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# In Vitro Antibacterial Activity of CE-156811, a Novel Analog Derived from Hygromycin A▽

Gregory G. Stone,[1]* Dennis Girard,[1] Steve Finegan,[1] Joan Duignan,[1] John Schafer,[1] Meghan Maloney,[1] Richard P. Zaniewski,[1] Steven J. Brickner,[1] Sarah K. Wade,[1] Phuong T. Le,[3] and Michael D. Huband[2]

*Pfizer Global Research & Development, Groton/New London Laboratories, Pfizer Inc., Groton, Connecticut 06340[1]; Pfizer Global Research & Development, Ann Arbor Laboratories, Pfizer Inc., Ann Arbor, Michigan 48105[2]; and Pfizer Global Research & Development, La Jolla Laboratories, Pfizer Inc., La Jolla, California 92121[3]*

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**We evaluated a novel truncated hygromycin A analog in which the furanose ring was replaced with a 2-fluoro-2-cyclopropylethyl substituent for its activity against multidrug resistant gram-positive bacteria and compared its activity to the activities of linezolid, quinupristin-dalfopristin, and vancomycin. CE-156811 demonstrated robust in vitro activity against gram-positive bacteria that was comparable to that of linezolid.**

The continuing emergence of resistance to existing antibacterial agents in gram-positive organisms has created the need for the discovery of novel antibacterial agents that possess activity against these resistant bacteria (1, 5, 9). The isolation of hygromycin A from *Streptomyces hygroscopicus* was first reported in 1953 (10), and it was demonstrated to possess weak activity against gram-positive organisms (6). The mechanism of action of hygromycin A has been demonstrated to be protein synthesis inhibition through interference with peptide bond formation (2).

The current study was designed to evaluate the in vitro antibacterial activity of CE-156811 against various gram-positive and gram-negative isolates in comparison to the activities of other marketed agents.

(This study was presented in part at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 27 to 30 September 2006 [4, 11].)

CE-156811 is a novel truncated hygromycin A analog with potent activity against multidrug-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), community-acquired MRSA, and vancomycin-intermediate *S. aureus*. The chemical structures of CE-156811 and hygromycin A are displayed in Fig. 1. All bacterial strains used in this study were from the Pfizer collection of clinical bacterial cultures and were obtained between 2000 and 2004 from several sources, including various surveillance studies and clinical trials. Transcription and translational inhibition studies of protein synthesis were performed as described previously (7). Susceptibility testing was performed by the broth microdilution method, as described by the CLSI (formerly the NCCLS) (8). In vitro killing kinetics studies were performed with 25-ml volumes of Mueller-Hinton broth (supplemented with 3% lysed horse blood for streptococci) with agitation. CE-156811 was tested at 1×, 2×, 4×, and 8× the MIC; and samples were plated for colony count determinations at 0, 2, 4, 8, and 24 h. A ≥3-log reduction from the original inoculum at 24 h was considered bactericidal. CE-156811 was obtained by alkylation of the phenol {3-(2,5-difluoro-4-hydroxy-phenyl)-2-methyl-*N*-[(3a*S*,4*R*,5*R*,6*S*,7*R*,7a*R*)-4,6,7-trihydroxy-hexahydro-benzo[1,3]dioxol-5-yl]-acrylamide} with (*R*)-methanesulfonic acid 2-cyclopropyl-2-fluoro-ethyl ester and potassium carbonate in anhydrous *N*,*N*-dimethylformamide at 75°C for 5 days. The phenol can be derived from the corresponding *O*-allyl ether (3) by a $Pd(Ph_3P)_4$ (where Ph is phenyl)-catalyzed deallylation with dimedone. CE-156811 was found to undergo slow hydrolysis at 25°C in water (to the extent of 4% decomposition over 24 h) to generate the corresponding cyclopropyl alcohol (validated by independent synthesis) resulting from the hydrolysis of the cyclopropyl alkyl

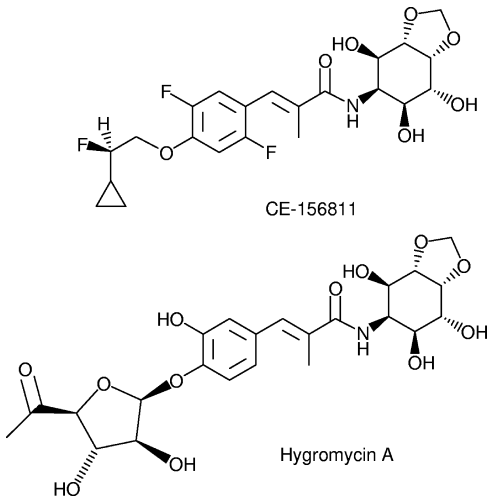


FIG. 1. Structures of hygromycin A and CE-156811.

* Corresponding author. Present address: Infection Biosciences, AstraZeneca Pharmaceuticals, 35 Gatehouse Drive, Waltham, MA 02451. Phone: (781) 839-4425. Fax: (781) 839-4800. E-mail: gregory.stone@astrazeneca.com.

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TABLE 1. Susceptibilities of gram-positive clinical isolates to CE-156811 and comparator agents

| Organism (no. of isolates) | Test agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Staphylococcus aureus*, methicillin susceptible (28) | CE-156811 | 0.25–1 | 0.5 | 1 |
| | Linezolid | 1–4 | 2 | 2 |
| | Quinupristin-dalfopristin | 0.25–2 | 0.5 | 0.5 |
| | Vancomycin | 0.25–2 | 0.5 | 1 |
| *Staphylococcus aureus*, methicillin resistant (18) | CE-156811 | 0.25–1 | 0.5 | 0.5 |
| | Linezolid | 1 | 1 | 1 |
| | Quinupristin-dalfopristin | 0.06–0.25 | 0.125 | 0.25 |
| | Vancomycin | 0.5–2 | 1 | 2 |
| *Staphylococcus aureus*, glycopeptide intermediate (8) | CE-156811 | 0.125–0.5 | | |
| | Linezolid | 0.5–1 | | |
| | Quinupristin-dalfopristin | 0.06–0.25 | | |
| | Vancomycin | 4–8 | | |
| *Staphylococcus epidermidis*, methicillin susceptible (23) | CE-156811 | 0.06–0.5 | 0.12 | 0.25 |
| | Linezolid | 0.25–4 | 0.5 | 1 |
| | Quinupristin-dalfopristin | 0.06–1 | 0.06 | 0.06 |
| | Vancomycin | 0.25–2 | 1 | 2 |
| Other coagulase-negative staphylococci (23) | CE-156811 | 0.5–2 | 1 | 2 |
| | Linezolid | 1–2 | 2 | 2 |
| | Levofloxacin | 0.06–64 | 0.25 | 32 |
| | Vancomycin | 1–4 | 2 | 2 |
| *Enterococcus faecalis*, vancomycin susceptible (19) | CE-156811 | 0.25–1 | 1 | 2 |
| | Linezolid | 0.5–2 | 2 | 2 |
| | Quinupristin-dalfopristin | 4–32 | 8 | 32 |
| | Vancomycin | 1–4 | 2 | 4 |
| *Enterococcus faecalis*, VRE (28) | CE-156811 | 0.5–2 | 1 | 2 |
| | Linezolid | 0.5–16 | 2 | 2 |
| | Quinupristin-dalfopristin | 4–16 | 8 | 16 |
| | Vancomycin | 8–>32 | >32 | >32 |
| *Enterococcus faecium*, vancomycin susceptible (13) | CE-156811 | 0.5–2 | 1 | 2 |
| | Linezolid | 2–4 | 2 | 2 |
| | Quinupristin-dalfopristin | 0.5–4 | 1 | 2 |
| | Vancomycin | 1 | 1 | 1 |
| *Enterococcus faecium*, VRE (49) | CE-156811 | 0.5–2 | 1 | 2 |
| | Linezolid | 1–4 | 2 | 4 |
| | Quinupristin-dalfopristin | 0.25–4 | 1 | 2 |
| | Vancomycin | 8–>32 | >32 | >32 |
| *Streptococcus pneumoniae*, penicillin susceptible (17) | CE-156811 | 0.12–0.5 | 0.25 | 0.5 |
| | Linezolid | 0.5–1 | 1 | 1 |
| | Quinupristin-dalfopristin | 0.06–0.5 | 0.12 | 0.25 |
| | Vancomycin | 0.25 | 0.25 | 0.25 |
| | Penicillin | 0.015–0.06 | 0.015 | 0.015 |
| *Streptococcus pneumoniae*, penicillin nonsusceptible (21) | CE-156811 | 0.06–0.5 | 0.25 | 0.5 |
| | Linezolid | 0.25–1 | 0.5 | 1 |
| | Quinupristin-dalfopristin | 0.12–1 | 0.25 | 1 |
| | Vancomycin | 0.12–0.25 | 0.25 | 0.25 |
| | Penicillin | 0.12–8 | 2 | 4 |
| *Streptococcus pyogenes* (28) | CE-156811 | 0.5–1 | 0.5 | 1 |
| | Linezolid | 1–4 | 2 | 2 |
| | Quinupristin-dalfopristin | 0.25–1 | 0.5 | 0.5 |
| | Vancomycin | 0.5–2 | 0.5 | 0.5 |
| | Levofloxacin | 0.25–2 | 0.5 | 2 |
| *Listeria monocytogenes* (10) | CE-156811 | 4–8 | 4 | 8 |
| | Linezolid | 2 | 2 | 2 |
| | Levofloxacin | 0.5–1 | 1 | 1 |
| | Vancomycin | 1 | 1 | 1 |

*Continued on facing page*

TABLE 1—*Continued*

| Organism (no. of isolates) | Test agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Corynebacterium* spp. (30) | CE-156811 | 0.5–1 | 1 | 1 |
| | Linezolid | 0.12–1 | 1 | 1 |
| | Levofloxacin | 0.25–64 | 0.5 | 32 |
| | Vancomycin | 0.25–1 | 0.5 | 1 |
| *Neisseria* spp. (16) | CE-156811 | 0.5–8 | 4 | 8 |
| | Levofloxacin | 0.004–0.125 | 0.008 | 0.008 |
| *Moraxella catarrhalis* (30) | CE-156811 | 0.25–4 | 2 | 4 |
| | Levofloxacin | 0.03–0.06 | 0.06 | 0.06 |
| | Amoxicillin | 0.015–16 | 1 | 8 |
| *Haemophilus influenzae* (67) | CE-156811 | 1–16 | 4 | 8 |
| | Levofloxacin | 0.004–0.5 | 0.015 | 0.25 |
| | Amoxicillin | 0.25–64 | 2 | 32 |
| *Enterobacteriaceae*[a] (214) | CE-156811 | 16–>64 | >64 | >64 |
| | Levofloxacin | 0.008–>64 | 0.06 | 8 |
| | Ceftazidime | 0.06–>64 | 0.5 | >64 |
| Gram-negative nonfermenters[b] (82) | CE-156811 | 16–>64 | >64 | >64 |
| | Levofloxacin | 0.06–>64 | 2 | 16 |
| | Ceftazidime | 1–>64 | 16 | >64 |
| *Legionella pneumophila* (10) | CE-156811 | 0.25–2 | 1 | 2 |
| | Levofloxacin | 0.002–0.015 | 0.004 | 0.015 |

[a] Ten *Citrobacter diversus*, 20 *Citrobacter freundii*, 17 *Enterobacter aerogenes*, 21 *E. cloacae*, 31 *E. coli*, 13 *Klebsiella oxytoca*, 30 *Klebsiella pneumoniae*, 14 extended-spectrum β-lactamase-producing *K. pneumoniae*, 15 *Serratia marcescens*, 11 *Proteus mirabilis*, 6 *Proteus vulgaris*, 13 *Morganella morganii*, 1 *Providencia rettgeri*, 6 *Salmonella* sp., and 6 *Shigella* sp. isolates.
[b] Twenty-three *P. aeruginosa*, 24 *Burkholderia cepacia*, 19 *Acinetobacter* sp., and 17 *Stenotrophomonas maltophilia* strains.

fluoride. As the hydrolysis presumably involves the intermediacy of the cyclopropyl methyl carbocation, a potential alkylator, this finding precluded our further development of this compound. The comparator agents were supplied from outside vendors (vancomycin was purchased from Sigma Chemical Co.; quinupristin-dalfopristin was provided by Sanofi-Aventis, France; and linezolid was purchased from Sequoia Research Products, Pangbourne, United Kingdom).

CE-156811 is structurally diverse from the original parent molecule of hygromycin A. However, determination of the inhibition of protein synthesis by a coupled transcriptional-translational inhibition assay demonstrated that the potency was very similar to that of hygromycin A. CE-156811 inhibited transcription and translation at 0.149 μM, and hygromycin A inhibited transcription and translation at 0.190 μM. Linezolid inhibited transcription and translation at 2.6 μM, indicating that CE-156811 may be a more potent inhibitor of ribosome function than linezolid. The in vitro activities of CE-156811 and the comparator agents are summarized in Table 1 as the range of MICs, the $MIC_{50}$s, and the $MIC_{90}$s. Overall, CE-156811 had activity similar to or greater than the activities of the comparator agents (linezolid, vancomycin, and quinupristin-dalfopristin) against the majority of gram-positive organisms. CE-156811 had limited activity against gram-negative organ-

TABLE 2. Susceptibilities of linezolid-resistant gram-positive clinical isolates to CE-156811 and comparator agents

| Organism | Strain | Mutant allele (G2576)[a] | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|---|---|
| | | | CE-156811 | Linezolid | Vancomycin | Q-D[b] | Chloramphenicol |
| *S. aureus* | ATCC 29213 | 0/5 | 1 | 2 | 0.5 | 0.06 | 8 |
| *S. aureus* | 01A1220 | 5/5 | 16 | >32 | 1 | 0.06 | >32 |
| *S. aureus* | 01A1221 | 5/5 | 16 | >32 | 1 | 0.06 | 32 |
| *E. faecalis* | ATCC 29212 | 0/4 | 1 | 2 | 2 | 4 | 8 |
| *E. faecalis* | 03A1128 | 4/4 | 16 | >32 | 1 | >32 | >32 |
| *E. faecium* | 03B1073 | 0/6 | 1 | 2 | >32 | 0.5 | 8 |
| *E. faecium* | 03B1077 | 1/6 | 2 | 8 | >32 | 0.125 | 8 |
| *E. faecium* | 03B1074 | 2/6 | 4 | 32 | >32 | 0.5 | 32 |
| *E. faecium* | 03B1075 | 2/6 | 4 | 32 | >32 | 0.5 | 32 |
| *E. faecium* | 03B1078 | 3/6 | 4 | 32 | >32 | 0.5 | 16 |
| *E. faecium* | 03B1076 | 5/6 | 8 | 32 | 0.5 | 0.25 | 32 |

[a] The data represent the number of mutant alleles at position G2576 of 23S rRNA/total number of possible mutant alleles.
[b] Q-D, quinupristin-dalfopristin.

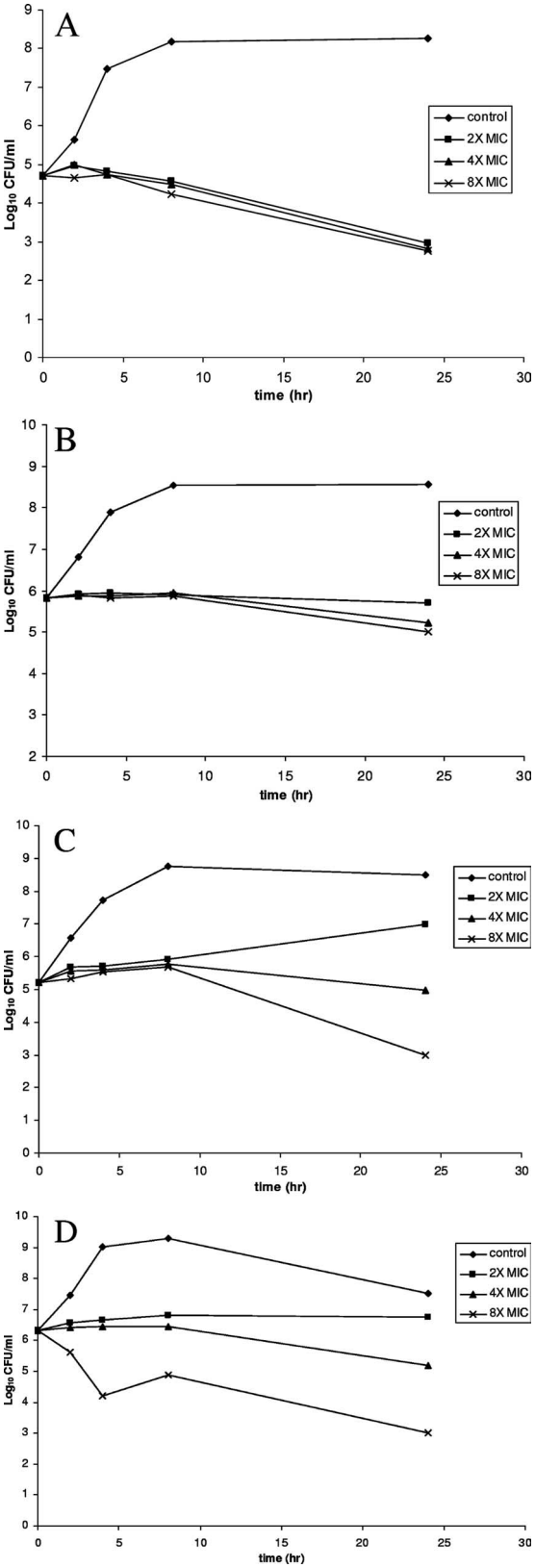


FIG. 2. Killing kinetics of CE-156811 against several gram-positive bacterial species: (A) MRSA 01A1095 (MIC, 1 μg/ml); (B) *E. faecium* 03B1022 (VRE; MIC, 1 μg/ml); (C) *S. pyogenes* 02C0203 (MIC, 0.25 μg/ml); and (D) *S. pneumoniae* 02J1046 (MIC, 0.125 μg/ml).

isms. Of the gram-negative members of the family *Enterobacteriaceae* and nonfermentative bacteria, no one particular species had MICs at the lower end of the range of MICs. One strain of *Acinetobacter* species, one strain of *Shigella* species, one strain of *Escherichia coli*, and one strain of *Enterobacter cloacae* had MICs in the 16- to 32-μg/ml range, whereas all the other strains had MICs of >64 μg/ml. We also investigated the activity of CE-156811 against linezolid-nonsusceptible staphylococci and linezolid-resistant enterococci. CE-156811 has reduced activity against linezolid-resistant *S. aureus* and linezolid-nonsusceptible enterococci (Table 2), indicating the potential for cross-resistance to the oxazolidinone class of antibacterial agents. The MICs were elevated approximately 16-fold in relation to the MIC for a wild-type strain of *S. aureus*. It should be noted that the two staphylococcal isolates were clearly distinct clones, as determined by pulsed-field gel electrophoresis (data not shown), and had the G2576U mutation at all five alleles. The linezolid-resistant enterococcal organisms used in this study were recent clinical isolates that had an identified number of mutant alleles at G2576 in the 23S rRNA gene. There were clear increases in the MICs of CE-156811 with an increasing number of mutant alleles in the enterococci; however, it is not quite clear if the loss in activity was due to the G2576U mutations alone or was due to other factors that may have a role in decreasing the activity of CE-156811.

The bactericidal activity of CE-156811 against several gram-positive bacterial strains was evaluated (Fig. 2). CE-156811 treatment resulted in an approximately 1.5-log reduction at 24 h against all the strains tested, indicating that CE-156811 is bacteriostatic.

Overall, CE-156811 has potent in vitro antibacterial activity that is similar to that of linezolid against a wide range of gram-positive pathogens. It also appears that mutations of G2576U in the 23S rRNA affect the activity of CE-156811.

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