

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 non-glandular 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)

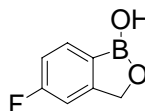


TABLE 1. Summary of AN2690 toxicity studies

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.