

Specific Anti-Staphylococcal Activity of AFN-1252, a Novel Fatty Acid Synthesis Inhibitor

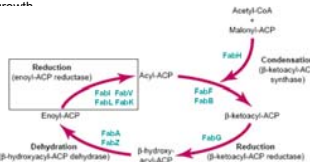
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Introduction and Objectives

AFN-1252 is a novel inhibitor of the bacterial fatty acid synthesis pathway (FASII) currently in clinical development for the treatment of infections due to susceptible and drug-resistant staphylococci. The FASII pathway utilizes several condensing and reducing enzymes including enoyl-ACP reductase (ENR) that catalyzes the final step in the chain elongation process, reducing enoyl-ACP to acyl-ACP. Enoyl-ACP reductase is known to have 4 distinct enzyme forms – FabI, FabK, FabL, and FabV. Complete inhibition of the enoyl-ACP reductase activity prevents growth of the acyl chain, and disrupts both saturated and unsaturated fatty acid biosynthesis, thus preventing bacterial growth.



The FabI target is conserved across some Gram-positive and -negative bacterial species, including staphylococci. However, some of the most efficient bacterial pathogens and typical commensal flora have an alternative form or multiple forms of the enzyme. Bacterial genera that possess enoyl-ACP reductase other than FabI or possess multiple forms of the enzyme are not expected to be inhibited by AFN-1252, defining a very specific bacterial spectrum for the agent. To analyze the genetic basis of this assumption, a phylogenetic analysis of publicly-available genomic databases was conducted. In addition, AFN-1252 was tested against clinical isolates of a variety of aerobic and anaerobic bacterial species in order to test the conclusions of the phylogenetic analysis and to define the spectrum of activity of AFN-1252.

Methods

For phylogenetic analyses all bacterial amino acid sequences were from the RefSeq database at the National Center for Biotechnology Information (NCBI). A total of 628 bacterial enoyl-ACP reductase sequences from 551 representative bacterial pathogen species were examined. Phylogenetic trees were constructed using the PHYLIP phylogeny inference package version 3.65 (Retief, J.D. *Phylogenetic analysis using PHYLIP. Methods Mol Biol.* 2000, 132:243-58) and visualized using Dendroscope (D.H. Huson et al., *Dendroscope: An interactive viewer for large phylogenetic trees. BMC Bioinformatics* 2007, 8:4.60).

Test strains included recent clinical isolates from 12 Canadian medical centers via the CANWARD 2007 in vitro surveillance study, and additional strains from the ATCC and the Micromyx strain collection. Aerobic and anaerobic MICs were determined using the recommended CLSI microdilution (M7-A7 2006) and agar dilution (M11-A7 2007) methods, respectively. CANWARD strains were tested at AFN-1252 concentrations of 0.008 – 4 µg/ml and anaerobic strains at 0.008 – 8 µg/ml.

AFN-1252 was supplied by Affinium Pharmaceuticals

Figure 1. Chemical structure of AFN-1252

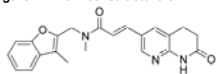


Figure 2. Phylogeny of Bacterial Enoyl-ACP Reductase delineates four distinct isoforms

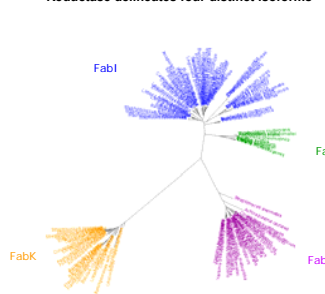


Figure 3. Phylogenetic Distance Map of Select Bacterial Enoyl-ACP Reductase sequences. Species depicted in bold have more than one form of enoyl-ACP reductase

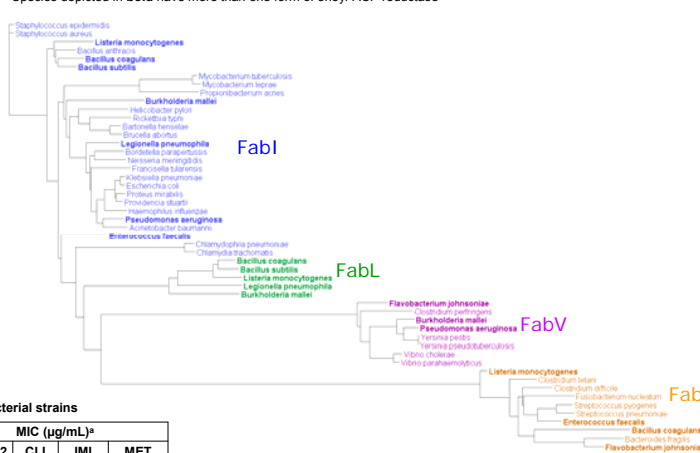


Table 2. Activity of AFN-1252 against select anaerobic bacterial strains

Organism	ENR isoform	MIC (µg/mL) ^a			
		AFN-1252	CLI	IMI	MET
Gram Positive					
<i>Bifidobacterium bifidum</i> 3965	Fas ^b	>8	≤ 0.03	≤ 0.03	1
<i>Bifidobacterium longum</i> 3968	Fas ^b	>8	0.06	0.06	4
<i>Clostridium perfringens</i> 3518	FabV	>8	>16	2.5	4
<i>Clostridium difficile</i> 3579	FabK	>8	16	4	>16
<i>Eubacterium lentum</i> 1274	FabK	>8	0.12	0.5	1
<i>Lactobacillus acidophilus</i> 0681	unknown	>8	8	0.12	>16
<i>Lactobacillus casei</i> 1722	FabK	>8	4	0.12	>16
<i>Peptostreptococcus anaerobius</i> 3531	FabK	>8	>16	0.06	0.5
<i>Peptostreptococcus micros</i> 3545	FabK	>8	4	≤ 0.03	>16
<i>Propionibacterium acnes</i> 1267	FabI	>8	>16	≤ 0.03	>16
<i>Streptococcus constellatus</i> 1202	FabK	>8	>8	2.5	>16
Gram Negative					
<i>Bacteroides fragilis</i> 3479	FabK	>8	4	0.25	0.5
<i>Bacteroides ovatus</i> 3503	FabK	>8	2	0.25	1
<i>Bacteroides thetaiotaomicron</i> 3496	FabK	>8	>16	0.5	1
<i>Bacteroides vulgatus</i> 3494	FabK	>8	0.5	0.5	0.25
<i>Eikenella corrodens</i> 1206	FabI	>8	0.5	0.25	4
<i>Fusobacterium necrophorum</i> 3963	FabK	>8	≤ 0.03	≤ 0.03	0.06
<i>Fusobacterium nucleatum</i> 3962	FabK	>8	≤ 0.03	≤ 0.03	0.06
<i>Porphyromonas asaccharolytica</i> 3557	FabK	>8	0.25	0.03	0.1
<i>Prevotella melaninogenica</i> 3437	FabK	>8	>16	≤ 0.03	0.25
<i>Prevotella</i> spp. 3568	FabK	>8	4	0.06	1
<i>Veillonella parvula</i> 1272	FabK	>8	>16	≤ 0.03	>16

^aCLI – Clindamycin; IMI – Imipenem; MET – Metronidazole
^bFatty acid synthase, similar to non-bacterial systems, with no homology to the FASII cycle

Results

Table 1. Amino Acid Identity Matrix for Select Bacterial Species that have FabI Only

	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>	Inhibitory effects of AFN-1252 (optimized against the <i>S. aureus</i> FabI)
<i>S. aureus</i>	--	93	88	Excellent FabI and whole cell inhibition
<i>S. epidermidis</i>	93	--	87	
<i>S. saprophyticus</i>	88	87	--	
<i>Bacillus anthracis</i>	63	62	62	Good FabI but poor whole cell inhibition
<i>Francisella tularensis</i>	45	45	44	
<i>Escherichia coli</i>	42	42	41	
<i>Helicobacter pylori</i>	41	42	39	Poor FabI and whole cell inhibition
<i>Acinetobacter</i> sp.	40	40	39	
<i>Propionibacterium acnes</i>	29	29	29	
<i>Mycobacterium tuberculosis</i>	30	30	29	
<i>Chlamydia trachomatis</i>	27	26	27	

Figure 4. AFN-1252 MIC distribution against CANWARD 2007 *S. aureus* and *E. coli* isolates

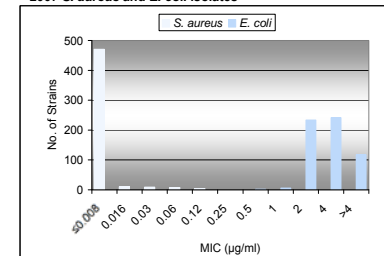


Table 3. Activity of AFN-1252 against CANWARD 2007 isolates

Species (n)	ENR isoform	MIC (µg/ml)		
		Range	MIC ₅₀	MIC ₉₀
Gram positive				
<i>S. aureus</i> (502)	FabI	≤ 0.008 - 0.12	≤ 0.008	≤ 0.008
MSSA (375)	FabI	≤ 0.008 - 0.12	≤ 0.008	≤ 0.008
MRSA (127)	FabI	≤ 0.008 - 0.06	≤ 0.008	≤ 0.008
<i>S. epidermidis</i> (55)	FabI	≤ 0.008 - 0.06	≤ 0.008	0.03
MSSE (42)	FabI	≤ 0.008 - 0.06	≤ 0.008	0.03
MRSE (9)	FabI	≤ 0.008	≤ 0.008	≤ 0.008
<i>S. pneumoniae</i> (489)	FabK	4 - >4	>4	>4
<i>E. faecalis</i> (81)	FabI + FabK	>4	>4	>4
<i>E. faecium</i> (38)	FabI + FabK	4 - >4	>4	>4
Gram negative				
Non-fermentors				
<i>A. baumannii</i> (15)	FabI	>4	>4	>4
<i>M. catarrhalis</i> (70)	FabI	>4	>4	>4
<i>P. aeruginosa</i> (137)	FabI + FabV	>4	>4	>4
Enterobacteriaceae				
<i>E. coli</i> (600)	FabI	0.5 - >4	4	>4
<i>E. cloacae</i> (72)	FabI	4 - >4	>4	>4
<i>K. pneumoniae</i> (199)	FabI	2 - >4	>4	>4

Conclusions

- AFN-1252 antibacterial activity was evident only in species with an exclusive FabI ENR enzyme
- Activity of AFN-1252, optimized against *S. aureus* FabI, decreases against FabI from other species as their amino acid identities diverge
- Potent *in vitro* activity was demonstrated only against staphylococci
- Poor or no activity was evident for other Gram-positive and -negative aerobic, facultative and anaerobic bacteria
- Species not inhibited include major human pathogens and commensal skin and gut flora
- Results highlight the narrow spectrum and potential safety benefits of AFN-1252, including reduced antibiotic associated adverse events