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Activity of F901318, a Member of the New Orotomide Class of Antifungal Agents, against Clinical *Aspergillus* Isolates from the UK and Austria.

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Abstract

Background: F901318 is the most advanced candidate from a novel series of antifungal agents, the orotomides. F901318 is currently being developed for the treatment of serious systemic fungal infections, in particular invasive aspergillosis. In this study the potency of F901318 was compared with established antifungal agents against a large panel of clinical *Aspergillus* isolates from the UK and the Tyrol region of Austria. M**ethods:** A total of 221 *Aspergillus* strains (*A. fumigatus* n= 80, *A. terreus* n = 45, *A. flavus* n=50 and *A. niger* n=46) were tested for susceptibility to F901318. The testing methods used are as outlined in CLSI document M38-A2. All organisms were tested in the microdilution format. Voriconazole, posaconazole, itraconazole and amphotericin B were tested as comparators. In an inhaled model of aspergillosis, mice were infected with a L98H/TR cyp51A mutant strain and treated with posaconazole 10mg/kg/day PO or F901318 8mg/kg IV TID. **Results:** All *Aspergillus* isolates tested were susceptible to F901318 with MICs < 0.03 mg/L. Retesting of strains at a lower drug range, (132 strains representing all four species) revealed on-scale MICs of 0.002- 0.008 mg/L for all isolates except two *A. niger* isolates with MICs of 0.016 mg/L. Fourteen isolates of *A. fumigatus* were resistant to one or more azoles because of known cyp51A mutations (M220K, G54E, G138C, G54R, Y431C, H147Y/G448, G434C, M222T, G54V and L98H/TR). In these isolates the MIC for F901318 was also 0.008mg/L, indicating a lack of cross resistance with the azoles. In an inhaled model of aspergillosis, mice infected with a L98H/TR cyp51A mutant and treated with F901318 showed 80% survival 10 days post infection compared to 0% survival for posaconazole at supra-pharmacological exposures. **Conclusion:** In this study, F901318 was shown to have potent activity against a large collection of the commonly isolated pathogenic *Aspergillus* species. Activity was also demonstrated against strains with eleven well characterised cyp51A mutations resistant to one or more azoles, indicating a lack of cross-resistance. F901318 also produced a significant survival benefit in pulmonary aspergillosis caused by an azole-resistant isolate.

Introduction

There is an urgent requirement for novel antifungal agents distinct from current classes of agent to combat the emergence of drug resistance particularly to the azole group of drugs. F901318 is currently being developed for the treatment of serious systemic fungal infections, in particular invasive aspergillosis. F901318 has a novel mechanism of action, namely selective inhibition of the enzyme DHODH a key enzyme in the pyrimidine biosynthesis pathway. In this study the potency of F901318 was compared with established antifungal agents against a large panel of clinical *Aspergillus* isolates from the UK and the Tyrol region of Austria including clinical azole resistant isolates. In addition the *in vivo* efficacy of F901318 was assessed against an azole resistant isolate of *A. fumigatus* in a murine model of pulmonary aspergillosis.

Methods

A total of 221 *Aspergillus* clinical isolates (*A. fumigatus,* n= 80, *A. terreus,* n = 45, *A. flavus,* n=50 and *A. niger,* n=46) were tested for susceptibility to F901318 using methods outlined in CLSI document M38-A2 (1). 110 isolates were from the UK and 111 were from Austria. Voriconazole, posaconazole, itraconazole and amphotericin B were tested as comparators.

In an inhaled murine model of pulmonary aspergillosis (2), mice were infected intranasally with conidia of a L98H/TR cyp51A mutant *A. fumigatus* strain and treated with posaconazole 10mg/kg/day PO or F901318 8mg/kg IV TID for 10 days and survival monitored throughout the study.

Results

Initial MIC testing revealed that all 221 isolates had MICs <0.03 mg/L when tested in the range 16-0.03 mg/L. Testing at lower dilution ranges showed very low F901318 MICs for the majority of isolates. The MICs for F901318 and comparator agents are shown in table 1. F901318 is much more potent than the other agents against all four species of *Aspergillus*.

Table 1 Geometric mean MIC and MIC range (mg/L) of F901318 and comparator drugs

		F901318	Itraconazole	Posaconazole	Voriconazole	Amphotericin B
A. fumigatus	Geo mean	0.008	1.00	0.30	0.46	0.68
(n=80)	Range	0.004-0.016	0.06-16	0.03-16	0.06-16	0.25-1
A. terreus	Geo mean	0.006	0.25	0.14	0.18	1.49
(n=45)	Range	0.002-0.008	0.06-1	0.06-2	0.03-0.5	0.125-4
A. flavus	Geo mean	0.007	0.21	0.087	0.26	0.79
(n=50)	Range	0.004-0.008	0.125-1	0.03-1	0.06-1	0.5-2
A. niger	Geo mean	0.007	0.62	0.16	0.51	0.46
(n=46)	Range	0.004-0.016	0.125-16	0.03-2	0.125-16	0.125-1

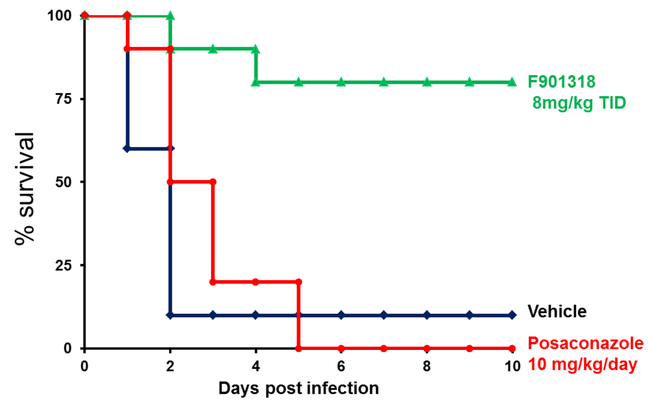
Fourteen isolates of *A. fumigatus* were resistant to one or more azoles and had known *cyp51A* mutations. Table 2 shows the F901318 and azole MICs of these isolates along with the the *cyp51A* mutation. It is clear that the F901318 MICs are very low for these isolates and there is no evidence of cross resistance.

Table 2 F901318 and Azole MICs (mg/L) for 14 isolates with known *cyp51A* mutations

Isolate	Cyp mutation	F901318	Itraconazole	Posaconazole	Voriconazole
F6919	M220K	0.008	16	2	2
F7075	G54E	0.008	16	16	0.5
F11628	G138C	0.008	16	2	8
F12219	G54R	0.008	16	2	0.5
F12636	G54E	0.008	16	2	0.25
F12776	Y431C	0.008	16	2	4
F13619	H147Y G448S	0.008	2	1	8
F13747	G434C	0.008	16	2	8
F14403	G54R	0.008	16	2	0.5
F14532	M222T	0.008	4	1	1
F16134	M220K	0.008	16	16	4
F16216	L98H +TR	0.008	16	2	8
F16157	G54V	0.008	16	2	0.5
F17294	L98H +TR	0.008	16	2	8

In vivo data

A severe model of murine pulmonary invasive aspergillosis was set up using an *A. fumigatus* isolate with an L98H/TR genotype. Mice were treated with either posaconazole 10mg/kg/day PO or F901318 8mg/kg IV TID for 10 days. The survival curves are shown in the figure. At the end of the study 80% of mice treated with F901318 survived whilst none of the mice treated with posaconazole survived. This clearly demonstrates the efficacy of F901318 against an azole-resistant *A. fumigatus* isolate in a relevant animal model.



Conclusions

F901318 has potent *in vitro* activity against the 4 main clinical *Aspergillus* species.

F901318 is highly active against isolates resistant to one or more azoles because of *cyp51A* mutations.

F901318 is highly active against *A. terreus* isolates which frequently demonstrate resistance to amphotericin B.

In a murine model of invasive pulmonary aspergillosis, F901318 produced a survival rate of 80% in mice infected with an azole-resistant *A. fumigatus.* In the same model, posaconazole at exposure levels above those seen in humans produced 0% survival. F901318 is an attractive candidate for treating infections caused by azole-resistant aspergilli.

References

1) Clinical Laboratory Standards Institute, 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

2) Howard SJ, Lestner JM, Sharp A. *et al*. Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: Clinical implications for antifungal therapy. J. Infect. Dis. 2011. **203:** 1324-1332.