Letters to the Editor

In Vitro Activity of API-1252, a Novel FabI Inhibitor, against Clinical Isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*[▽]

API-1252 (Fig. 1), an inhibitor of bacterial enoyl-acyl carrier protein (enoyl-ACP) reductase (FabI), is being developed by Affinium Pharmaceuticals, Inc. (Toronto, Canada), in both oral and intravenous formulations, for single-agent and combination treatment of antimicrobial-susceptible and -resistant staphylococcal infections, particularly *Staphylococcus aureus* infections.

To assess the in vitro activity of API-1252 against recent clinical isolates of *S. aureus* and *Staphylococcus epidermidis*, we selected 350 isolates of methicillin-susceptible *S. aureus* (MSSA), 154 isolates of methicillin-resistant *S. aureus* (MRSA), 50 isolates of methicillin-susceptible *S. epidermidis* (MSSE), and 50 isolates of methicillin-resistant *S. epidermidis* (MRSE) collected from May 2005 to June 2006 as part of an ongoing 19-center national surveillance study, the Canadian Intensive Care Unit study. Clinical and Laboratory Standards Institute (CLSI)-specified broth microdilution testing was performed using frozen panels (prepared in-house) containing API-1252, ciprofloxacin, gentamicin, oxacillin, and vancomycin (2, 6). API-1252 was tested over a doubling dilution concentration range from 0.001 to 2 µg/ml, and its MICs were read following 20 to 24 h of incubation at 35°C in ambient air (2). The reference strains *S. aureus* ATCC 29213 and *S. aureus* ATCC 33592 (MRSA) reproducibly demonstrated MICs to API-1252 of 0.015 and 0.008 µg/ml, respectively. The methicillin-resistant phenotype of each isolate of staphylococci was confirmed using a 30-µg cefoxitin disk (2). MICs for ciprofloxacin, gentamicin, oxacillin, and vancomycin were interpreted using CLSI M100-S15 guidelines (2).

The genotypes of isolates of MRSA were established using a previously described *mecA* and *nuc* multiplex PCR assay (5), and each isolate of MRSA was subtyped using a standardized, previously described pulsed-field gel electrophoresis (PFGE) protocol (4, 5, 9). MRSA isolates identified by PFGE profile as community-acquired strains (i.e., CMRSA-7 [USA-400]; CMRSA-10 [USA-300]) were tested by PCR to detect *lukF-PV*, *lukS-PV*, *eta*, and *etb* toxin genes (5), and their staphylococcal chromosome cassette *mec* type was determined, as previously described (7).

Table 1 depicts the concentrations of API-1252 that inhibited 50% (MIC₅₀) and 90% (MIC₉₀s) of isolates and MIC ranges. The MIC₉₀s of API-1252 for MSSA and MRSA were 0.015 µg/ml; all isolates of *S. aureus* were inhibited by API-1252 at a concentration of 0.125 µg/ml. No difference in API-1252 in vitro activity was observed for hospital-associated and community-associated isolates of MRSA. The MIC₉₀s of API-1252 for MSSE and MRSE were 0.06 and 0.03 µg/ml,

TABLE 1. Activity of API-1252 against staphylococci with various phenotypic and genotypic profiles

<i>Staphylococcus</i> species	Phenotype (no. of isolates tested)	API-1252 MIC (µg/ml)		
		Range	50%	90%
<i>S. aureus</i>	Methicillin-susceptible (350)	0.004–0.125	0.008	0.015
	Pansusceptible ^a (308)	0.004–0.125	0.008	0.015
	Methicillin-resistant (154)	0.002–0.125	0.008	0.015
	Multidrug-resistant ^b (26)	0.002–0.125	0.004	0.015
	Community-associated ^c methicillin-resistant (16)	0.004–0.015	0.008	0.008
	Hospital-associated ^d methicillin-resistant (138)	0.002–0.125	0.008	0.015
<i>S. epidermidis</i>	Methicillin-susceptible (50)	0.008–0.5	0.015	0.06
	Pansusceptible (17)	0.008–0.5	0.015	0.015
	Methicillin-resistant (50)	0.008–0.5	0.015	0.03
	Multidrug-resistant (23)	0.008–0.06	0.015	0.03

^a Pansusceptible was defined as susceptible to oxacillin, ciprofloxacin, gentamicin, and vancomycin.

^b Multidrug-resistant isolates of staphylococci were defined as those isolates resistant to ciprofloxacin and gentamicin and included both methicillin-susceptible and methicillin-resistant isolates.

^c Community-associated MRSA isolates were defined as isolates with CMRSA-7 (USA-400) or CMRSA-10 (USA-300) PFGE profiles. All community-associated MRSA isolates harbored *lukF-PV* and *lukS-PV* toxin genes and were staphylococcal cassette chromosome *mec* type IV. None of the community-associated MRSA isolates harbored *eta* or *etb* toxin genes (4, 5, 7, 9). All isolates with CMRSA-7 (USA-400) PFGE profiles were susceptible to ciprofloxacin, gentamicin, and vancomycin. All isolates with CMRSA-10 (USA-300) PFGE profiles were resistant to ciprofloxacin and susceptible to gentamicin and vancomycin.

^d Hospital-associated MRSA isolates were defined as isolates with one of the following PFGE profiles: CMRSA-1, CMRSA-2, CMRSA-4, CMRSA-6, CMRSA-8, and CMRSA-9 (4, 5, 9); 5.1, 81.2, and 100% of hospital-associated MRSA isolates were susceptible to ciprofloxacin, gentamicin, and vancomycin, respectively.

respectively; all isolates of *S. epidermidis* were inhibited by API-1252 at a concentration of 0.5 µg/ml.

FabI is an essential enzyme that catalyzes the reduction of *trans*-2-enoyl-ACP to acyl-ACP in the final step of each elongation cycle in bacterial fatty acid biosynthesis (3, 8). FabI is a selective antibacterial target for *S. aureus* and *S. epidermidis*, as well as for *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Escherichia coli*; it is the sole enoyl-ACP reductase present in each of these bacterial species (8). No alternative enzyme or rescue pathway has been identified for FabI in staphylococci, suggesting that resistance to FabI inhibitors, such as API-1252, will not readily emerge with therapy (1).

In conclusion, API-1252 demonstrated potent in vitro activity against recent clinical isolates of MSSA and MRSA (MIC₉₀, 0.015 µg/ml), MSSE (MIC₉₀, 0.06 µg/ml), and MRSE (MIC₉₀, 0.03 µg/ml) from patients with serious hospital infections. API-1252 is a potentially promising advance in the treatment of patients with staphylococcal infections known or suspected to be resistant to conventional therapies both in hospitals and in the community.

FIG. 1. Chemical structure of API-1252.

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REFERENCES

1. Campbell, J. W., and J. E. Cronan, Jr. 2001. Bacterial fatty acid biosynthesis: targets for antibacterial drug discovery. *Annu. Rev. Microbiol.* **55**:305–332.
2. Clinical and Laboratory Standards Institute. 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI M100-S15, vol. 25, no. 1. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Heath, R. J., S. W. White, and C. O. Rock. 2002. Inhibitors of fatty acid synthesis as antimicrobial chemotherapeutics. *Appl. Microbiol. Biotechnol.* **58**:695–703.
4. Mulvey, M. R., L. Chiu, J. Ismail, L. Louie, C. Murphy, N. Chang, M. Alfa, and the Canadian Committee for the Standardization of Molecular Methods. 2001. Development of a Canadian standardized protocol for subtyping methicillin-resistant *Staphylococcus aureus* using pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **39**:3481–3485.
5. Mulvey, M. R., L. MacDougall, B. Cholin, G. Horsman, M. Fidyk, and S. Woods. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*, Canada. *Emerg. Infect. Dis.* **11**:844–850.
6. National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A6, 6th ed., vol. 23, no. 2. National Committee for Clinical Laboratory Standards, Wayne, PA.
7. Oliveira, D. C., and H. de Lencastre. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **46**:2155–2161.
8. Payne, D. J., W. H. Miller, V. Berry, J. Brosky, W. J. Burgess, E. Chan, W. E. Wolfe, Jr., A. P. Fosberry, R. Greenwood, M. S. Head, D. A. Heerding, C. A. Janson, D. D. Jaworski, P. M. Keller, P. J. Manley, T. D. Moore, K. A. Newlander, S. Pearson, B. J. Polizzi, X. Qiu, S. F. Rittenhouse, C. Slater-Radosti, K. L. Salyers, M. A. Seefeld, M. G. Smyth, D. T. Takata, I. N. Uzinskas, K. Vaidya, N. G. Wallis, S. B. Winram, C. C. K. Yuan, and W. F. Huffman. 2002. Discovery of a novel and potent class of FabI-directed antibacterial agents. *Antimicrob. Agents Chemother.* **46**:3118–3124.
9. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.

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