

Antifungal Activity and Mechanism of Action of a Benzoxaborole, AN2718, which is in Development for the Treatment of Tinea Pedis

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Introduction

AN2718 (1) is a member of a new class of antifungals, benzoxaboroles, which inhibits fungal growth by blocking protein synthesis (2) using the oxaborole tRNA trapping (OBORT) mechanism. AN2718 is being developed for the topical treatment of tinea pedis including the hard to treat moccasin-type, which at present is only treatable with oral antifungals.

Methods

MICs were determined according to CLSI guidelines (3). Most of the data supplied for Table 1 were generated by NAEJA Pharmaceuticals, Alberta, Canada. The *Candida albicans* and *Aspergillus fumigatus* cytoplasmic leucyl-tRNA synthetases (LeuRS) were over-expressed in *E. coli* as N-terminal six-histidine-tagged proteins and purified by standard techniques using a nickel column. Enzyme inhibition was determined by the inhibition of leucine incorporation into crude baker's yeast tRNA measured by TCA precipitation. To obtain a crystal structure for the *C. albicans* cytoplasmic leucyl-tRNA synthetase we over-expressed the C-terminal six-histidine-tagged editing domain. AN3018-AMP bound crystals were collected on the European Synchrotron Radiation Facility beamlines. The AN3018-AMP complex structure was solved by molecular replacement with PHASER.

Results

Table 1. Broad-spectrum Antifungal Activity (µg/mL)

Compound	Yeast				Molds		Dermatophytes			
	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Candida parapsilosis</i>	<i>Cryptococcus neoformans</i>	<i>Fusarium solani</i>	<i>Aspergillus fumigatus</i>	<i>Epidermophyton floccosum</i>	<i>Microsporum canis</i>	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubrum</i>
AN 2718	1	0.25	4	2	4	1	2	4	1	0.5
Fluconazole	0.25	8	2	2	>128	>128	4	2	16	2
Terbinafine	1	64	0.5	0.125	64	4	0.015	0.03	0.015	0.008

Table 2. *Trichophyton spp.* MIC₉₀

	AN2718 (µg/mL)			Terbinafine (µg/mL)		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
<i>T. rubrum</i> (n=100)	0.25 - 1	0.5	0.5	≤0.001 - 16	0.008	0.008
<i>T. mentagrophytes</i> (n=100)	0.25 - 1	0.5	1	0.002 - 0.06	0.008	0.015

Data supplied by Center for Medical Mycology, OH

Table 3. *Trichophyton spp.* MFC₉₀

	AN2718 (µg/mL)			Terbinafine (µg/mL)		
	Range	MFC ₅₀	MFC ₉₀	Range	MFC ₅₀	MFC ₉₀
<i>T. rubrum</i> (n=100)	0.5 - 64	8	32	0.004 - >64	0.015	0.03
<i>T. mentagrophytes</i> (n=100)	0.5 - >32	8	16	0.002 - >0.5	0.03	0.12

Data supplied by Center for Medical Mycology, OH

Results (cont'd)

Table 4. Biochemical inhibition of fungal cytoplasmic LeuRS

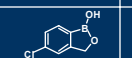
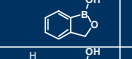
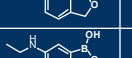
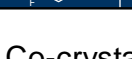
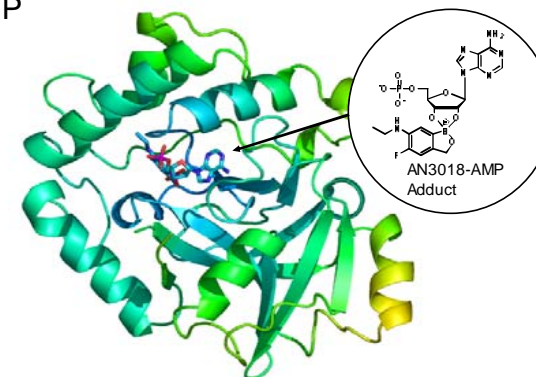
Compound	Structure	<i>C. albicans</i>		<i>A. fumigatus</i>	
		IC ₅₀ (µM)	MIC (µg/mL)	IC ₅₀ (µM)	MIC (µg/mL)
AN2718		4.2	1	2	1
AN2679		5.7	1	2.6	2
AN3009		4.3	8	4.1	>64
AN3018		38	2	19.4	64

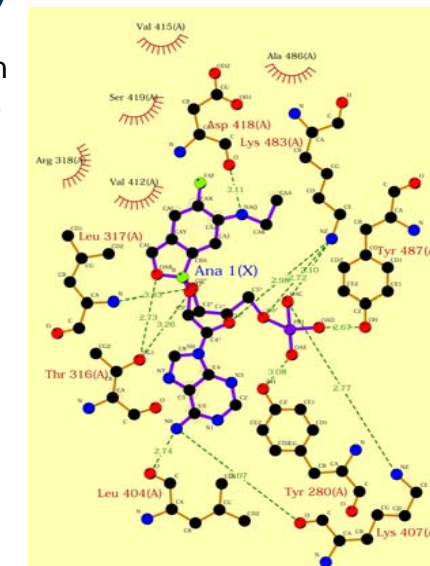
Figure 1. Co-crystal structure of *C. albicans* LeuRS editing domain with AN3018 and AMP



Data set collected at 2.2 Å,
space group $P2_12_12_1$

Results (cont'd)

Figure 2. *C. albicans* LeuRS editing domain complex with AN3018 and AMP



Conclusions

- AN2718 inhibits LeuRS by the OBORT (2) mechanism of trapping tRNA^{Leu} in the editing site of LeuRS
- AN2718 has good MIC₉₀ activity against the dermatophytes, *T. rubrum* and *T. mentagrophytes*
- These data support the further evaluation of AN2718 as a topical agent to treat tinea pedis

References

- J Med Chem. 2006 49:4447-50. Discovery of a new boron-containing antifungal agent, 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole, for the potential treatment of onychomycosis. Baker SJ et al.
- Science. 2007 316:1759-61. An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. Rock FL, et al.
- CLSI/NCCLS. 2002. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: approved standard. NCCLS document M38-A. NCCLS, Wayne, Pa.