

A Novel Borinic Acid Ester With Antibacterial Activity Against *Propionibacterium acnes*

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ABSTRACT

AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity. *Propionibacterium acnes* (*P. acnes*) is a common bacterial organism found on human skin and is a major causative agent of inflammatory acne. Many current topical antibiotics show only modest efficacy in standard treatment regimens, and antibiotic resistance of the organism due to overuse of these drugs is becoming an increasing problem (Leyden and Levy, 2001). The development of new agents for the treatment of acne is therefore warranted. We have identified a picolinate borinic acid ester, AN0128 (3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane), that has *in vitro* bactericidal activity against *P. acnes*.

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 *P. acnes* and its causal role in acne vulgaris. The rise in antibiotic resistance of *P. acnes* to standard antibiotics necessitates the development of new treatment agents. AN0128 is a good candidate for a topical antibiotic and is currently being developed by Anacor as a novel therapeutic for acne and atopic dermatitis.

METHODS

MIC Determination. Minimum Inhibitory Concentrations (MICs) were determined by macrobroth dilution in reinforced Clostridium broth in accordance with CLSI (formerly NCCLS) guidelines. The MIC was defined as the lowest concentration that resulted in over 90% reduction of growth, as compared to a vehicle (drug-free) control.

Time-kill Assay. An inoculum of *P. acnes* ATCC 6919 in log phase was prepared at 10⁸ CFU/mL in reinforced Clostridium broth and diluted 1:10 in 2 mL to obtain a final test concentration of 10⁷ CFU/mL in each test tube containing AN0128 at concentrations representing 1x, 2x, 5x, or 10x multiples of the MIC. Retinaldehyde, Tetracycline HCl, and Erythromycin were each tested as comparator drugs at 2x and 4x their respective MICs and set up identically to the AN0128 tubes. All tubes were incubated at 37°C under anaerobic conditions for 48 hours, during which time 100 µL samples were removed at 0, 24, and 48 hours for plating on Brain Heart Infusion (BHI) plates to obtain viable counts.

Post-antibiotic Effect (PAE) Study. A 10⁷ CFU/mL suspension of *P. acnes* ATCC 6919 in log phase growth was prepared in 10 mL reinforced Clostridium broth supplemented with AN0128 at 4 µg/mL and incubated at 37°C for 2 hours under anaerobic conditions. This concentration is 2x the MIC and was determined from the time-kill assay to be sub-lethal to *P. acnes* ATCC 6919. A vehicle control (no AN0128) was also set up and incubated under identical conditions. Following treatment, the bacteria were centrifuged and washed twice with 10 mL of broth to remove the drug. The washed bacteria from both tubes were resuspended in 20 mL of fresh broth and incubated at 37°C under anaerobic conditions for 52 hours. Samples were removed from each tube at 4, 8, 24, 28, 46, and 52 hours for absorbance readings and plating onto BHI plates for viable counts. The PAE was defined as the time for the drug-treated culture to increase by one log₁₀ CFU/mL, as compared with the time needed for the control.

Figure 1. AN0128 Chemical Structure

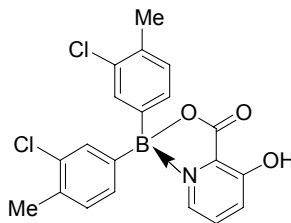


Table 1. MIC of AN0128 Against Select Gram Positive Bacteria

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

Figure 2. AN0128 Is Cidal Against *P. acnes* ATCC 6919 at 5X and 10X MIC

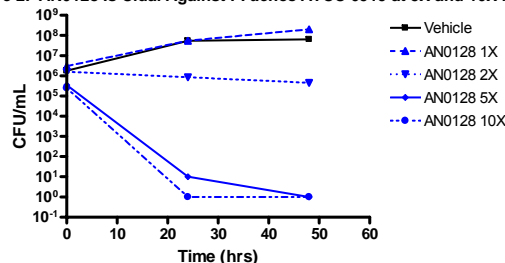


Figure 3. Erythromycin, Tetracycline HCl, and Retinaldehyde Are Bacteriostatic Against *P. acnes* ATCC 6919

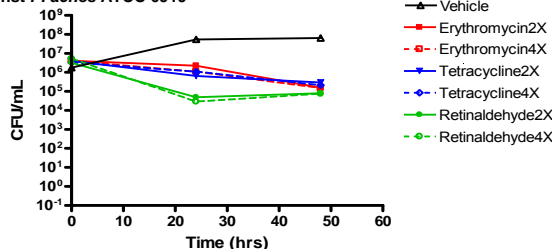
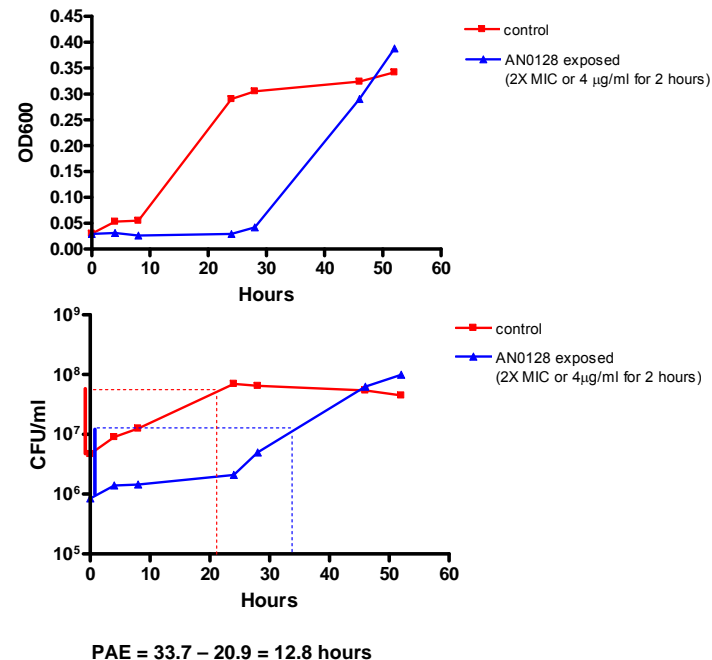


Figure 4. Pre-exposure of *P. acnes* ATCC 6919 to a Sub-lethal Concentration of AN0128 Significantly Retards Growth



RESULTS AND CONCLUSIONS

The MIC was determined to be 2 µg/mL. Time-kill studies showed that AN0128 was bactericidal at concentrations equal to or greater than 10 µg/mL, whereas all reference compounds were bacteriostatic at all equivalent concentrations tested. Exposure of *P. acnes* ATCC 6919 to AN0128 to 4 µg/mL for 2 h caused a delayed return to the normal growth rate. Drug-exposed organisms required 33.7 h to increase one log₁₀ whereas non-exposed organisms required 20.9 h to increase one log₁₀ (PAE = 12.8 h). AN0128 is a novel compound with antibacterial activity against *P. acnes* and is in development as a topical antibiotic for acne.

REFERENCES

Leyden J and Levy S. The Development of Antibiotic Resistance in *Propionibacterium acnes*. *Cutis*. 2001;67:21-24.