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AN0128, A Novel Borinic Acid Ester in Development for the Topical Treatment of Atopic Dermatitis and Acne Vulgaris

Poster Number 145

- Clinical Safety of AN0128, a Novel Borinic Acid Ester
Authors: J.J. Leyden, K. Kaidbey, K. Maples, K. Beutner

Poster Number 108

- AN0128, A Novel Borinic Acid Ester with *In Vitro* and *In Vivo* Anti-Inflammatory Activity
Authors: E. Ip, C. Bellinger-Kawahara, Y. Freund, K. Maples

Poster Number 122

- Preclinical Toxicology of AN0128, A Novel Borinic Acid Ester with Combined Antimicrobial and Anti-Inflammatory Activity
Authors: E. Ip, C. Wheeler, K. Maples

Poster Number 103

- A Novel Borinic Acid Ester With Antibacterial Activity Against *Propionibacterium acnes*
Authors: R. Kimura , C. Bellinger-Kawahara, K. Maples

Poster Number 800

- A Novel Borinic Acid Ester with Antibacterial Activity Against *Staphylococcus aureus*
Authors: R. Kimura, C. Bellinger-Kawahara, K. Maples

Poster Number 104

- A Novel Synthetic Borinic Acid Ester with a Broad Spectrum of Antibacterial Activity
Authors: M.R.K. Alley, W. Mao



K. Beutner^a, J. J. Leyden^b, K. Kaidbey^b, and K. Maples^a,^aAnacor Pharmaceuticals, 1060 E. Meadow Circle, Palo Alto, CA 94303; ^bIvy Laboratories (KGL, Inc.), 505 Parkway, Broomall, PA 19008-4204**ABSTRACT**

AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity. Its minimum inhibitory concentration *in vitro* against *S. aureus* *P. acnes* is 2 mcg/mL for both bacteria. At a concentration of 10 mcg/mL, AN0128 significantly inhibits the release of pro-inflammatory cytokines TNF-alpha, IL-1beta, IL-6, and IL-8 with no significant effect on Th1 and Th2 cytokine release. Systemic and dermal toxicology studies in rats and minipigs for up to 28 days of continuous dosing at concentrations exceeding 10 mg/mL revealed no organ toxicities that would prohibit clinical investigation of a 1% AN0128 Cream applied topically to skin.

Clinical safety and antibacterial activity of 1% AN0128 Cream versus its vehicle was evaluated in a randomized, double-blind, parallel study of 50 normal subjects who presented with baseline *P. acne* colonization of the forehead of >6 log₁₀/cm². This poster will focus only on the clinical safety of 1% AN0128 Cream. Subjects applied the test agents bid for 28 consecutive days with daily clinical evaluations for local tolerance of the tested agents. Treatment emergent systemic adverse events were also monitored daily.

AN0128 Cream and its vehicle were well tolerated with no evidence of erythema or other skin irritation. There were no treatment emergent adverse events or serious adverse events associated with either test agent.

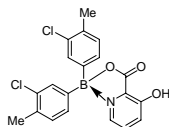
In this first in man study of 1% AN0128 Cream, this new chemical entity was safe with no clinical evidence of adverse systemic effects and excellent local tolerance at the site of topical application.

INTRODUCTION

AN0128 is a novel compound with combined antibacterial and anti-inflammatory activity that is currently being evaluated for clinical efficacy in atopic dermatitis trials. The anti-inflammatory activity, the antibacterial activity, and the preclinical toxicology of AN0128 are the subject of other posters being presented at this meeting (see list at right). Summarized here are the clinical safety data from the first Phase I study completed for AN0128. The purpose of the investigation was to evaluate the safety and tolerance of the test product when applied twice daily for 4 weeks to the forehead of volunteers.

AN0128

3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane)

**METHODS**

This study was a double-blind, randomized trial in which AN0128 Cream, 1% was compared to AN0128 Cream Vehicle in two parallel groups of subjects. Each group consisted of twenty five (25) normal, healthy adult males and females 18 years of age and older. Each volunteer was treated once daily under supervision by a technician at Ivy Laboratories in a standardized manner for 4 weeks. The topical test material was applied by the subjects (unsupervised) at home once daily at bedtime and twice on Saturdays and Sundays. At each visit, a sufficient amount of the test product (about 0.5 mL) was applied to the entire forehead area and rubbed in for about 30 seconds. Erythema, defined as abnormal redness of the skin, was assessed at post screening visits after both 2 and 4 weeks of application. Erythema was scored on a scale of 0-3.

Erythema Scoring Scale

Score	Grade	Guideline
0	None	No redness present
1	Mild	Faintly detectable erythema; very light pink
2	Moderate	Dull red, clearly distinguishable
3	Severe	Deep, dark red

Inclusion Criteria:

- Healthy, adult male and/or female volunteers 18 years of age and older with no past or present history of any significant internal disease (e.g. cardiovascular, pulmonary, renal, etc.)
- All females must have been using a highly effective method of contraception
- Subjects had to be willing to refrain from sunbathing or excessive sun exposure
- Subjects had to be compliant and able to return to Ivy Laboratories as instructed once daily for four weeks

Exclusion Criteria:

- Any volunteer who exhibited any skin disorders of an acute or chronic nature including psoriasis, eczema, etc.
- History of any significant internal disease
- Females who were pregnant, planning a pregnancy or breastfeeding
- Subjects who were known to be allergic to any of the test product(s) or any components in the test product(s)
- Past or present history of drug abuse
- AIDS or AIDS Related Complex
- Any subject not able to meet the study attendance requirements

RESULTS AND CONCLUSIONS

Erythema was not observed in any subject at any screening visit. AN0128 was safe and well tolerated at the site of topical application.

RELATED POSTERS ON AN0128 BEING PRESENTED AT THIS MEETING

AN0128, A Novel Borinic Acid Ester with *In Vitro* and *In Vivo* Anti-Inflammatory Activity. Emily Ip, Carole Bellinger-Kawahara, Yvonne Freund, Kirk Maples

Preclinical Toxicology of AN0128, A Novel Borinic Acid Ester with Combined Antimicrobial and Anti-Inflammatory Activity. Emily Ip, Conrad Wheeler, Kirk Maples

A Novel Borinic Acid Ester With Antibacterial Activity Against *Propionibacterium acnes*. Richard Kimura, Carole Bellinger-Kawahara, Kirk Maples

A Novel Borinic Acid Ester with Antibacterial Activity Against *Staphylococcus aureus*. Richard Kimura, Carole Bellinger-Kawahara, Kirk Maples

A Novel Synthetic Borinic Acid Ester with a Broad Spectrum of Antibacterial Activity. MRK Alley, Weimin Mao

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ABSTRACT

AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity. AN0128 inhibited the release of pro-inflammatory cytokines TNF α , IL-1 β , IL-6, and IL-8 from human peripheral blood mononuclear cells (PBMCs) and THP-1 cells challenged with lipopolysaccharide (LPS), concanavalin A (ConA), or phyto-hemagglutinin (PHA). AN0128 had no effect on Th1 and Th2 cytokine release (IFN γ , IL-2, IL-4, and IL-10) from human PBMCs. AN0128 displayed dose-responsive anti-inflammatory effects in a phorbol-ester induced ear edema model in mice. However, AN0128 failed to produce any observable effect on dinitrofluorobenzene (DNFB)-induced ear swelling in a model of contact hypersensitivity in mice. In this skin sensitization model for which Th1 and Th2 responses are more dominant, AN0128 did not block the dermal immune hypersensitivity response, which is consistent with the *in vitro* results.

INTRODUCTION

Atopic dermatitis is a common skin disorder that is characterized by erythema, edema, pruritis, and dry skin. Pro-inflammatory cytokines and chemokines are important in the recruitment of inflammatory cells and the selective recruitment of Th2 cells into lesional skin¹. *S. aureus* colonization has associated with lesional skin in atopic dermatitis^{2,3}. *S. aureus* colonization can trigger the inflammatory response in the disease. Staphylococcal exotoxins such as superantigens and alpha toxin can activate T cells and stimulate cytokine release⁴. AN0128 is a novel compound in development for the topical treatment of atopic dermatitis. Its combined antibacterial and anti-inflammatory activity make it a good candidate for topical therapy. In *in vitro* assays, AN0128 has shown to be active against microorganisms commonly found colonizing the skin that may be involved in the pathology of acne and atopic dermatitis (please see poster entitled "A Novel Borinic Acid Ester with Antibacterial Activity Against *Staphylococcus aureus*"). In addition, AN0128 inhibits the release of pro-inflammatory cytokines while not affecting the normal Th1/Th2 response. Thus, AN0128 can prevent the release of IL-1 β and TNF α , pro-inflammatory cytokines implicated in the progression of multiple inflammatory skin diseases (atopic dermatitis, psoriasis, acne) without altering the innate immune response.

FIGURE 1. AN0128 [3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane]

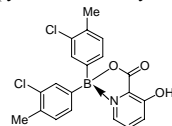


TABLE 1. Cytokine release inhibition from PBMCs or THP-1 cells challenged with LPS, ConA, or PHA at 25 μ M.

Cell Type	Cytokine Release Inhibition (%)							
	TNF α	IL-1 β	IL-6	IL-8	IFN γ	IL-2	IL-4	IL-10
PBMC	100	99	80	122	-20	-9	-21	3
THP-1	57	48	85	56	-	-	-	-

TABLE 2. IC₅₀ values for cytokine release inhibition from human peripheral blood mononuclear cells.

IC ₅₀ Values (μ M)			
TNF α	IL-1 β	IL-6	IL-8
8.9	11	2.8	2.9

FIGURE 2. Effect of AN0128 on inhibition of PMA-induced ear edema in male CD-1 mice. Values (mean \pm SEM, n=5) shown are ear thickness minus baseline thickness (ear without PMA treatment). Percent inhibition of induced edema shown above bars in red. AN0128 significantly inhibited PMA-induced edema, to a level comparable to that achieved by a topical corticosteroid.

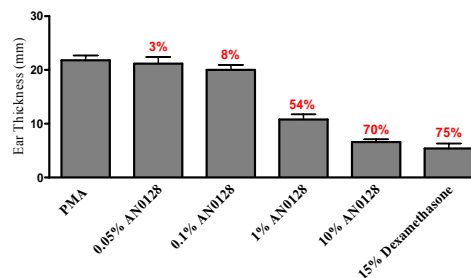
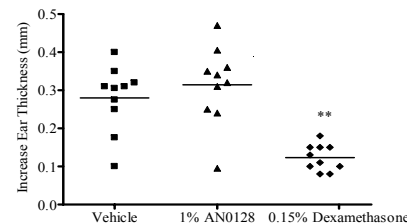


FIGURE 3. Effect of AN0128 on inhibition of DNFB-induced contact hypersensitivity in female BALB/c mice. Values shown are increase in ear thickness, with means indicated by the horizontal bars. AN0128 had no effect on contact hypersensitivity in this model.



METHODS

Cytokine Release. Human PBMCs were stimulated with either LPS, ConA, or PHA. ELISA kits were used for the measurement of pro-inflammatory cytokines (TNF α , IL-1 β , and IL-6, IL-8, Th1 cytokines (IL-2 and IFN γ), or Th2 cytokines (IL-4 and IL-10). For single concentration testing, the final concentration of AN0128 was 25 μ M, and the final DMSO concentration was 0.1%. For IC₅₀ value determinations, concentrations ranged from 10 nM to 25 μ M. PBMCs were stimulated at 37 $^{\circ}$ C with either 1 μ g/mL LPS for 24 h (TNF α , IL-1 β , IL-2, IL-6, and IL-8), 20 μ g/mL ConA for 48 h (IL-4), 2 μ g/mL PHA for 24 h (IFN γ), or 3 μ g/mL PHA for 48 h (IL-10). THP-1 cells (TIB-202, ATCC) were stimulated for 6 h (TNF α) or 24 h (IL-1 β , IL-6, and IL-8) with 1 μ g/mL LPS. AN0128 was evaluated at a concentration of 25 μ M.

Phorbol-Ester (PMA)-Induced Ear Edema. Ear swelling was induced by topical application of phorbol ester 12-myristate 13-acetate (PMA) to the right ears of male CD-1 mice. Vehicle and test substances were applied 30 minutes before and 15 minutes after PMA challenge at a volume of 20 μ L/ear. Five mice were used for each treatment group. Dexamethasone, in a vehicle of ethanol:acetone (1:1), was used as the positive internal reference control. AN0128, also in a vehicle of ethanol:acetone (1:1), was tested at concentrations of 0.05%, 0.1%, 1%, and 10%. PMA (4 μ g/20 μ L of acetone) was topically applied to the anterior and posterior surfaces of the right ear of each animal. A control group was treated with PMA only. Six hours after the PMA challenge, the thickness of the right and left ears was measured using a micrometer gauge. Inhibition of \geq 30% was considered significant anti-inflammatory activity.

Contact Hypersensitivity. Female BALB/c mice were sensitized to DNFB by topical application to the abdomen on two consecutive days. After 5 days, the mice were challenged by a further topical application of DNFB to the right ear and DNFB vehicle to the left ear as an internal control. The right (challenge) ears were dosed with topical vehicle (acetone/olive oil), 200 μ g AN0128, or 30 μ g dexamethasone (as an internal reference control) 1 h before challenge. The total dose volume was 20 μ L/ear. Twenty four h after challenge the thickness of the left and right ears was measured and the difference between them calculated.

CONCLUSIONS

- AN0128 is a novel compound which is effective in both *in vitro* and *in vivo* anti-inflammatory studies.
- Affects pro-inflammatory cytokine release without affecting innate immunity.
- A novel therapeutic which exhibits both anti-inflammatory and antibacterial activity such as AN0128 may be highly effective in the treatment of multiple inflammatory skin diseases (atopic dermatitis, psoriasis, acne). AN0128 is currently being evaluated in clinical trials for efficacy in atopic dermatitis.

REFERENCES

¹Leung DYM, Boguniewicz M, Howell MD, *et al*. New insights into atopic dermatitis. The Journal of Clinical Investigation 2004;113(5):651-657.
²Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. British Journal of Dermatology 1974;90:525-530.
³Aly R, Maibach HI, Shinefield HR. Microbial flora of atopic dermatitis. Arch Dermatol 1977;113:780-785.
⁴Leung DYM. Infection in atopic dermatitis. Curr Opin Pediatr 2003;15:399-404.

Preclinical Toxicology of AN0128, A Novel Borinic Acid Ester with Combined Antimicrobial and Anti-Inflammatory Activity

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ABSTRACT

AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity that is currently being evaluated for clinical efficacy in atopic dermatitis trials (please see poster entitled, "Clinical Safety of AN0128, A Novel Borinic Acid Ester"). Summarized here are the preclinical toxicity studies with AN0128.

METHODS AND RESULTS

Rat oral toxicity studies

Acute. Six male and 6 female Sprague-Dawley rats were treated with AN0128 at 300, 1000, or 2000 mg/kg (preliminary study), and 25 male and 25 female rats were treated with AN0128 at 0, 100, 300, 1000, and 2000 mg/kg (main study). Parameters included mortality, clinical signs, body weight, and post-mortem examination (gross pathology).
Results. The maximum tolerated dose (MTD) was greater than 2000 mg/kg.

7-Day. Twenty male and 20 female Sprague-Dawley rats were treated with AN0128 at 0, 30, 100, or 500 mg/kg/day for 7 days. Parameters included mortality, clinical signs, body weights, food consumption, clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), and post-mortem examination (gross pathology, organs weights, and histopathology).
Results. The no-observed-effect-level (NOEL) was 500 mg/kg/day.

28-Day. Sixty-eight male and 68 female Sprague-Dawley rats were treated with AN0128 at 0, 10, 30, or 100 mg/kg/day for 28 days, with a recovery period of 14 days. Parameters included mortality, clinical signs, body weights, food consumption, ophthalmology, clinical pathology, and post-mortem examination. Blood samples for toxicokinetic analysis were collected on Days 1 and 28.
Results. The NOEL was 100 mg/kg/day. Mean concentration-time profiles increased with increasing dose. For each dose group, AUC values on Days 1 and 28 were similar.

13-Week. Seventy-four male and 74 female Sprague-Dawley rats were treated with AN0128 at 0, 10, 30, or 100 mg/kg/day for 13 weeks, with a recovery period of 4 weeks. Parameters included mortality, clinical signs, body weights, food consumption, ophthalmology, clinical pathology, and post-mortem examination. Blood samples for toxicokinetic analysis were collected on Day 1 and 91.
Results. The NOEL was 100 mg/kg/day. Mean concentration-time profiles increased with increasing dose. Systemic differences in AUC values on Days 1 and 91 were not observed.

Genotoxicity studies

Bacterial reverse mutation. AN0128 was tested against four *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and one *Escherichia coli* strain (WP2 uvrA) using the plate incorporation method in the presence and absence of S9 activation. In the initial toxicity-mutation assay, toxicity was observed beginning at 500, 1500, or 5000 µg per plate, and precipitate was observed beginning at 1500 µg per plate. In the confirmatory mutagenicity assay, AN0128 was plated in triplicate at dose levels ranging from 5 to 5000 µg per plate.
Results. AN0128 demonstrated no mutagenic activity in the presence or absence of S9 activation.

Chromosome aberration. AN0128 was tested for its clastogenic potential in human peripheral blood lymphocytes in the presence and absence of S9 activation. In the preliminary toxicity study, toxicity was observed beginning at 40 µg/mL, and precipitate was observed beginning at 400 µg/mL. In the chromosome aberration assay, AN0128 was tested at 2.5-45 µg/mL, and microscopic examination of at least 200 metaphase spreads was performed for the 5, 10, and 25 µg/mL dose levels.
Results. AN0128 was negative for the induction of structural and numerical chromosome aberrations in the presence or absence of S9 activation using human peripheral blood lymphocytes.

Rat micronucleus. In the definitive micronucleus study, 35 male and 35 female Sprague-Dawley rats were dosed orally with AN0128 at 500, 1000, or 2000 mg/kg and sacrificed at 24 h or at 2000 mg/kg and sacrificed at 48 h. Femurs were removed and the bone marrow extracted. The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes, and 2000 polychromatic erythrocytes were scored for the presence of micronuclei on each slide (for a total of 10,000 per treatment group).
Results. AN0128 was negative for the induction of micronucleated polychromatic erythrocytes in rats.

FIGURE 1. AN0128 [3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane]

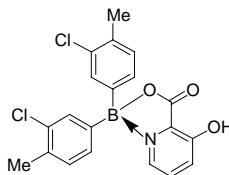


TABLE 1. Summary of AN0128 toxicity studies

Study	Species	AN0128 Doses Tested	Results
Systemic Safety Studies (All GLP)			
Acute Safety	Rat	100, 300, 1,000 & 2,000 mg/kg AN0128	Safe at All Doses
7-Day Safety	Rat	30, 100 & 500 mg/kg/day AN0128	Safe at All Doses
28-Day Safety	Rat	10, 30 & 100 mg/kg/day AN0128	Safe at All Doses
13-Week Safety	Rat	10, 30 & 100 mg/kg/day AN0128	Safe at All Doses
Genotoxicity Studies (All GLP)			
Bacterial Reverse Mutation	<i>in vitro</i>	5 - 5,000 µg/plate AN0128	Safe at All Doses
Chromosome Aberration	<i>in vitro</i>	5 - 25 µg/mL AN0128	Safe at All Doses
Rat Micronucleus	Rat	500, 1,000 & 2,000 mg/kg AN0128	Safe at All Doses
Dermal Safety Studies (All GLP)			
14-Day Safety	Swine	1%, 2.5% & 5% AN0128 Cream	Safe at All Doses
1-Month Safety	Minipig	1%, 2.5% & 5% AN0128 Cream	Safe at All Doses
3-Month Safety	Minipig	1%, 2.5% & 5% AN0128 Cream	Safe at All Doses
Local Tolerance Studies (All GLP)			
Eye Irritation	Rabbit	1% AN0128 Cream	Safe at All Doses
Skin Irritation	Rabbit	1% AN0128 Cream	Safe at All Doses
Nasal Irritation	Rabbit	1%, 2.5% & 5% AN0128 Cream	Safe at All Doses
Sensitization	Guinea Pig	1% AN0128 Cream	Safe at All Doses
Phototoxicity	Guinea Pig	1%, 2.5% & 5% AN0128 Cream	Safe at All Doses

CONCLUSIONS

- Based on preclinical studies, AN0128 is exceptionally safe after both topical and systemic dosing.

METHODS AND RESULTS (continued)

Pig dermal toxicity studies

14-Day. Ten male and 10 female Yorkshire swine were treated with twice daily dermal application of 1%, 2.5%, or 5% AN0128 Cream or AN0128 Cream Vehicle for 14 days to approximately 7% or 10% of the total body surface area (BSA). Following each dermal application, the test site was covered by a stockinette sleeve (non-occlusive binding) secured at both ends using porous athletic tape. Parameters included mortality, clinical signs, body weight, ophthalmology, clinical pathology, and post-mortem examination.

Results. No test article-related effects were observed following twice daily dermal application of 0, 1.76, 4.4, and 8.8 mg/kg/day AN0128 for 14 days to Yorkshire swine, and these dose levels were selected for the 1-month study.

1-Month. Twenty male and 20 female Hanford minipigs were treated with twice daily dermal application of 1%, 2.5%, or 5% AN0128 Cream or AN0128 Cream Vehicle for 30 days to approximately 10% of the total BSA. Parameters included mortality, clinical signs, body weight, ophthalmology, clinical pathology, electrocardiograms, blood pressure, and post-mortem examination. Blood samples for toxicokinetic analysis were collected on Days 2 and 28.

Results. No test article-related effects were observed following twice daily dermal application of 0, 1.76, 4.4, and 8.8 mg/kg/day AN0128 for 30 days to Hanford minipigs. The no-observed-adverse-effect level (NOAEL) was 8.8 mg/kg/day. AN0128 was not detected in any plasma sample (<0.25 µg/mL).

3-Month. Twenty male and 20 female Hanford minipigs were treated with twice daily dermal application of 1%, 2.5%, or 5% AN0128 Cream or AN0128 Cream Vehicle for 90 days to approximately 10% of the total BSA, with a recovery period of 28 days. Parameters included mortality, clinical signs, body weight, ophthalmology, clinical pathology, electrocardiograms, blood pressure, and post-mortem examination. Blood samples for toxicokinetic analysis were collected on Day 88.

Results. Twice daily dermal administration of AN0128 for 90 days was well-tolerated and produced no localized or systemic toxicity when administered to Hanford minipigs. The NOAEL was 8.8 mg/kg/day. AN0128 was not detected in any plasma sample (<0.25 µg/mL).

Local tolerance studies

Eye irritation. Twelve male New Zealand White rabbits were treated with 0.1 mL AN0128 Cream Vehicle or 1% AN0128 Cream to the right eye and rinsed after 24 h. At 24, 48, and 72 h after application, the eyes were examined for evidence of corneal ulceration or opacity, inflammation of the iris, or redness and chemosis of the conjunctiva and scored according to the Draize rating system for ocular lesions.

Results. AN0128 Cream Vehicle and 1% AN0128 Cream did not cause any positive irritation responses. AN0128 Cream Vehicle was classified as non-irritating (mean score of 0), and 1% AN0128 Cream was classified as practically non-irritating (mean score of 1.3).

Skin irritation. Twelve male New Zealand White rabbits were treated with AN0128 Cream Vehicle or 1% AN0128 Cream. Each animal received two 0.5 mL doses under occlusion to one intact skin site and one abraded skin site. The test sites were uncovered and rinsed after 24 h. Signs of edema, erythema, and/or eschar formation were scored according to the Draize rating system for skin reactions, and a Primary Irritation Score (PIS) was calculated.
Results. Neither AN0128 Cream Vehicle nor 1% AN0128 Cream were primary irritants. Erythema was observed in both treatment sites (intact and abraded) at 24 h and 72 h for both AN0128 Cream Vehicle and 1% AN0128 Cream. No signs of edema or eschar formation were seen in any group. The Primary Irritation Scores for AN0128 Cream Vehicle and 1% AN0128 Cream were 2.1 and 1.7, respectively.

Nasal irritation. Twenty male New Zealand White rabbits were treated twice daily with 100 µL of 1%, 2.5, or 5% AN0128 Cream or AN0128 Cream Vehicle to the external nares for 28 days, with a recovery period of 28 days. Parameters included mortality, clinical signs, body weight, food consumption, and post-mortem examination.

Results. No test article-related signs of irritation or systemic toxicity were observed in this study.

Sensitization. Forty-five male and female Hartley albino guinea pigs were used in the guinea pig maximization test. The intradermal induction phase utilized a 10% dilution of AN0128 Cream Vehicle and 1% AN0128 Cream in cottonseed oil. Undiluted test articles were used in the dermal induction and challenge phases.

Results. AN0128 Cream Vehicle and 1% AN0128 Cream were not contact sensitizers.

Phototoxicity. Fifteen male albino hairless guinea pigs were evaluated for primary irritancy and phototoxicity of 1%, 2.5%, or 5% AN0128 Cream or AN0128 Cream Vehicle. For primary irritancy evaluation, 0.3 mL test article was administered using Hilltop® chambers and occluded with dental dam and elastic wrap for 2 h. Animals were evaluated at 15 min, 1, 4 h, and 1, 2, and 3 days. For phototoxicity evaluation, 0.3 mL test article was administered using Hilltop® chambers for 2 h. Animals were then exposed to solar simulated light, including UVR, for 2.25 h at approximately 2.5 human minimal erythema doses and evaluated at 15 min, 1 and 4 h, and 1, 2, and 3 days.
Results. No skin responses indicative of primary irritancy or phototoxicity were observed in any animals.

Poster will be available at www.anacor.com after March 7, 2006

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 10^8 CFU/mL in reinforced Clostridium broth and diluted 1:10 in 2 mL to obtain a final test concentration of 10^7 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 μ L 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 10^7 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 μ g/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

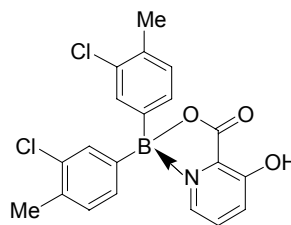


Table 1. MIC of AN0128 Against Select Gram Positive Bacteria

AN0128 MIC (μ g/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	1	0.25	< 0.12

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

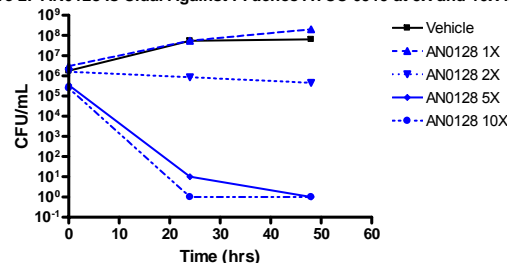


Figure 3. Erythromycin, Tetracycline HCl, and Retinaldehyde Are Bacteriostatic Against *P. acnes* ATCC 6919

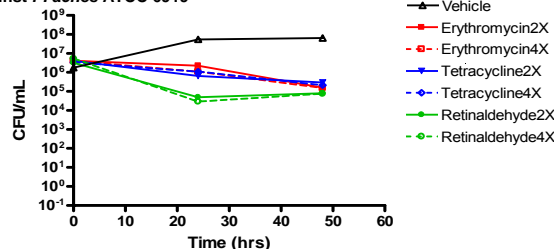
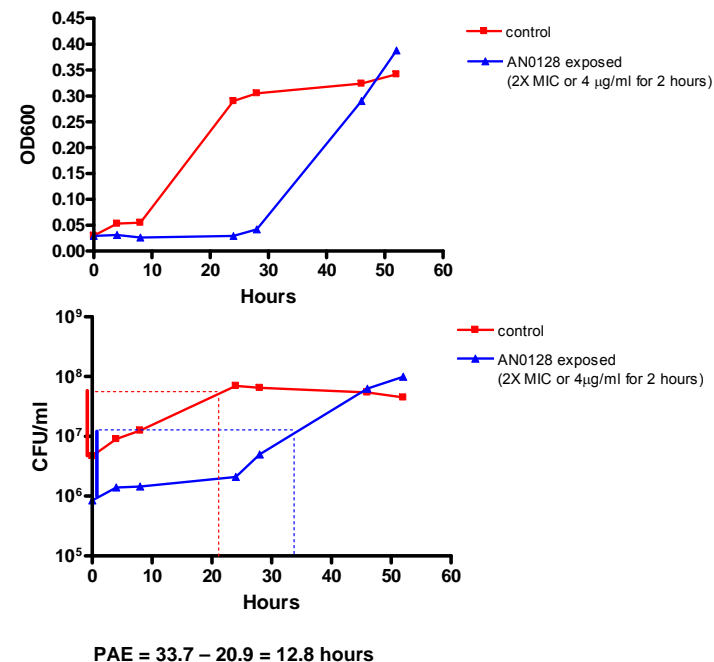


Figure 4. Pre-exposure of *P. acnes* ATCC 6919 to a Sub-lethal Concentration of AN0128 Significantly Retards Growth



RESULTS AND CONCLUSIONS

The MIC was determined to be 2 μ g/mL. Time-kill studies showed that AN0128 was bactericidal at concentrations equal to or greater than 10 μ g/mL, whereas all reference compounds were bacteriostatic at all equivalent concentrations tested. Exposure of *P. acnes* ATCC 6919 to AN0128 to 4 μ g/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.

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

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 picolinic 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

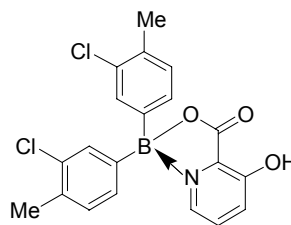


Table 1. MIC of AN0128 Against Select Gram Positive Bacteria

	<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

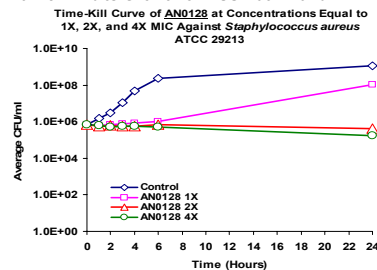


Figure 3. AN0128 Inhibits Growth of MRSA at 2X and 4X MIC

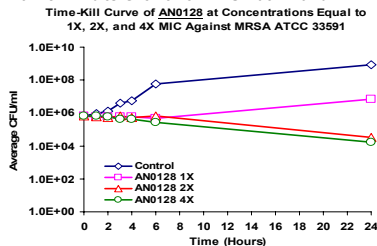
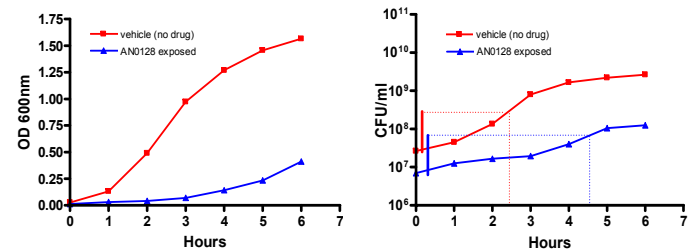


Figure 4. Pre-exposure of *S. aureus* ATCC 29213 to a Bacteriostatic Concentration of AN0128 Significantly Retards Growth



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

This study was conducted with financial support from DARPA.

REFERENCES

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

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ABSTRACT

AN0128 is a novel boronic acid ester with combined antimicrobial and anti-inflammatory activity. This poster reports the following results:

- AN0128 has broad-spectrum antibacterial activity against low GC Gram-positive bacteria with MIC values ranging from 0.125-2 µg/mL.
- AN0128 has good activity against Gram-negative bacteria of the Bacteroides class with MIC values of 0.125-2 µg/mL.
- AN0128 has an MIC₉₀ value of 4 µg/mL against *Propionibacterium acnes* and *P. granulosum* regardless of clindamycin, erythromycin or tetracycline susceptibility.
- AN0128 has an MIC₉₀ value of 4 µg/ml against *S. epidermidis* regardless of clindamycin or erythromycin susceptibility.
- AN0128 has an MIC₉₀ value of 1 µg/mL against MSSA and MRSA *Staphylococcus aureus*.

INTRODUCTION

The continual battle to maintain the efficacy of antibiotics in the face of the rapid rise of bacterial resistance necessitates the need to develop novel classes of antibacterial compounds. In this study we have tested AN0128 (3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane) against a broad panel of Gram-positive and Gram-negative bacteria.

METHODS

Minimal inhibitory concentrations (MIC) were determined as outlined by the CLSI (formerly NCCLS) guidelines with exception that streptococcal MICs were performed in the absence of lysed horse blood. MICs against *Propionibacterium spp* were obtained in the absence of laked sheep blood.

RESULTS and DISCUSSION

AN0128 is a member of a new class of antibiotics that has activity against Gram-positive and anaerobic Gram-negatives from the Bacteroides class (Table 1). The activity is not affected by vancomycin resistance in enterococci (Table 1) or macrolide and tetracycline resistance in *Propionibacterium spp* (Table 2). The presence of macrolide or methicillin resistance in staphylococci also does not affect the MIC₉₀ values for AN0128 (Table 3 and 4). Furthermore the spontaneous frequency of resistance to AN0128 is extremely low, less than $< 1.3 \times 10^{-11}$ for *S. aureus* and $< 8.8 \times 10^{-11}$ for *P. acnes*.

CONCLUSIONS

AN0128, a novel compound in development for the topical treatment of acne vulgaris and atopic dermatitis exhibits:

- Broad-spectrum antibacterial activity with MIC₉₀ values against *Staphylococcus spp* and *Propionibacterium spp* ranging from 1-4 µg/mL.
- MIC₉₀ values for AN0128 are not affected by *Staphylococcus spp* and *Propionibacterium spp* resistance to macrolides, tetracycline, ciprofloxacin or methicillin.

FIGURE 1. Structure of AN0128 (3-hydroxypyridine-2-carboxyloxy-bis(3-chloro-4-methylphenyl)-borane)

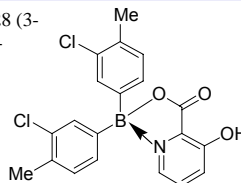


TABLE 1. MIC (µg/mL) values of AN0128 against wide panel of both Gram-negative and Gram-positive bacteria.

Bacteria		AN0128	
Gram-positive	Low GC	<i>Bacillus cereus</i> ATCC 1178	0.5
		<i>Clostridium difficile</i> ATCC 9689	1
		<i>Enterococcus faecalis</i> ATCC 19433	2
		<i>Enterococcus faecalis</i> ATCC 700802 VanR	2
		<i>Enterococcus faecium</i> CT-26	2
		<i>Enterococcus faecium</i> ATCC 700221 VanR	2
	High GC	<i>Eubacterium nodatum</i> ATCC 33099	0.25
		<i>Staphylococcus aureus</i> ATCC 29213	1
		<i>Staphylococcus epidermidis</i> ATCC 12228	2
		<i>Streptococcus mutans</i> ATCC 25175	1
		<i>Streptococcus pneumoniae</i> ATCC 6301	≤ 0.12
		<i>Streptococcus pyogenes</i> ATCC 19615	0.25
Gram-negative	Bacteroides	<i>Mycobacterium tuberculosis</i> ATCC 25177	16
		<i>Mycobacterium avium</i> ATCC 25291	> 32
		<i>Propionibacterium acnes</i> ATCC 6919	2
	Bacteroides	<i>Propionibacterium granulosum</i> NCTC 11865	2
		<i>Bacteroides fragilis</i> ATCC 25285	2
		<i>Porphyromonas gingivalis</i> ATCC 33277	≤ 0.12
		<i>Prevotella intermedia</i> ATCC 25611	0.25
		<i>Tannerella forsythensis</i> ATCC 43037	≤ 0.12
		<i>Escherichia coli</i> ATCC 25922	>64
		<i>Haemophilus influenzae</i> ATCC 49766	>64
<i>Helicobacter pylori</i> 26695	8		
<i>Pseudomonas aeruginosa</i> ATCC 27853	>64		
<i>Treponema denticola</i> ATCC 35405	8		

VanR = vancomycin resistant

TABLE 2. MIC₉₀ (µg/mL) values of AN0128 compared with clindamycin against sensitive and resistant *P. acnes* and *P. granulosum*.

Organism	AN0128			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>P. acnes</i>	33	2.0	2.0	2.0
<i>P. acnes</i> ErmR or TetR or ClnR	59	2.0 - 4.0	2.0	2.0
<i>P. granulosum</i>	4	2.0 - 4.0	2.0	4.0
<i>P. granulosum</i> ErmR	14	2.0 - 4.0	2.0	4.0
Organism	Clindamycin			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>P. acnes</i>	33	0.06-0.125	0.06	0.125
<i>P. acnes</i> ErmR or TetR or ClnR	59	0.125-512	16	512
<i>P. granulosum</i>	4	0.0625-0.5	0.0625	0.5
<i>P. granulosum</i> ErmR	14	2.0-512	256	512

ErmR = erythromycin resistant, TetR = tetracycline resistant, ClnR = clindamycin resistant

TABLE 3. MIC₉₀ (µg/mL) values of AN0128 compared with clindamycin against sensitive and resistant *S. epidermidis*.

Organism	AN0128			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>S. epidermidis</i>	30	1.0-4.0	2.0	4.0
<i>S. epidermidis</i> ErmR and ClnR	30	1.0-16	2.0	4.0
Organism	Clindamycin			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>S. epidermidis</i>	30	0.06-0.125	0.125	0.125
<i>S. epidermidis</i> ErmR and ClnR	30	>32	>32	>32

ErmR = erythromycin resistant, ClnR = clindamycin resistant

TABLE 4. MIC₉₀ (µg/mL) values of AN0128 compared with ciprofloxacin against *S. aureus* MSSA and MRSA strains.

Organism	AN0128			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>S. aureus</i> MSSA	17	1.0	1.0	1.0
<i>S. aureus</i> MRSA	15	0.5-1.0	1.0	1.0
Organism	Ciprofloxacin			
	Total	MIC Range	MIC ₅₀	MIC ₉₀
<i>S. aureus</i> MSSA	17	<0.125-0.25	0.25	0.25
<i>S. aureus</i> MRSA	15	<0.125->64	>64	>64

AN2690, A New Antifungal Agent in Development for the Topical Treatment of Onychomycosis

Poster Number 1611

- Preclinical Toxicology of AN2690, A New Antifungal Agent in Development for the Topical Treatment of Onychomycosis

Authors: E. Ip, S. Baker, C. Bellinger-Kawahara, K. Maples

Poster Number 1607

- Initial Characterization of Resistant Mutants to AN2690, A New Antifungal Agent in Development for the Topical Treatment of Onychomycosis, Predicts a Novel Mechanism of Action

Authors: M.R.K. Alley and W. Mao

Poster Number 1608

- Microbiological Activity of AN2690, a New Antifungal Agent in Development for the Topical Treatment of Onychomycosis

Authors: V. Sanders, M.R.K. Alley, M.A. Ghannoum, A.K. Gupta, N. Isham, J. Khan, M. Kully, W. Mao, K. Maples, J. Singh, M. Zaman and S.J. Baker



Preclinical Toxicology of AN2690, A New Antifungal Agent in Development for the Topical Treatment of Onychomycosis

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ABSTRACT

AN2690 is a new antifungal agent in development for the topical treatment of onychomycosis. Summarized here are the preclinical toxicity studies with AN2690.

METHODS AND RESULTS

Rat oral toxicity study

28-Day. Eighty-two male and eighty-two female Sprague-Dawley rats were treated orally with AN2690 (in 1% carboxymethylcellulose) at 0, 30, 50, 100, or 200 mg/kg/day for 28 days. Parameters included mortality, clinical signs, body weights, food consumption, ophthalmoscopy, hematology, coagulation, clinical chemistry, urinalysis, and post-mortem examination (gross pathology, organs weights, and histopathology). Blood samples for toxicokinetic analysis were collected on Days 1 and 28.

Results. There were no test article-related clinical or ophthalmologic findings or effects on body weight or clinical pathology parameters. Microscopic findings of the nonglandular stomach were observed at all dose levels, implying that the no-observed-effect-level (NOEL) was less than 30 mg/kg. Mean plasma concentration-time profiles increased with increasing dose. C_{max} and AUC values on Day 28 were substantially lower than on Day 1. C_{max} values on Day 1 ranged from 0.61-23.41 $\mu\text{g/mL}$, and C_{max} values on Day 28 ranged from 0.18-4.33 $\mu\text{g/mL}$.

Safety pharmacology studies

Receptor binding, CYP450, and HERG channel. AN2690 was tested at 10 μM against a panel of 50 transmembrane and soluble receptors, ion channels, and monoamine transporters and against 5 cytochrome P450 isoforms. AN2690 was tested at 1 μM against the HERG channel.

Results. AN2690 did not significantly inhibit any of the receptors, ion channels, or transporters tested. AN2690 also did not significantly inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4. Based on its HERG channel tail current inhibition, AN2690 was classified as a low-potency HERG-channel blocker (lowest category).

Cardiovascular. Four male Beagle dogs were dosed orally with 0, 30, 100, or 200 mg/kg AN2690 in a latin-square design. Telemetry recording were continuously collected on each treatment day for at least 1.5 pre-dosing and 24 h post-dosing. Parameters included systolic, diastolic, and mean arterial pressures, pulse rate, electrocardiograms, locomotor activity, and body temperature.

Results. No effects were observed at 30 mg/kg. Given at 100 or 200 mg/kg, AN2690 caused a dose-related, reversible, mild to marked hypotension with transient increases in heart and pulse rates.

Functional observational battery. Twenty-four male Sprague-Dawley rats were dosed orally with 0, 30, 100, or 200 mg/kg AN2690. Behavioral, neurological (sensorimotor)/neuromuscular, and autonomic observations were recorded before dosing and at approximately 15 min, 1, 2, and 3 h post-dosing.

Results. AN2690 produced no pharmacologically relevant effects on behavioral, neurological/neuromuscular, or autonomic activities at any dose tested.

Genotoxicity studies

Bacterial reverse mutation. AN2690 was tested against four *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and one *Escherichia coli* strain (WP2 uvrA) using the plate incorporation method in the presence and absence of S9 activation. In the initial toxicity-mutation assay, toxicity was observed beginning at 50 or 150 μg per plate, and no precipitate was observed at any dose level. In the confirmatory mutagenicity assay, AN2690 was plated in triplicate at dose levels ranging from 0.5 to 500 μg per plate.

Results. AN2690 demonstrated no mutagenic activity in the presence or absence of S9 activation.

FIGURE 1. AN2690 (5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole)

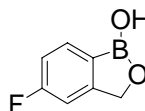


TABLE 1. Summary of AN2690 toxicity studies

Study	Species	AN2690 Doses Tested	Results
Systemic Safety Study (GLP)			
28-Day Safety	Rat	30, 50, 100, & 200 mg/kg/day oral	No Effects on Clinical Signs, Ophthalmologic Findings, Body Weight, or Clinical Pathology at Any Dose Stomach Epithelial Effects At All Doses by Histology
Safety Pharmacology Studies (GLP, except as noted)			
Receptor Binding, CYP450, & HERG Channel (non-GLP)	<i>in vitro</i>	1 or 10 μM	No Significant Inhibition of 50 Receptor Panel No Significant Inhibition of CYP450s Low Potency HERG-Channel Inhibition
Cardiovascular	Dog	30, 100, & 200 mg/kg	Safe at 30 mg/kg Reversible Mild to Marked Hypotension, Increased Heart and Pulse Rates at 100 and 200 mg/kg
Functional Observational Battery	Rat	30, 100, & 200 mg/kg	Safe at All Doses
Genotoxicity Studies (GLP)			
Bacterial Reverse Mutation	<i>in vitro</i>	0.5 - 500 $\mu\text{g/plate}$	Safe at All Doses
Chromosome Aberration	<i>in vitro</i>	2.5, 5, & 10 $\mu\text{g/mL}$	Safe at All Doses
Rat Micronucleus	Rat	250, 500, & 1000 mg/kg	Safe at All Doses
Dermal Safety Studies (GLP)			
28-Day Safety	Minipig	1%, 7%, 10%, & 15% AN2690 Solution over 5% body surface area	Dose-Responsive, Reversible Skin Irritation NOAEL for Local Tolerance = 1%; No Systemic Toxicity at Any Dose NOAEL for Systemic Safety = 15%
Local Tolerance Studies (GLP)			
Eye Irritation	Rabbit	10% AN2690 Solution	AN2690 Vehicle and 10% AN2690 Solution Were Irritants
Skin Irritation	Rabbit	10% AN2690 Solution	Not Primary Irritant
Skin Sensitization	Guinea Pig	10% AN2690 Solution	Not Sensitizer

CONCLUSION

• AN2690, a novel compound in development for the topical treatment of onychomycosis, exhibits excellent systemic safety and supports topical dosing up to 7.5%.

METHODS AND RESULTS (continued)

Genotoxicity studies (continued)

Chromosome aberration. AN2690 was tested for its clastogenic potential in human peripheral blood lymphocytes in the presence and absence of S9 activation. In the preliminary toxicity study, toxicity was observed beginning at 15.1 $\mu\text{g/mL}$. In the chromosome aberration assay, AN2690 was tested at 1.25-25 $\mu\text{g/mL}$, and microscopic examination of at least 200 metaphase spreads was performed for the 2.5, 5, and 10 $\mu\text{g/mL}$ dose levels.

Results. AN2690 was negative for the induction of structural and numerical chromosome aberrations in the presence or absence of S9 activation using human peripheral blood lymphocytes.

Rat micronucleus. Based on pilot toxicity, toxicity, and supplemental toxicity studies, the high dose for the definitive study was set at 1000 mg/kg, the estimated maximum tolerated dose. In the definitive micronucleus study, 35 male and 35 female Sprague-Dawley rats were dosed orally with AN2690 at 250, 500, or 1000 mg/kg and sacrificed at 24 h or at 1000 mg/kg and sacrificed at 48 h. Femurs were removed and the bone marrow extracted. The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes, and 2000 polychromatic erythrocytes were scored for the presence of micronuclei on each slide (for a total of 10,000 per treatment group).

Results. AN2690 was negative for the induction of micronucleated polychromatic erythrocytes in rats.

Minipig dermal toxicity study

28-Day. Twenty-six male and twenty-six female Göttingen minipigs were treated with daily dermal application of 0%, 1%, 7%, 10%, or 15% AN2690 Solution (in propylene glycol/ethanol, 20/80, v/v) for 28 days to approximately 5% of the total body surface area (BSA). Parameters included mortality, clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, and post-mortem examination. Blood samples for toxicokinetic analysis were collected on Days 1 and 28.

Results. The no-observed-adverse-effect level (NOEL) for systemic toxicity was 15% (13.8 mg/kg/day). The NOAEL for local irritation was 1% (0.9 mg/kg/day). Due to skin irritation, dosing for some animals at 10% and all animals at 15% was stopped on Day 21. AN2690 was detected in plasma samples from all dose groups except 1%. C_{max} and AUC values on Days 21 and 28 were higher than on Day 1. C_{max} values on Day 1 ranged from 0.12-0.30 $\mu\text{g/mL}$, and C_{max} values on Days 21/28 ranged from 0.40-0.90 $\mu\text{g/mL}$.

Local tolerance studies

Eye irritation. Six male New Zealand White rabbits were treated with 0.1 mL AN2690 Vehicle or 10% AN2690 Solution to the right eye and rinsed after 24 h. At 1, 24, 48, and 72 h after application, the eyes were examined for evidence of corneal ulceration or opacity, inflammation of the iris, or redness and chemosis of the conjunctivae and scored according to the Draize rating system for ocular lesions.

Results. Both AN2690 Vehicle and 10% AN2690 Solution were ocular irritants, and contact of AN2690 Solution with eyes should be avoided.

Skin irritation. Twelve female New Zealand White rabbits were treated with AN2690 Vehicle or 10% AN2690 Solution. Each animal received two 0.5 mL doses under occlusion to one intact skin site and one abraded skin site. The test sites were uncovered and rinsed after 24 h. Signs of edema, erythema, and/or eschar formation were scored according to the Draize rating system for skin reactions, and a Primary Irritation Score (PIS) was calculated.

Results. Neither AN2690 Vehicle nor 10% AN2690 Solution were primary irritants. Mild erythema was observed in both groups. The Primary Irritation Scores for AN2690 Vehicle and 10% AN2690 Solution were 1.1 and 1.4, respectively.

Sensitization. Forty-one female Hartley albino guinea pigs were used in the guinea pig maximization test. The intradermal induction phase utilized a 3% dilution of AN2690 Vehicle and 10% AN2690 Solution in cottonseed oil. A 0.5% dilution of AN2690 Vehicle and 10% AN2690 Solution was used in the challenge phase.

Results. AN2690 Vehicle and 10% AN2690 Solution were not contact sensitizers.

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ABSTRACT

AN2690 is a new class of antifungal agent that is in development for the topical treatment of onychomycosis. AN2690 has broad-spectrum activity against filamentous fungi and yeast. Since the genome sequence of *Trichophyton rubrum* is unknown, and there are no genetic tools available in this microorganism, we therefore used the yeasts *Saccharomyces cerevisiae* and *Candida albicans* to get a better understanding of how AN2690 works. We isolated resistant mutants to both AN2690, and AN1677, a structurally related analog to AN2690, and found the frequency of resistance was similar to that of amphotericin B. Finally, we tested the MIC of resistant isolates against common antifungal agents including azoles, terbinafine, ciclopirox and amphotericin B. We found that both AN2690 and AN1677 mutants were cross-resistant to each other but none of the mutants conferred resistance to any of these common antifungal agents.

INTRODUCTION

Onychomycosis is a common disease affecting 50% of people by the age of 70. The fungal pathogens include dermatophytes, molds, and yeast. The two dermatophytes most commonly implicated in onychomycosis are *T. rubrum* and *T. mentagrophytes*, accounting for more than 90% of the cases of onychomycosis. The lack of a genomic sequence or any genetic tools for *Trichophyton* spp. makes it difficult to study the mechanism of action of AN2690 in either *Trichophyton* spp. therefore, we used the yeasts *S. cerevisiae* and *C. albicans*. We report here the isolation and genetic characterization of *S. cerevisiae* and *C. albicans* mutants resistant to AN2690 and AN1677.

METHODS

Chemicals and strains

AN2690 (1,3-dihydro-5-fluoro-1-hydroxy-2,1-benzoxaborole) and AN1677 (1,3-dihydro-5-fluoro-1-phenyl-2,1-benzoxaborole) were obtained from Anacor Pharmaceuticals, Inc. (Palo Alto, CA, USA) (Figure 1). *S. cerevisiae* strain ATCC201388 (MATa *his3D1 leu2D0 met5D0 ura3D0*) was obtained from ATCC (Manassas, VA, USA). *Candida albicans* (ATCC90028) was kindly provided by Dr. Olga Lomovskaya.

Determination of MICs

The minimal inhibitory concentration (MIC) was essentially performed following the CLSI (formerly NCCLS) guidelines outlined in the M27 protocol with the exception of using YPD or synthetic defined media (SDM).

Isolation of spontaneous resistant mutants

The wild type haploid *S. cerevisiae* ATCC201388, or wild type *C. albicans* ATCC90028 was grown overnight in YPD broth (BD, NJ USA) at 30°C and 1 ml of cells was plated out onto YPD agar plates (YPD broth+1.5% Bacto-agar, BD, NJ USA) containing either 1.6, 3.2 or 6.4 µg/ml AN2690/AN1677 (equivalent to 4x, 8x, 16x MIC of AN2690/AN1677). Resistant mutants appeared after 2 days incubation at 30°C. Frequency of resistance was determined by dividing the number of resistant mutants by the total number of cells plated as determined by plating dilutions of the overnight culture on YPD plates.

RESULTS and DISCUSSION

AN2690 has excellent activity against fungi and yeast, with MICs ranging from 0.5 µg/ml for yeast *S. cerevisiae*/*C. albicans* to 1 µg/ml for the filamentous fungi *T. rubrum* (Table 1). AN2690, AN1677 spontaneous resistant mutants isolated from *S. cerevisiae*, *C. albicans* showed 16-128 fold increased resistance (Table 3,4). The frequency of resistance was comparable to that of well-known anti-fungal agent amphotericin B (Table 2). AN2690, AN1677 mutants were also shown to be cross-resistant to each other, suggesting that both compounds may act on the same target (Table 3,4). We further tested resistant mutants against various antifungal agents with known mechanisms of action. Our mutants did not show any resistance to these compounds (Table 5,6), which suggests that AN2690, AN1677 act very differently from these antifungal agents.

FIGURE 1. Structure of AN2690 (1,3-dihydro-5-fluoro-1-hydroxy-2,1-benzoxaborole) and AN1677 (1,3-dihydro-5-fluoro-1-phenyl-2,1-benzoxaborole)

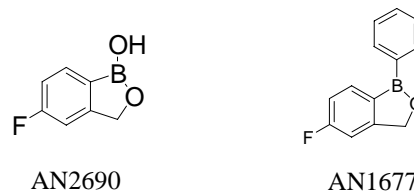


Table 1. MIC (µg/ml) against *T. rubrum*, *S. cerevisiae*, *C. albicans*

Anti-fungal agent	<i>T. rubrum</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>
Amphotericin B	1	2	0.25-1
AN1677	1	0.5	0.5
AN2690	1	0.25	0.5

Table2. Frequency of resistance

Anti-fungal agent	<i>S. cerevisiae</i>	<i>C. albicans</i>
Amphotericin B	6.0×10^{-7}	-
AN1677	2.2×10^{-7}	1.0×10^{-7}
AN2690	4.0×10^{-7}	5.5×10^{-8}

Table 3. MIC (µg/ml) of *S. cerevisiae* resistant mutants

Anti-fungal agent	<i>S. cerevisiae</i>		
	wild type	AN1677 mutant	AN2690 mutant
AN1677	0.5	8	8
AN2690	0.25	16	16

Table 4. MIC (µg/ml) of *C. albicans* resistant mutants

Anti-fungal agent	<i>C. albicans</i>		
	wild type	AN1677 mutant	AN2690 mutant
AN1677	0.5	64	64
AN2690	0.5	64	64

Table 5. *S. cerevisiae* mutant MICs to various antifungal agents (µg/ml)

Anti-fungal agents	<i>S. cerevisiae</i>		
	wild type	AN1677 mutant	AN2690 mutant
AN2690	0.5	8	16
Amphotericin B	0.125	0.125	0.125
Cerulenin	0.5	0.5	0.5
Itraconazole	0.125	0.25	0.125
Aculeacin A	4	4	4
Ciclopirox	0.5	0.5	0.5
Terbinafine	4	8	4
Nikkomycin Z	64	64	64
Tunicamycin	8	8	8

Table 6. *C. albicans* mutant MICs to various antifungal agents (µg/ml)

Anti-fungal agents	<i>C. albicans</i>		
	wild type	AN1677 mutant	AN2690 mutant
AN2690	0.5	64	64
Amphotericin B	0.5	0.5	0.5
Cerulenin	2	2	2
Itraconazole	0.5	0.5	0.5
Aculeacin A	0.5	1	0.25
Ciclopirox	0.5	0.5	0.5
Terbinafine	16	16	16
Nikkomycin Z	2	4	4
Tunicamycin	4	4	4

Amphotericin B: complexes with ergosterol in the cell membrane, which leads to destabilization and changes in the permeability of the membrane.
 Cerulenin: inhibit fatty acid biosynthesis.
 Terbinafine: inhibits ergosterol biosynthesis via inhibition of squalene epoxidase.
 Aculeacin A: inhibit β-glucan synthesis (cell wall)
 Ciclopirox: iron chelation
 Itraconazole: inhibits ergosterol biosynthesis via inhibition of cytochrome P450 14a-demethylase.
 Nikkomycin Z: inhibit chitin synthesis (cell wall)
 Tunicamycin: inhibit glycosylation of proteins

CONCLUSIONS

- AN2690 has unique mechanism of action.
- AN2690 represents a new class of antifungal compounds.
- AN2690 has a frequency of resistance similar to amphotericin B.

Microbiological Activity Of AN2690, A New Antifungal Agent In Development For The Topical Treatment Of Onychomycosis

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ABSTRACT

AN2690 is a new class of antifungal agent in development for the topical treatment of onychomycosis. This poster reports the following results:

- AN2690 has broad-spectrum antifungal activity with MIC values ranging from 0.25-8 µg/mL.
- The MIC of AN2690 against 100 clinical isolates each of *T. rubrum* and *T. mentagrophytes* ranged from 1-8 µg/mL and the MFC ranged from 8->128 µg/mL
- The MIC of AN2690 against *T. rubrum* was unaffected by the presence of 5% powdered keratin.
- AN2690 showed an excellent ability to penetrate full thickness human nails at concentrations above it's MIC.

In conclusion, the antifungal activity of AN2690 and it's exceptional ability to penetrate human nails make it a promising candidate for the topical treatment of onychomycosis.

INTRODUCTION

We are developing a novel boron-containing small molecule, AN2690, (Figure 1) as a topical treatment for onychomycosis, a fungal infection of the toe and fingernails and nail bed. In order to treat the disease, the drug must have the following attributes:

- Activity against the major pathogenic dermatophytes
- Activity within the nail plate
- Ability to penetrate through the nail plate to the nail bed

In this poster, we report the results of our studies to test AN2690 for these criteria

METHODS

MIC values and MFC values were obtained using standard NCCLS guidelines. MIC in the presence of keratin was determined following literature procedure.¹ Turchub studies were conducted by MedPharm. Ltd.²

RESULTS and DISCUSSION

Antifungal activity

AN2690 was screened for antifungal activity against dermatophytes, yeast and filamentous fungi and the results of these are shown in Table 1. In this study AN2690 was compared with ciclopirox and from the results, AN2690 showed broad-spectrum activity against all fungal strains tested with MIC's ranging from 0.25-8 µg/mL.

MIC and MFC study against *T. rubrum* and *T. mentagrophytes*

AN2690 was screened for activity against 100 clinical isolates each of *T. rubrum* and *T. mentagrophytes* and compared with ciclopirox (Table 2). AN2690 showed an MIC₉₀ of 8 µg/mL against both strains. Furthermore, AN2690 showed cidal activity against these dermatophytes with an MFC₉₀ of 64 µg/mL against *T. rubrum* and MFC₉₀ of 64 µg/mL against *T. mentagrophytes*. Ciclopirox was not cidal at the concentrations tested (16 µg/mL).

TABLE 1. MIC (µg/mL) values of AN2690 compared with ciclopirox against dermatophytes, yeast and filamentous fungi

Isolate	AN2690	Ciclopirox
<i>T. rubrum</i>	1-8	1
<i>T. mentagrophytes</i>	2-8	0.5
<i>T. tonsurans</i>	2-4	≤ 0.5
<i>E. floccosum</i>	≤ 0.5	≤ 0.5
<i>M. audouinii</i>	2	1
<i>M. canis</i>	2	≤ 0.5
<i>M. gypseum</i>	2	≤ 0.5
<i>A. fumigatus</i>	0.25	nt
<i>F. solani</i>	≤ 0.5	4
<i>C. albicans</i>	1	0.5
<i>C. glabrata</i>	≤ 0.5	≤ 0.5
<i>C. krusei</i>	1	≤ 0.5
<i>C. neoformans</i>	0.25	nt
<i>C. parapsilosis</i>	≤ 0.5	≤ 0.5
<i>C. tropicalis</i>	≤ 0.5	≤ 0.5
<i>M. furfur</i>	1	≤ 0.5
<i>M. pachydermatis</i>	1	≤ 0.5
<i>M. sympodialis</i>	1	≤ 0.5

FIGURE 1. Structure of AN2690 (5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole)

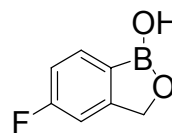


FIGURE 2. Turchub cells designed so that formulations pass through human nails prior to contact with the test organism.²



TABLE 2. MIC (µg/mL) and MFC (µg/mL) values of AN2690 compared with ciclopirox against 100 isolates each of *T. rubrum* and *T. mentagrophytes*

Isolate	AN2690					
	MIC Range	MIC ₅₀	MIC ₉₀	MFC Range	MFC ₅₀	MFC ₉₀
<i>T. rubrum</i>	1.0-8.0	4	8	8.0-128	64	64
<i>T. mentagrophytes</i>	4.0-8.0	4	8	16->128	64	>128
Isolate	Ciclopirox					
	MIC Range	MIC ₅₀	MIC ₉₀	MFC Range	MFC ₅₀	MFC ₉₀
<i>T. rubrum</i>	0.06-1.0	0.25	0.5	0.5->16	>16	>16
<i>T. mentagrophytes</i>	0.125-0.5	0.25	0.5	1.0->16	>16	>16

TABLE 3. MIC (µg/mL) values of AN2690 compared with ciclopirox in the presence of keratin

Isolate	AN2690	Ciclopirox
<i>T. rubrum</i>	1	1
<i>T. rubrum</i> + 5% keratin powder	2	1

RESULTS and DISCUSSION (continued)

Activity in the presence of keratin

It has been reported that the MIC of terbinafine decreased 32 fold in the presence of 5% powdered keratin,¹ suggesting it is less active within the nail plate. We used the method reported to determine if the activity of AN2690 was affected by keratin and found essentially no difference in the MIC in the presence or absence of keratin (Table 3).

Nail penetration and perungual activity

Finally, we tested AN2690 for its ability to penetrate full thickness human nails using turchub cells shown in Figure 2.² In this study, the test articles were applied to the top receiver well and the antifungal agent must first pass through full thickness human nail plates to show a zone-of-inhibition (ZOI) in the receiver well. AN2690 (10% w/v in ethyl acetate, propylene glycol 1:1) was compared to ciclopirox (8% w/w in commercial lacquer) and amorolfine (5% w/w in commercial lacquer). The test articles were dosed daily for five days at a concentration of 40 µL/cm² nail plate. The results of this study are shown in Figures 3-6. The vehicle showed no ZOI and neither did ciclopirox nor amorolfine in their commercial lacquers demonstrating that, at the concentration applied to the nail plate, neither drug penetrated full thickness nail plates at concentrations above their MIC's. By contrast, AN2690 showed a significant ZOI as shown in Figure 4, demonstrating that, at the concentration dosed, AN2690 was able to penetrate full thickness nail plates and disseminate through a large area of the receiver cells at concentrations above its MIC to prevent the growth of the dermatophyte.

FIGURE 3. Turchub result of placebo:



FIGURE 5. Turchub result of ciclopirox, 8% w/w in commercial lacquer



FIGURE 4. Turchub result of AN2690, 10% w/v in ethyl acetate, propylene glycol 1:1



FIGURE 6. Turchub result of amorolfine, 5% w/w in commercial lacquer



CONCLUSIONS

AN2690, a novel compound in development for the topical treatment of onychomycosis exhibits:

- Broad-spectrum antifungal activity with MIC's ranging from 0.25-8 µg/mL
- Antifungal activity in the presence of nail keratin
- Ability to penetrate the human nail plate significantly better than ciclopirox and amorolfine in their commercial vehicles

References

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