F1-1348

Activity of AFN-1252, a FabI Inhibitor with Potent Activity against *S. aureus* (SA) and Coagulase-Negative *Staphylococcus* spp. (CoNS), Including Multidrug-Resistant Strains

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ICAAC 2011
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ABSTRACT

Background: SA and CoNS are responsible for a wide variety of human infections. AFN-1252 specifically targets SA and CoNS without significant activity against other Gram-positive/-negative species. AFN-1252 inhibits FabI, an enzyme critical to fatty acid biosynthesis in staphylococci. The activity of AFN-1252 against CoNS, methicillin-susceptible (MSSA) and -resistant (MRSA) SA, including significant clones, was determined.

Methods: A globally diverse collection (35 countries) of 574 patient isolates was tested that included contemporary CoNS (6 species, 103 strains), MSSA (154), MRSA (163) and molecularly characterized (MC) strains (including *spa* typed MRSA clones; 154). Isolates were susceptibility (S) tested by CLSI broth microdilution methods against AFN-1252 and 10 comparators. S rates for comparators were determined using CLSI and EUCAST criteria.

Results: All SA and CoNS were inhibited by AFN-1252 concentrations of ≤0.12 and ≤0.5 µg/ml, respectively. MIC_{50} values for MSSA, MRSA and MC-MRSA strains were 0.004 µg/ml and MIC_{90} values ranged from 0.008 - 0.03 µg/ml. MIC values were higher for CoNS isolates ($MIC_{50/90}$, 0.015/0.12 µg/ml). Among SA, resistance (R) was common for erythromycin (61.6%), clindamycin (27.6%), levofloxacin (49.0%), tetracycline (15.7%) and trimethoprim/sulfamethoxazole (7.0%).

Conclusions: AFN-1252 demonstrated potent activity against MSSA, MRSA and CoNS. AFN-1252 showed significantly greater activity overall (MIC_{50}, 0.004 µg/ml) where compared to other agents tested against these staphylococcal species that included dominant MRSA clones and strains R to currently utilized antimicrobials.

INTRODUCTION

AFN-1252 is a novel Fab inhibitor that specifically targets *Staphylococcus* spp. FabI is an essential enzyme that is needed for the final step in the elongation cycle of bacterial fatty acid biosynthesis. AFN-1252 is currently in clinical development for treatment of staphylococcal infections. This compound has demonstrated very little activity against other species of bacteria including streptococci, entercocci, *Enterobacteriaceae* and nonfermentative Gram-negative species. The narrow spectrum of AFN-1252 is potentially beneficial as it may minimize the effect on normal flora and hence have reduced antibiotic associated adverse events such as overgrowth of resistant commensals, diarrhea, colitis and Candidiasis.

Antimicrobial resistance among both *S. aureus* and coagulase-negative staphylococci (CoNS) has been observed in many countries, including against current therapeutic agents used to treat these pathogens. There is an identified need for a novel class of agents with high potency and potential clinical efficacy against *S. aureus* and CoNS.

In this study, AFN-1252 was evaluated for activity against a large collection of contemporary pathogens of *Staphylococcus* spp., including *S. aureus* and coagulase-negative *Staphylococcus* spp. (CoNS). This study showed similar activity for AFN-1252 against *Staphylococcus* spp. compared to previous investigator results when tested in vitro.

MATERIALS AND METHODS

Susceptibility Test Methods: AFN-1252 was supplied by the sponsor and was tested over 12 log_2 dilutions (0.001 – 2 µg/ml). Rifampin; acquired from Sigma Chemical Co. (St. Louis, MO, USA), was used as a control agent [12 log_2 dilutions (0.001 – 2 µg/ml)]. Additional comparator antimicrobial agents included the following previously tested antimicrobial agents; oxacillin, erythromycin, clindamycin, daptomycin, vancomycin, linezolid, levofloxacin, tetracycline and trimethoprim/sulfamethoxazole. Broth microdilution frozen-form panels were supplied by TREK Diagnostics (Cleveland, OH, USA) using cation-adjusted Mueller-Hinton broth. The study design followed the CLSI M07-A8 (2009) guideline. Quality control (QC) ranges and interpretive criteria for comparator compounds were as published in CLSI M100-S21 [2011] and for AFN-1252 as approved by CLSI (CLSI meeting minutes January 2011); tested QC strains included *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Organism Collection: A collection of 574 isolates were used to determine the activity of AFN-1252. These included 317 geographically dispersed contemporary (2010) *S. aureus* isolates. This collection included MSSA/MRSA (154/163) from North America (60/66), Latin America (30/32), Europe (32/31) and the Asia-Western Pacific (32/34). In addition, 154 genetically defined *S. aureus*, including strains representative of the major circulating global clones (details in Table 1). Strains were obtained from the JMI Laboratories bacterial strain collection and the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA).

Coagulase-negative staphylococci (103) included the following species, *S. epidermidis* (56), *S. haemolyticus* (20), *S. hominis* (11), *S. xylosus* (five), *S. warneri* (five) and *S. saprophyticus* (six) all collected from the SENTRY Antimicrobial Surveillance Program. All strains were identified to species level using BactiStaph® Latex 150 followed by confirmatory tube latex agglutination with coagulase plasma (Remel, Lenexa, KS, USA) and the Vitek II identification system (BioMerieux, Hazelwood, MO, USA). Resistance phenotypes were determined by reference broth microdilution tests followed by confirmation as required or specified by CLSI M100-S21 criteria.

RESULTS

- AFN-1252 was very active against *S. aureus* (Table 1). All *S. aureus* isolates were inhibited by AFN-1252 MIC values of ≤0.12 µg/ml with MIC_{50} and MIC_{90} values of 0.004 µg/ml and 0.008 µg/ml, respectively.
- The collection of the clinical subset of *S. aureus* isolates included 51.4% MRSA against which AFN-1252 ($MIC_{50/90}$, 0.004/0.008 µg/ml) was two-fold more active than rifampin ($MIC_{50/90}$, 0.008/0.015 µg/ml) and significantly more potent than the other comparator agents (Table 2).
- The molecularly characterized *S. aureus* isolates were nearly all resistant to oxacillin (95.5%) and AFN-1252 retained similar potency compared to the clinical MRSA strains (Table 2).
- Against the all isolate collection, AFN-1252 was 64-, 128-fold and 256-fold more active compared to daptomycin (MIC_{90}, 0.5 µg/ml), vancomycin (MIC_{90}, 1 µg/ml) and linezolid (MIC_{90}, 2 µg/ml), respectively.
- Among the *S. aureus* isolates overall (471 isolates), resistance to other antimicrobial classes (Table 2) was high, including erythromycin (61.6 – 62.2%), clindamycin (27.6 – 28.0%), levofloxacin (49.0%), tetracycline (15.7 – 17.0%) and trimethoprim/sulfamethoxazole (6.6 – 7.0 %).
- CoNS isolates tended to have higher AFN-1252 MIC values compared to *S. aureus* (Tables 2 and 3). This species group was represented by the most common species isolated from human infections (Table 3). AFN-1252 MIC_{50} and MIC_{90} values for these species were 0.015 µg/ml (four-fold higher than *S. aureus*) and 0.12 µg/ml (16-fold higher than *S. aureus*), respectively with the highest reproducible MIC observed at 0.5 µg/ml (one *S. epidermidis* isolate).

Table 1. AFN-1252 MIC frequency distributions when tested against 574 isolates of *Staphylococcus* spp.

	No. (cum. %) of isolates inhibited at AFN-1252 MIC (µg/ml)								
Species/phenotype (no. tested)	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5
S. aureus (471)	4 (0.9)	297 (63.9)	142 (94.1)	9 (96.0)	6 (97.2)	11 (99.6)	2 (100.0)	-	-
MSSA (154)	2 (1.3)	88 (58.4)	48 (89.6)	0 (89.6)	6 (93.5)	9 (99.4)	1 (100.0)	-	-
MRSA (163)	1 (0.6)	102 (63.2)	52 (95.1)	8 (100.0)	-	-	-	-	-
Molecularly characterized (154)[a]	1 (0.7)	107 (70.1)	42 (97.4)	1 (98.1)	0 (98.1)	2 (99.4)	1 (100.0)	-	-
Coagulase-negative *Staphylococcus* spp. (103)	0 (0.0)	4 (3.9)	23 (26.2)	28 (53.4)	20 (72.8)	12 (84.5)	12 (96.1)	3 (99.0)	1 (100.0)
MS-CoNS (86)	0 (0.0)	4 (4.7)	18 (25.6)	27 (57.0)	13 (72.1)	11 (84.9)	11 (97.7)	2 (100.0)	-
MR-CoNS (17)	0 (0.0)	0 (0.0)	5 (29.4)	1 (35.3)	7 (76.5)	1 (82.4)	1 (88.2)	1 (94.1)	1 (100.0)

a. Spa types included ST239 (Hungarian/Brazilian clone ; SCCmec III), ST8 (USA300; SCCmec IV), ST22; (EMRSA-15 ; SCCmec IV), ST5 (Cordoes/Chilean clone, SCCmec I), USA100 to 1100 clones, linezolid and tigecycline-resistant strains, vancomycin-intermediate strains (VISA) and strains positive for several toxin genes, including Panton-Valentine Leukocidin (PVL).

Table 2. Activity of AFN-1252 and comparator antimicrobial agents when tested against 471 isolates of *Staphylococcus aureus* (all regions).

Antimicrobial agent (no. tested)	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %R	EUCAST[b] %S / %R
All isolates (471)					
AFN-1252	0.004	0.008	0.002 – 0.12	- / -	- / -
Rifampin	0.008	0.015	0.004 – >2	93.0 / 4.5	- / -
Oxacillin	>2	>2	≤0.25 – >2	34.2 / 65.8	34.2 / 65.8
Erythromycin	>2	>2	≤0.25 – >2	37.6 / 61.6	37.6 / 62.2
Clindamycin	≤0.25	>2	≤0.25 – >2	72.0 / 27.6	71.5 / 28.0
Daptomycin	0.25	0.5	≤0.06 – 4	98.7 / -	98.7 / 1.3
Vancomycin	1	1	≤0.12 – 8	98.3 / 0.0	98.3 / 1.7
Linezolid	1	2	0.5 – >8	99.2 / 0.8	99.2 / 0.8
Levofloxacin	2	>4	≤0.5 – >4	49.7 / 49.0	49.7 / 49.0
Tetracycline	≤2	>8	≤2 – >8	83.4 / 15.7	82.0 / 17.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5 – >2	93.0 / 7.0	93.0 / 6.6
MSSA (154)					
AFN-1252	0.004	0.03	0.002 – 0.12	- / -	- / -
Rifampin	0.008	0.008	0.004 – >2	98.1 / 0.6	- / -
Oxacillin	≤0.25	≤0.25	≤0.25 – 0.5	100.0 / 0.0	100.0 / 0.0
Erythromycin	≤0.25	>4	≤0.25 – >4	77.3 / 22.1	77.3 / 22.1
Clindamycin	≤0.25	≤0.25	≤0.25 – >2	94.2 / 5.8	94.2 / 5.8
Daptomycin	0.25	0.5	≤0.06 – 1	100.0 / -	100.0 / 0.0
Vancomycin	1	1	0.25 – 2	100.0 / 0.0	100.0 / 0.0
Linezolid	1	1	0.5 – 2	100.0 / 0.0	100.0 / 0.0
Levofloxacin	≤0.5	≤0.5	≤0.5 – >4	92.2 / 7.1	92.2 / 7.1
Tetracycline	≤0.25	2	≤0.25 – >8	90.3 / 8.4	89.6 / 9.7
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5 – >4	98.7 / 1.3	98.7 / 1.3
MRSA (163)					
AFN-1252	0.004	0.008	0.002 – 0.015	- / -	- / -
Rifampin	0.008	0.015	0.004 – >2	92.0 / 6.7	- / -
Oxacillin	>2	>2	>2	0.0 / 100.0	0.0 / 100.0
Erythromycin	>4	>4	≤0.25 – >4	18.4 / 79.8	18.4 / 81.6
Clindamycin	>2	>2	≤0.25 – >2	47.9 / 52.1	47.9 / 52.1
Daptomycin	0.25	0.5	0.12 – 1	100.0 / -	100.0 / 0.0
Vancomycin	1	1	0.5 – 2	100.0 / 0.0	100.0 / 0.0
Linezolid	1	1	0.5 – 2	100.0 / 0.0	100.0 / 0.0
Levofloxacin	>4	>4	≤0.5 – >4	19.6 / 77.3	19.6 / 77.3
Tetracycline	≤0.25	>8	≤0.25 – >8	81.6 / 17.2	79.1 / 18.4
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5 – >4	93.3 / 6.7	93.3 / 5.5
Molecularly characterized *S. aureus* (154)					
AFN-1252	0.004	0.008	0.002 – 0.12	- / -	- / -
Rifampin	0.008	2	0.004 – >2	89.0 / 5.8	- / -
Oxacillin	>2	>2	≤0.25 – >2	4.5 / 95.5	4.5 / 95.5
Erythromycin	>2	>2	≤0.25 – >2	18.2 / 81.8	18.2 / 81.8
Clindamycin	≤0.25	>2	≤0.25 – >2	75.3 / 23.4	74.0 / 24.7
Daptomycin	0.5	0.5	0.25 – 4	96.1 / -	96.1 / 3.9
Vancomycin	1	1	≤0.12 – 8	94.8 / 0.0	94.8 / 5.2
Linezolid	1	2	0.5 – >8	97.4 / 2.6	97.4 / 2.6
Levofloxacin	4	>4	≤0.5 – >4	39.0 / 61.0	39.0 / 61.0
Tetracycline	≤2	>8	≤2 – >8	78.6 / 21.4	77.3 / 22.7
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5 – >2	87.0 / 13.0	87.0 / 13.0

a. Criteria as published by the CLSI [2011] and EUCAST [2011]. β-lactam susceptibility should be directed by the oxacillin test results.

Table 3. Activity of AFN-1252 and comparator antimicrobial agents when tested against 103 isolates of coagulase-negative staphylococci[a] (all regions).

Antimicrobial agent (no. tested)	MIC_{50}	MIC_{90}	Range	CLSI[b] %S / %R	EUCAST[b] %S / %R
All isolates (103)					
AFN-1252	0.015	0.12	0.004 – 0.5	- / -	- / -
Rifampin	0.008	0.015	≤0.001 – >2	97.1 / 2.9	- / -
Oxacillin	≤0.25	1	≤0.25 – >2	83.5 / 16.5	83.5 / 16.5
Erythromycin	≤0.25	>2	≤0.25 – >2	51.5 / 48.5	51.5 / 48.5
Clindamycin	≤0.25	>2	≤0.25 – >2	88.3 / 11.7	85.4 / 11.7
Daptomycin	0.25	0.5	≤0.06 – 1	100.0 / -	100.0 / 0.0
Vancomycin	1	2	0.25 – 2	100.0 / 0.0	100.0 / 0.0
Linezolid	0.5	1	≤0.12 – 1	100.0 / 0.0	100.0 / 0.0
Levofloxacin	≤0.5	>4	≤0.5 – >4	76.7 / 22.3	76.7 / 22.3
Tetracycline	≤2	>8	≤2 – >8	86.3 / 12.7	82.4 / 13.7
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5 – >2	86.4 / 13.6	86.4 / 10.7
MS-CoNS (86)					
AFN-1252	0.015	0.12	0.004 – 0.25	- / -	- / -
Rifampin	0.008	0.015	≤0.001 – >2	97.7 / 2.3	- / -
Oxacillin	≤0.25	≤0.25	≤0.25	100.0 / 0.0	100.0 / 0.0
Erythromycin	≤0.25	>4	≤0.25 – >4	52.3 / 47.7	52.3 / 47.7
Clindamycin	≤0.25	0.5	≤0.25 – >2	91.9 / 8.1	88.4 / 8.1
Daptomycin	0.25	0.5	0.12 – 1	100.0 / -	100.0 / 0.0
Vancomycin	1	2	0.25 – 2	100.0 / 0.0	100.0 / 0.0
Linezolid	0.5	1	≤0.12 – 1	100.0 / 0.0	100.0 / 0.0
Levofloxacin	≤0.5	>4	≤0.5 – >4	77.9 / 22.1	77.9 / 22.1
Tetracycline	≤0.25	>8	≤0.25 – >8	87.1 / 11.8	83.5 / 12.9
Trimethoprim/sulfamethoxazole	≤0.5	4	≤0.5 – >4	88.4 / 11.6	88.4 / 9.3
MS-CoNS (17)					
AFN-1252	0.03	0.25	0.008 – 0.5	- / -	- / -
Rifampin	0.015	0.015	≤0.001 – >2	94.1 / 5.9	- / -
Oxacillin	1	>2	0.5 – >2	0.0 / 100.0	0.0 / 100.0
Erythromycin	>2	>2	≤0.25 – >2	47.1 / 52.9	47.1 / 52.9
Clindamycin	≤0.25	>2	≤0.25 – >2	70.6 / 29.4	70.6 / 29.4
Daptomycin	0.25	0.5	≤0.06 – 0.5	100.0 / -	100.0 / 0.0
Vancomycin	1	2	0.5 – 2	100.0 / 0.0	100.0 / 0.0
Linezolid	1	1	0.5 – 1	100.0 / 0.0	100.0 / 0.0
Levofloxacin	≤0.5	4	≤0.5 – >4	70.6 / 23.5	70.6 / 23.5
Tetracycline	≤2	>8	≤2 – >8	82.4 / 17.6	76.5 / 17.6
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5 – >2	76.5 / 23.5	76.5 / 17.6

a. Includes: *Staphylococcus epidermidis* (56 strains), *Staphylococcus haemolyticus* (20 strains), *Staphylococcus hominis* (11 strains), *Staphylococcus saprophyticus* (6 strains), *Staphylococcus warneri* (5 strains), and *Staphylococcus xylosus* (5 strains).
b. Criteria published by the CLSI [2011] and EUCAST [2011]. β-lactam susceptibility should be directed by the oxacillin test results.

CONCLUSIONS

- In this study, AFN-1252 was shown to have significant activity against a diverse collection of staphylococcal pathogens, including endemic MRSA strains that are currently circulating in the hospital and community environments worldwide.
- Clinical isolates of *Staphylococcus* spp. were highly susceptible to AFN-1252 with slightly higher MIC_{50} and MIC_{90} values observed among CoNS.
- AFN-1252 retained excellent potency against molecularly characterized *S. aureus* isolates with MIC_{50} and MIC_{90} values identical to the MRSA clinical isolates.
- The remarkably consistent activity against these common Gram-positive pathogens, including those with increasingly prevalent resistant mechanisms, is a promising feature of this novel agent and warrants its further development for serious staphylococcal infections.

ACKNOWLEDGEMENTS

This study was sponsored by Affinium Pharmaceuticals, Toronto, Ontario, Canada.

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