

A Novel Borinic Acid Ester With Antibacterial Activity Against *Staphylococcus aureus*

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ABSTRACT

Background. AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity. *Staphylococcus aureus* (*S. aureus*) is a Gram positive aerobic bacterium of the human skin flora and is a common contributor to various dermatological infections. While the use of currently available topical antibiotics for the treatment of skin infections has been successful, the frequent and sometimes indiscriminate use of these drugs inevitably leads to the development of resistance in the causative organisms and other bacterial species. Clearly, new compounds that are effective against these organisms are needed. We have identified a picolinate borinic acid ester, AN0128 (3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane), that has *in vitro* bacteriostatic activity against *S. aureus*.

INTRODUCTION

AN0128 (3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane) is a novel compound that contains a boron atom within a borinic acid complex (Figure 1). AN0128 has broad spectrum activity against a wide variety of Gram positive bacteria, including many that are known skin colonizers (Table 1). Of particular importance is *S. aureus* and its causal role in atopic dermatitis (AD). AN0128 is a good candidate for a topical antibiotic and is currently being developed by Anacor as a novel therapeutic for AD.

METHODS

MIC Determination. Minimum inhibitory concentrations (MIC's) and minimum bactericidal concentrations (MBC's) were determined in 96-well plates in accordance with NCCLS guidelines. The MIC was defined as the lowest concentration that resulted in over 90% reduction of growth, as compared to a drug-free control. The MBC was defined as the lowest concentration that resulted in over 99.9% killing.

Time-kill Studies. Test tubes containing 10 mL volumes of MHB were supplemented with AN0128 at concentrations equal to 1X, 2X, and 4X multiples of the MIC. Test tubes were also supplemented with Amphotericin B at 1 µg/mL to suppress fungal growth. Bacteria were added to the each tube at a final inoculum size of 0.5×10^5 CFU/mL and incubated with shaking at 37°C. Ten samples of 10 µL volumes were collected from each tube at T = 0, 2, 4, 8, 12, and 24 h post inoculation and plated onto MH agar to determine colony forming units (CFU's).

Post-antibiotic Effect (PAE). An inoculum of *S. aureus* ATCC 29213 (MSSA) in log phase growth was prepared at 5×10^7 CFU/mL in 10 mL of MHB supplemented with 4 µg/mL of AN0128 and incubated at 37°C with shaking for 2 h. A vehicle control was also incubated under identical conditions. The drug was then removed and the bacteria were resuspended in LB broth at a concentration of 3×10^7 CFU/mL. The growth of the bacteria at 37°C with shaking was monitored for 6 h, during which time 100 µL samples were removed every hour for OD600 readings and plating for CFU's. The PAE was defined as the time for the drug-treated culture to increase by one log₁₀, as compared to the time needed for the control.

Figure 1. AN0128 Structure

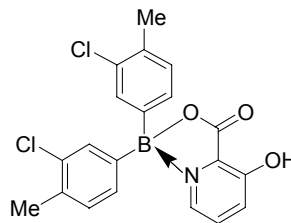


Table 1. MIC of AN0128 Against Select Gram Positive Bacteria

| | <i>S. aureus</i> (MSSA) ATCC 29213 | <i>S. aureus</i> (MRSA) ATCC 33591 | <i>S. aureus</i> (MRSA) ATCC 49521 | <i>S. aureus</i> ATCC 13709 | <i>S. aureus</i> ATCC 12228 | <i>S. epidermidis</i> ATCC 12228 | <i>S. pyogenes</i> | <i>S. pneumoniae</i> | <i>P. aeruginosa</i> ATCC 6919 |
|--------------------|---------------------------------------|---------------------------------------|---------------------------------------|--------------------------------|--------------------------------|-------------------------------------|--------------------|----------------------|-----------------------------------|
| AN0128 MIC (µg/mL) | 1 | 1 | 1 | 1 | 1 | 0.25 | < 0.12 | < 0.12 | 2 |

AN0128 MBC vs. *S. aureus* = ~10 µg/mL

Figure 2. AN0128 Inhibits Growth of MSSA at 2X and 4X MIC

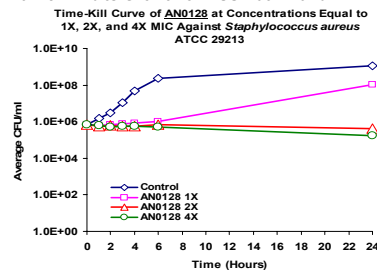


Figure 3. AN0128 Inhibits Growth of MRSA at 2X and 4X MIC

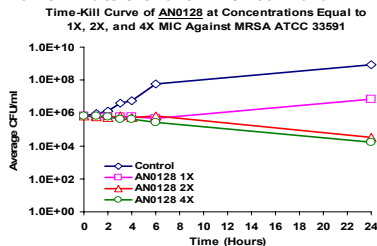
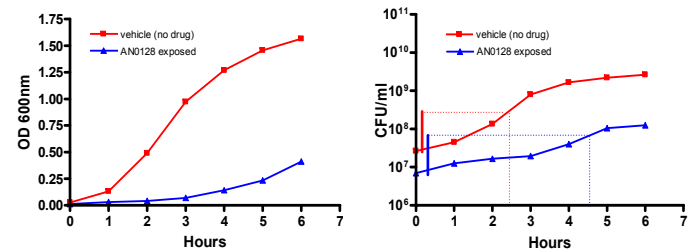


Figure 4. Pre-exposure of *S. aureus* ATCC 29213 to a Bacteriostatic Concentration of AN0128 Significantly Retards Growth



PAE = 4.6 hours - 2.4 hours = 2.2 hours

Table 3. AN0128 Is Similar To Other Antibiotics in Delaying Growth Rate of *S. aureus* ^a

| Drug | Concentration (µg/mL) | PAE (h) |
|---------------|-----------------------|---------|
| AN0128 | 4 | 2.2 |
| Vancomycin | 2 | 2.2 |
| Erythromycin | 0.5 | 3.1 |
| Clindamycin | 0.5 | 2.9 |
| Tetracycline | 0.5 | 2.4 |
| Methicillin | 10 | 1.9 |
| Ciprofloxacin | 0.5 | 2 |
| Rifampin | 0.025 | 2.8 |

^a Data from Craig and Gudmundsson, Antibiotics in Laboratory Medicine

RESULTS AND CONCLUSIONS

• AN0128 is a novel compound which is effective in inhibiting the growth of both MSSA, MRSA, and other bacteria commonly found on the skin.

• AN0128 has an MBC of ~10 µg/mL against *S. aureus* and is cidal at higher concentrations

• AN0128 causes a 2.2 h delay in return to normal growth rate for *S. aureus* ATCC 29213 after pre-exposure to bacteriostatic concentrations.

ACKNOWLEDGEMENTS

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REFERENCES

William A. Craig and Sigurdur Gudmundsson. Antibiotics in Laboratory Medicine, Fourth Edition, Chapter 8: p. 304. Baltimore: Wilkens and Wilkens, 1996