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In vitro activity of the novel antifungal compound F901318 against difficult-to-treat *Aspergillus* isolates

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Background: F901318 is a new antifungal agent with a novel mechanism of action with activity against *Aspergillus* species. We investigated the *in vitro* activity of F901318 against a collection of *Aspergillus* isolates.

Methods: A total of 213 *Aspergillus* isolates were used in this study. A total of 143 *Aspergillus fumigatus sensu stricto* isolates were used, of which 133 were azole resistant [25 TR_{34}/L98H; 25 TR_{46}/Y121F/T289A; 33 *A. fumigatus* with *cyp51A*-associated point mutations (25 G54, 1 G432 and 7 M220); and 50 azole-resistant *A. fumigatus* without known resistance mechanisms]. Ten azole-susceptible *A. fumigatus* isolates were used as WT controls. The *in vitro* activity was also determined against *Aspergillus calidoustus* (25 isolates), *Aspergillus flavus* (10), *Aspergillus nidulans* (10) and *Aspergillus tubingensis* (25). F901318 activity was compared with that of itraconazole, voriconazole, posaconazole, isavuconazole, amphotericin B and anidulafungin. Minimum effective concentrations and MICs were determined using the EUCAST broth microdilution method.

Results: F901318 was active against all tested isolates: *A. fumigatus* WT, MIC_{90} 0.125 mg/L (range 0.031–0.125); TR_{34}/L98H,TR_{46}/Y121F/T289A and azole resistant without known resistance mechanisms, MIC_{90} 0.125 mg/L (range 0.031–0.25); *A. fumigatus* with *cyp51A*-associated point mutations, MIC_{90} 0.062 mg/L (range 0.015–0.125); and other species, *A. calidoustus* MIC_{90} 0.5 mg/L (range 0.125–0.5), *A. flavus* MIC_{90} 0.062 mg/L (range 0.015–0.62), *A. nidulans* MIC_{90} 0.125 mg/L (range 0.062–0.25) and *A. tubingensis* MIC_{90} 0.062 mg/L (range 0.015–0.25).

Conclusions: F901318 showed potent and consistent *in vitro* activity against difficult-to-treat *Aspergillus* spp. with intrinsic and acquired antifungal resistance due to known and unknown resistance mechanisms, suggesting no significant implications of azole resistance mechanisms for the mode of action of F901318.

Introduction

Aspergillus species account for the majority of invasive mould infections.[1] Invasive aspergillosis is a life-threatening disease mainly affecting immunocompromised patients. Only two classes of antifungal agents are licensed for the primary therapy of invasive aspergillosis, namely the triazoles and amphotericin B. Since 2002, voriconazole has been the mainstay of therapy for invasive aspergillosis.[2] Since then, also because of improved diagnostics and supportive care management, the survival rate of patients with invasive aspergillosis improved from <30% to >70% in recent years.[3,4]

The improved survival rate of patients with invasive aspergillosis is threatened by the worldwide emergence of azole resistance in *Aspergillus fumigatus*. The prevalence of azole resistance in *A. fumigatus* isolates ranges between 1% and >10%, depending on the geographical region.[5] However, studies performed in high-risk patient groups, such as haematology or ICU patients, indicate that these prevalence rates may be up to 30%,[6,7] but these high resistance rates are probably restricted to hospitals in areas with high prevalence of azole resistance. Azole-resistant invasive aspergillosis seems to be mainly due to resistance mutations associated with environmental selection, i.e. TR_{34}/L98H or TR_{46}/Y121F/T289A, and these mutations account for 67% of resistance in Belgium and >80% of resistance cases in the Netherlands.[8] Importantly, case series indicate that azole-resistant invasive aspergillosis is associated with treatment failure.[8] Emerging species like *Aspergillus tubingensis* and *Aspergillus calidoustus* often have increased MICs of one or multiple antifungal agents.[9,10] Therefore, the clinical development of new antifungal drug classes is critical to overcome the current and future challenges in the management of invasive aspergillosis.

F901318 (F2G Limited) is a novel antifungal compound. It belongs to the new orotomide class targeting dihydroorotate dehydrogenase (DHODH), an important enzyme for pyrimidine biosynthesis.[11] This is a novel target, and F901318 has activity against *Aspergillus*, *Scedosporium* and *Fusarium* species and endemic fungi.[11,12] Pharmacokinetic studies in mice indicate good tissue distribution and the agent is now under evaluation in Phase I trials in both oral and intravenous formulations.[11] In this study, we evaluated the *in vitro* activity of F901318 against a collection of phenotypic azole-resistant *A. fumigatus* isolates. In addition, the activity against a collection of other difficult-to-treat *Aspergillus* species, including *Aspergillus nidulans*, *Aspergillus flavus* complex, *Aspergillus calidoustus* and *Aspergillus tubingensis* was investigated.

Materials and methods

Isolates

A total of 213 *Aspergillus* isolates were used in this study. A total of 143 *A. fumigatus sensu stricto* isolates were used, of which 133 were azole resistant [25 TR_{34}/L98H; 25 TR_{46}/Y121F/T289A, 33 were *A. fumigatus* with *cyp51A*-associated point mutations (25 G54, 1 G432 and 7 M220) and 50 were azole-resistant *A. fumigatus* without known resistance mechanisms]. Ten azole-susceptible *A. fumigatus* isolates were used as WT controls. Furthermore, a collection of *A. calidoustus* (25 isolates), *Aspergillus flavus* (10), *Aspergillus nidulans* (10) and *A. tubingensis* (25) were tested. All isolates were from the fungal culture collections of the Radboud University Medical Center and the Canisius Wilhelmina Hospital, Nijmegen, the Netherlands. The identification of isolates was performed by sequencing the β-tubulin gene (*A. fumigatus*, *A. nidulans*, *A. flavus* and *A. tubingensis*) or the calmodulin gene (*A. calidoustus*). The full *cyp51A* gene of *A. fumigatus sensu stricto* was sequenced as previously described.[13]

Susceptibility testing

The susceptibility of *Aspergillus* isolates to F901318 was compared with their susceptibility to voriconazole, itraconazole, isavuconazole, posaconazole, amphotericin B and anidulafungin. Susceptibility testing of voriconazole, itraconazole, isavuconazole, posaconazole, amphotericin B and anidulafungin was performed according to the EUCAST broth microdilution reference method (E.Def 9.2). F901318 was dissolved in DMSO. The concentration range of all drugs was 0.016–16 mg/L. Endpoints were determined after 48 h of incubation at 100% inhibition compared with growth control except for anidulafungin, for which the endpoint was defined as the lowest concentration in which abnormal, short and branched hyphal elements were observed after 24 h of incubation.

Statistics

Susceptibility testing was performed in duplicate for F901318 and the mean MIC was used for comparison and calculation of geometric mean (GM) MICs. For the other antifungals a single MIC value was determined. For F901318, when the mean of two measurements was between two dilutions, the next higher dilution was used to calculate the MIC_{50} and MIC_{90}. Calculations were performed by the use of GraphPad Prism (version 5.03). For comparing the GM MICs for *A. fumigatus* isolates, Kruskal–Wallis analysis of variance was used.

Results

MIC distributions of F901318 are shown in Figure 1. F901318 was most active against *A. flavus* and least active against *A. calidoustus*. The highest MIC found for *A. fumigatus*, *A. nidulans* and *A. tubingensis* was 0.25 mg/L. The highest MICs for *A. flavus* and *A. calidoustus* were 0.125 and 0.5 mg/L, respectively. The essential agreement between the duplicates was 91%. The agreement within one dilution was 100%.

The MIC_{50}, MIC_{90} and MIC ranges of all tested antifungals are shown in Table 1, grouped based on *Aspergillus* species and based on their azole resistance mechanism. The MICs of F901318 were in general lower than the MICs of the different azoles and amphotericin B. To test whether the observed differences in F901318 MICs (Figure 1) for the *A. fumigatus* subgroups were statistically significant, the GM MICs for these isolates were compared with the GM MICs for phenotypically WT *A. fumigatus* isolates. For TR_{34}/L98H isolates the F901318 MIC results were in the same range as for phenotypically WT *A. fumigatus* isolates. In contrast, the F901318 GM MIC for TR_{46}/Y121F/T289A isolates was one dilution higher than that for the WT isolates ($P < 0.01$), although this did not result in a higher MIC_{90}. The F901318 GM MIC for *A. fumigatus* with *cyp51A*-associated point mutations was one dilution step lower than for WT isolates (for both G54 and M220 mutations when analysed individually) ($P < 0.01$). The F901318 GM MIC for other subgroups was not significantly different from the F901318 GM MIC for WT isolates.

Some *A. flavus* isolates had reduced susceptibility to azoles and/or amphotericin B. F901318 showed potent activity against all of these isolates. Several *A. tubingensis* isolates had a high MIC of voriconazole while isolates of *A. nidulans* had a slightly increased MIC of amphotericin B compared with *A. fumigatus* WT isolates. The MIC of F901318 was consistently low (for both *A. tubingensis* and *A. nidulans*). *A. calidoustus* had higher MICs of the azoles, amphotericin B and anidulafungin compared with *A. fumigatus*. The MIC_{50} and MIC_{90} of F901318 were also higher compared with *A. fumigatus*, but an MIC >0.5 mg/L was not found.

Discussion

Despite optimal antifungal treatment, invasive aspergillosis remains a difficult-to-manage disease, especially when *Aspergillus* species with intrinsic or acquired resistance are involved. Therefore, development of new antifungal compounds is paramount. *In vitro* and *in vivo* activity of F901318 has been demonstrated against *A. fumigatus* previously.[11] Here, we demonstrated that F901318 has a potent *in vitro* activity against *A. fumigatus* isolates with intrinsic and acquired azole resistance. All *A. fumigatus* isolates had an F901318 MIC of ≤0.25 mg/L. No substantial implications of the specific azole resistance mechanism for the activity of F901318 were demonstrated.

Subgroup comparisons of *A. fumigatus* isolates showed small differences in the GM MIC when isolates with TR_{46}/Y121F/T289A and single-point mutations were compared with phenotypically WT isolates. The differences in GM MIC cannot be explained by the azole resistance mechanism, as the target of F901318 is different from the target of azoles. It is possible that the differences for TR_{46}/Y121F/T289A can be explained by the recent emergence of these TR_{46}/Y121F/T289A mutants and their related linage separate from the WT isolates, rather than by the mutation itself.[14] However, it is unlikely that such minor differences in susceptibility are relevant for the clinical management of patients with *Aspergillus* diseases.

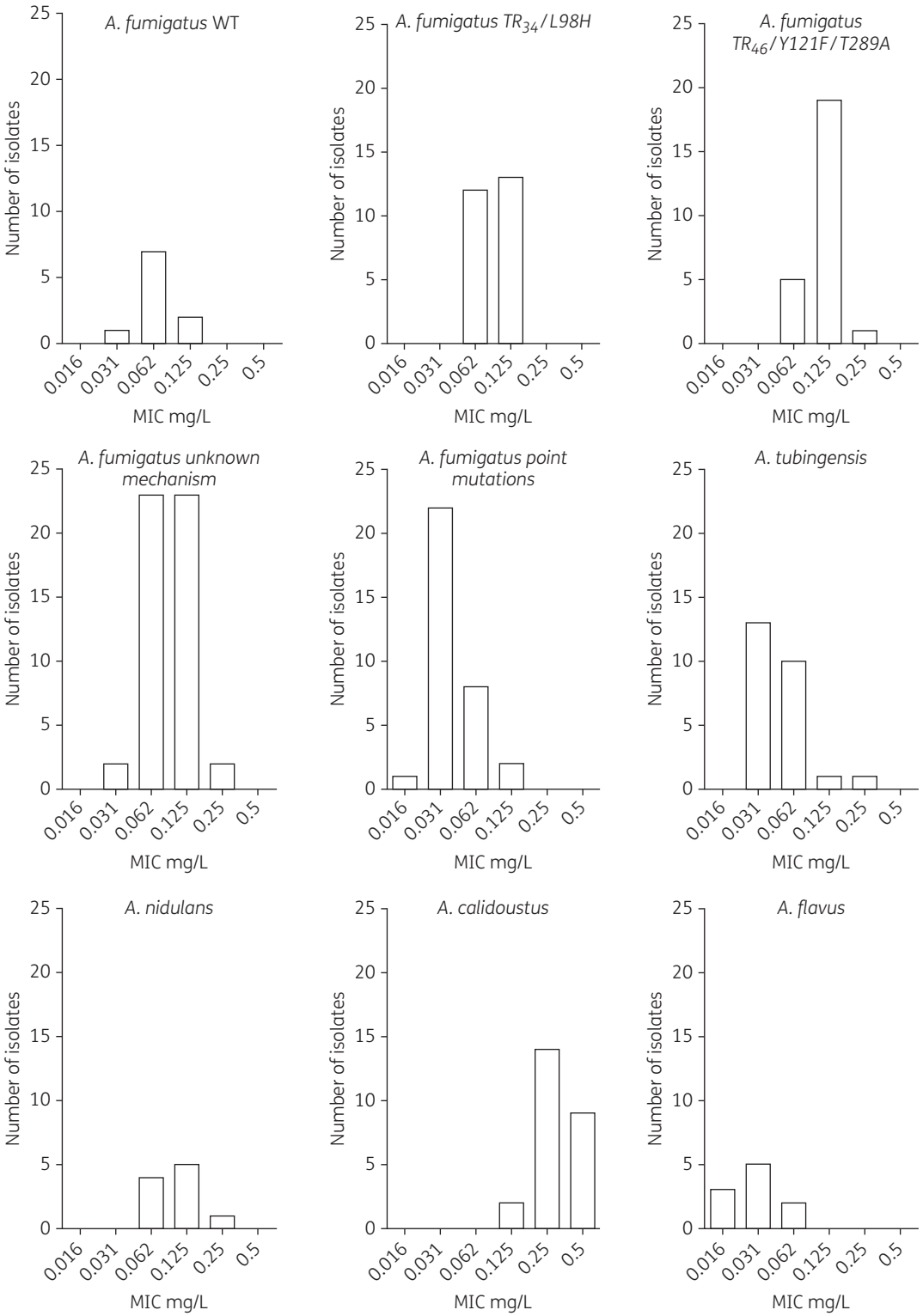

Figure 1. MIC distributions of F901318 for *Aspergillus* species grouped based on species and for *A. fumigatus* based on resistance mechanism. Susceptibility testing of F901318 was performed using the EUCAST methodology, with 100% growth inhibition as endpoint. Susceptibility testing was performed in duplicate; the highest MIC for the duplicates was used to draw the figures.

Table 1. Activity of F901318 and six comparator antifungal agents against 213 azole-resistant and difficult-to-treat *Aspergillus* isolates, determined by EUCAST methodology

			MIC_{50}/MIC_{90} (MIC range) (mg/L)						
Species	Mutation	Isolates	itraconazole	voriconazole	posaconazole	isavuconazole	amphotericin B	anidulafungin	F901318
A. fumigatus	WT	10	0.25/0.5 (0.25–0.5)	0.5/0.5 (0.25–1)	0.063/0.063 (0.031–0.063)	0.5/1 (0.5–2)	0.5/1 (0.5–1)	0.016/0.031 (0.016–0.031)	0.063/0.125 (0.031–0.125)
A. fumigatus	TR_{34}/L98H	25	>16/>16 (16 to >16)	4/16 (2–16)	0.5/2 (0.25–16)	4/16 (4 to >16)	0.5/1 (0.25–1)	0.016/0.031 (<0.016–0.063)	0.125/0.125 (0.031–0.125)
A. fumigatus	TR_{46}/Y121F/T289A	25	>16/>16 (0.5 to >16)	>16/>16 (>16)	0.5/1 (0.25–2)	>16/>16 (>16)	0.5/1 (0.5–1)	0.016/0.063 (<0.016–0.063)	0.125/0.125 (0.062–0.25)
A. fumigatus	point mutations	33	>16/>16 (0.125 to >16)	0.5/4 (0.125 to >16)	4/>16 (0.031 to >16)	1/4 (0.125–4)	1/1 (0.25–2)	0.031/0.125 (<0.008–2)	0.031/0.063 (0.016–0.125)
A. fumigatus	unknown mechanism	50	>16/>16 (0.25 to >16)	2/>16 (1 to >16)	0.25/1 (0.063 to >16)	2/16 (0.25 to >16)	1/2 (0.25–2)	0.031/0.125 (<0.016–0.25)	0.063/0.125 (0.031–0.25)
A. calidoustus		25	>16/>16 (1 to >16)	8/16 (8–16)	>16/>16 (>16)	4/8 (4–8)	1/2 (0.5–2)	1/4 (0.125–16)	0.25/0.5 (0.125–0.5)
A. flavus		10	0.25/1 (0.125–4)	4/>16 (1 to >16)	0.125/0.25 (0.063–0.5)	2/4 (1–16)	1/4 (1 to >16)	0.016/0.031 (0.016–0.031)	0.031/0.063 (0.016–0.063)
A. nidulans		10	0.25/0.5 (0.125–0.5)	0.5/0.5 (0.125–0.5)	0.125/0.25 (0.063–0.5)	0.25/0.5 (0.125–1)	1/2 (0.5–4)	0.031/0.063 (<0.016–0.125)	0.125/0.125 (0.063–0.25)
A. tubingensis		25	1/2 (0.5 to >16)	2/2 (1–4)	0.25/0.25 (0.063–0.25)	2/4 (1–8)	0.25/0.25 (0.125–0.5)	0.031/0.063 (<0.016–0.063)	0.031/0.063 (0.016–0.25)

Isolates are grouped based on species; *A. fumigatus* was further grouped based on resistance mechanism.
MIC testing (and minimum effective concentration testing for anidulafungin) were performed in accordance with EUCAST broth microdilution.

In addition to *A. fumigatus* isolates, we have tested several other, difficult-to-treat *Aspergillus* species with high MICs of several antifungals. *A. flavus* is globally the second most common species causing invasive aspergillosis.[15] Most isolates of *A. flavus* are susceptible to triazoles and echinocandins, but have amphotericin B MICs between 1 and 4 mg/L.[16] Although still rare, resistance to azoles was recently described.[17] Invasive *A. nidulans* infections are primarily associated with chronic granulomatous disease, with a higher mortality compared with *A. fumigatus*.[18] Notably, *A. nidulans* isolates are less susceptible to amphotericin B *in vitro*.[19] *A. tubingensis* is a black *Aspergillus* species, morphologically identical to *A. niger*. It is the fifth most identified species and has reduced susceptibility to azoles compared with *A. niger*.[9] *A. calidoustus* is a relatively rare cause of *Aspergillus* disease, but is increasingly seen in lung transplant patients on mould-active azole prophylaxis.[20] It shows resistance to medical triazoles *in vitro* and has reduced susceptibility to amphotericin B compared with *A. fumigatus*.[10,20] *In vitro* evaluation of F901318 revealed potent activity against all the non-*fumigatus Aspergillus* species evaluated in this study.

In conclusion, F901318 showed excellent *in vitro* activity against all isolates tested in this study and is a much-needed new antifungal class for the treatment of invasive aspergillosis, in a setting of emerging azole resistance.

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Transparency declarations

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