μg/mL streptavidin–allophycocyanin conjugate (ProZyme, San Leandro, CA), 410 mM KF, 4.1 mM EDTA in 0.5×PBS). The mixture was incubated overnight before reading in a Wallac Victor\textsuperscript{TM} plate reader (excitation at 340 nm, emission at 665 nm).


