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Brief Report

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# *In vitro* activity of the novel antifungal compound F901318 against Australian *Scedosporium* and *Lomentospora* fungi

**Chayanika Biswas[1,*], Derek Law[2], Michael Birch[2], Catriona Halliday[1], Tania C. Sorrell[3], John Rex[2], Monica Slavin[4] and Sharon C-A Chen[1,3]**

[1]Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead Hospital, Sydney, Australia, [2]F2G Limited, Manchester, UK, [3]Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, Australia and [4]Victorian Comprehensive Cancer Centre, Melbourne, and the Department of Infectious Diseases, Peter MacCallum Cancer Centre, Melbourne, Australia.

*To whom correspondence should be addressed. Dr Chayanika Biswas, PhD, Centre for Infectious Diseases and Microbiology Laboratory Services, 3rd Level ICPMR Building, Westmead Hospital Darcy Road, Westmead, New South Wales 2145, Australia. Tel: +61 2 9845 6255; Fax: + 61 2 9893 8659; E-mail: chayanika.biswas@health.nsw.gov.au

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## Abstract

We determined the *in vitro* activity of the novel orotomide antifungal, F901318, against 30 *Lomentospora prolificans,* 20 *Scedosporium apiospermum*, 7 *S. aurantiacum,* and 3 *S. boydii,* isolates in comparison with standard antifungals. Against *L. prolificans*, F901318 was the most potent compound ($MIC_{90}$ 0.25 $\mu$g/ml); the geometric mean MIC (0.26 $\mu$g/ml) was significantly lower (23–80-fold) than those of itraconazole, voriconazole, posaconazole, and isavuconazole (all $P < .001$), and amphotericin B ($P < .05$). F901318 also had good activity against *S. apiospermum*, *S. aurantiacum,* and *S. boydii*, comparable to that of voriconazole and posaconazole but was more active than isavuconazole for all three species.

**Key words:** *Scedosporium, Lomentospora*, antifungal susceptibility, F901318, orotomide.

There has been a dearth of new drug classes reaching the clinic since the echinocandins in 2001. Limitations of current antifungals include drug toxicity, variable pharmacokinetics and increasing resistance, where resistance to the azoles in *Aspergillus fumigatus* approaches 30%.[1] Further, pathogenic moulds inherently resistant to antifungal drugs are increasingly encountered.[2,3]

F901318 (F2G Limited, Manchester, UK) is an orotomide antifungal compound that inhibits the fungal pyrimidine biosynthesis enzyme, dihydroorotate dehydrogenase.[4] It has potent *in vitro* activity against *Aspergillus* species including against azole-resistant strains, and demonstrates favorable pharmacokinetics in mice.[4–6] F901318 was also active against *Scedosporium* species and seven *Lomentospora* (previously *Scedosporium*) *prolificans* isolates.[7] Study of larger numbers of *Scedosporium*/*Lomentospora* strains from different locales is essential as there are geographic differences in susceptibility patterns.[2,8] Here we determined the *in vitro* activity of F901318 against these fungi in comparison with standard anti-mould agents

including isavuconazole. These fungi are the second most common (30%) cause of non-*Aspergillus* mould infections in Australia[3] and are resistant to many current antifungals.

Fifty clinical isolates were studied, comprising 30 *L. prolificans* and 20 *S. apiospermum* species complex isolates (10 *S. apiospermum* sensu stricto, 3 *S. boydii*, 7 *S. aurantiacum*), identified as before.[9] Three clinical *A. fumigatus* strains were tested for comparison. *Candida parapsilosis* ATCC 22019 was the quality control strain and *A. fumigatus* ATCC 204305, the reference strain.[10]

F901318 pure substance[4] and isavuconazole (ISA) powder (Carbosynth Limited, Berkshire, UK) was provided by F2G Limited. Stock solutions were prepared in DMSO and drug dilutions prepared in RPMI-1640 medium. The drug concentration range tested was 0.008–4 μg/ml for F901318 and 0.03–32 μg/ml for ISA, and susceptibility testing performed according to CLSI M38-A2 methodology.[10] MICs read as 100% inhibition of growth after 48 h incubation. Susceptibility to amphotericin B (AMB), itraconazole (ITC), voriconazole (VOR), posaconazole (POS), caspofungin (CAS), micafungin (MFG), and anidulafungin (AFG) was determined using the Sensititre® YeastOne® YO10 system (Trek Diagnostics, Cleveland, OH, USA). MICs (for AMB, azoles) and minimum effective concentrations (MECs; for echinocandins) determined.[10] Experiments were performed on two separate occasions.

Geometric mean (GM) MICs/MECs and $MIC_{90}$/$MEC_{90}$ values were ascertained for species where at least 10 strains of a species were tested. To establish GM values, the low (<) and high (>) off-scale MIC/MEC values were converted to the next lowest or highest two-fold drug concentrations, respectively.[11] Differences between GM MICs were assessed for significance by the Mann–Whitney *U*-test using Statistical Analysis software SAS version 9.4.

Antifungal susceptibility results and MIC distributions according to species are shown in Table 1. The MICs of standard antifungals for *A. fumigatus* ATCC 204305 were as expected.[12] The F901318 MIC was 0.125 μg/ml for all *A. fumigatus* isolates. Azole MICs ranged from 0.03–0.5 μg/ml except for ISA (0.5–2 μg/ml).

F901318 exhibited potent *in vitro* activity against *Scedosporium*/*Lomentospora* isolates. Notably, it was the most active compound against all 30 *L. prolificans* isolates ($MIC_{90}$ 0.25 μg/ml, MIC range 0.125–0.5 μg/ml). The GM MIC of 0.26 μg/ml for F901318 was 23–80-fold (Table 1) lower than those of the azoles (all $P < .001$) and 40-fold lower than that of AMB ($P < .05$). Additionally, the F901318 $MIC_{90}$ value was 32–128-fold lower than those for ITC, VOR, POS, and ISA (Table 1). Of the azoles, VOR exhibited the lowest GM MIC (5.66 μg/ml) against *L. prolificans* with a broad MIC range where two strains had MICs of 0.5 μg/ml and 1.0 μg/ml, respectively. The MIC distribution of all agents against *L prolfiicans* illustrates the greater activity of F901318 (Fig. 1).

GM MICs of F901318 against *S. apiospermum* and the MIC range against *S. aurantiacum* and *S. boydii* were similar to those of *L. prolificans* (Table 1), and for all compounds, the susceptibility profiles of the three species were similar. Overall, GM MICs for F901318 were similar to those of POS, VOR, and ITC but were up to 32-fold lower than those of ISA (Table 1), The next most active agent against *Scedosporium* species was VOR (GM MICs 0.11–0.125 μg/ml) followed by POS (GM MICs 0.37–0.55 μg/ml). Compared with AMB, F901318 was more active against all three species. For all *Scedosporium* and *Lomentospora* strains, GM MICs of AMB were highest for *L. prolificans* (11.31 μg/ml) (Table 1).

MICs for ISA ranged between 2 and 32 μg/ml and were highest for *L. prolificans* (GM MIC 16.76 μg/ml; Table1) followed by *S. apiospermum* (GM MIC 5.28 μg/ml). MICs of the echinocandins were high and comparable for CAS, AFG, and MFG. A single *S. apiospermum* isolate had a CAS MIC of 0.125 μg/ml.

F901318 is a novel compound with established activity against *Aspergillus* spp.[4,6] The results herein extend its promise as an antifungal agent to *Scedosporium*/*Lomentospora* fungi, with particular clinical relevance in regions where infections due to these pathogens are relatively common.[3]

As a “quality check" all three clinical *A. fumigatus* isolates had low F901318 MICs (0.125 μg/ml) athough these were twofold higher than the modal MIC of 0.062 μg/ml in wild-type *A. fumigatus* strains in the Netherlands.[5] Buil et al. employed the EUCAST broth microdilution (E.Def 9.2)[13] and not the CLSI M38-A2, method, but the two methodologies generally produce comparable susceptibility results for moulds.[14]

The key finding of our study was that F901318 exhibited potent *in vitro* activity against all three species of *Scedosporium* and *L. prolificans*. In particular, F901318 was the only agent to show low MICs (0.125–0.5 μg/ml) against a relatively large number ($n = 30$) of *L. prolificans* isolates (Fig. 1). One study reported F901318 MICs of ≤0.25 μg/ml for this species but for only seven strains.[7] While *in vivo* efficacy data of F901318 against invasive scedosporiosis have not been published, the MICs of F901318 for *Scedosporium* and *Lomentospora* spp. were similar to those for *A. fumigatus* where F901318 has potent activity in animal models.[4,6] Importantly, our data also suggests that F901318 susceptibility results are generalizable across countries.

In contrast, none of the other antifungals had appreciable *in vitro* activity against *L. prolificans*. GM MIC values of ITC, POS, VOR, and ISA were >20-fold higher than that of F901318. VOR was the next most active agent

**Table 1.** Antifungal susceptibilities of nine antifungal compounds for 50 *Scedosporium* and *Lomentospora prolificans* isolates

| Fungus (no. of isolates) | Antifungal ($\mu$g/ml) | Range ($\mu$g/ml) | [a]$MIC_{50}/MEC_{50}$ ($\mu$g/ml) | [a]$MIC_{90}/MEC_{90}$ ($\mu$g/ml) | [a]GM MIC/MEC ($\mu$g/ml) |
|---|---|---|---|---|---|
| *Scedosporium apiospermum* (n=10) | F901318 | 0.12–0.5 | 0.12 | 0.25 | 0.16 |
| | AMB | 2–16 | 4 | 16 | 6.06 |
| | CAS | 0.12–16 | 8 | 16 | 6.03 |
| | AFG | 2–16 | 4 | 16 | 6.94 |
| | MFG | 4–16 | 16 | 16 | 10.56 |
| | ITC | 0.25–1 | 0.5 | 1 | 0.43 |
| | VOR | 0.06–0.5 | 0.12 | 0.25 | 0.15 |
| | POS | 0.25–1 | 0.5 | 1 | 0.57 |
| | ISA | 2–8 | 4 | 8 | 5.28 |
| *Scedosporium aurantiacum* (n=7) | F901318 | 0.12–0.5 | – | – | – |
| | AMB | 2–16 | – | – | – |
| | CAS | 2–16 | – | – | – |
| | AFG | 4–16 | – | – | – |
| | MFG | 0.5–16 | – | – | – |
| | ITC | 0.12–32 | – | – | – |
| | VOR | 0.03–0.25 | – | – | – |
| | POS | 0.12–1 | – | – | – |
| | ISA | 4–16 | – | – | – |
| *Scedosporium boydii* (n=3) | F901318 | 0.12–0.25 | – | – | – |
| | AMB | 4–8 | – | – | – |
| | CAS | 8 | – | – | – |
| | AFG | 16 | – | – | – |
| | MFG | 16 | – | – | – |
| | ITC | 0.5 | – | – | – |
| | VOR | 0.12 | – | – | – |
| | POS | 0.5 | – | – | – |
| | ISA | 4–8 | – | – | – |
| *Lomentospora prolificans* (n=30) | F901318 | 0.12–0.5 | 0.25 | 0.25 | 0.26 |
| | AMB | 4–16 | 16 | 16 | 11.31 |
| | CAS | 4–16 | 8 | 16 | 7.64 |
| | AFG | 2–16 | 8 | 16 | 7.29 |
| | MFG | 2–16 | 8 | 16 | 8.39 |
| | ITC | 32 | 32 | 32 | 32 |
| | VOR | 0.5–16 | 8 | 16 | 5.66 |
| | POS | 16 | 16 | 16 | 16 |
| | ISA | 8–32 | 16 | 16 | 16.76 |
| *Aspergillus fumigatus* (n=3) | F901318 | 0.12 | – | – | – |
| | AMB | 0.25–0.5 | – | – | – |
| | CAS | 0.008–0.015 | – | – | – |
| | AFG | 0.015–0.03 | – | – | – |
| | MFG | 0.008–0.015 | – | – | – |
| | ITC | 0.06–0.5 | – | – | – |
| | VOR | 0.03–0.12 | – | – | – |
| | POS | 0.03–0.5 | – | – | – |
| | ISA | 0.5–2 | – | – | – |

**Abbreviations**: MIC, minimum inhibitory concentration; MEC, minimum effective concentration; AMB, amphotericin B; AFG, anidulafungin; CAS, caspofungin; ISA, isavuconazole; ITC, itraconazole; MFG, micafungin; POS, posaconazole; VOR, voriconazole.

MIC/MECs of F901318 and isavuconazole determined by the CLSI M38-A2 reference method [10]. MICs/MECs of the remaining antifungal agents determined using the Sensititre® YeastOne® Y010 panel as described in the Methods.

[a]The $MIC_{50}/MEC_{50}$, $MIC_{90}/MEC_{90}$ and GM MICs/MECs values were ascertained for species where at least 10 isolates, respectively were tested.

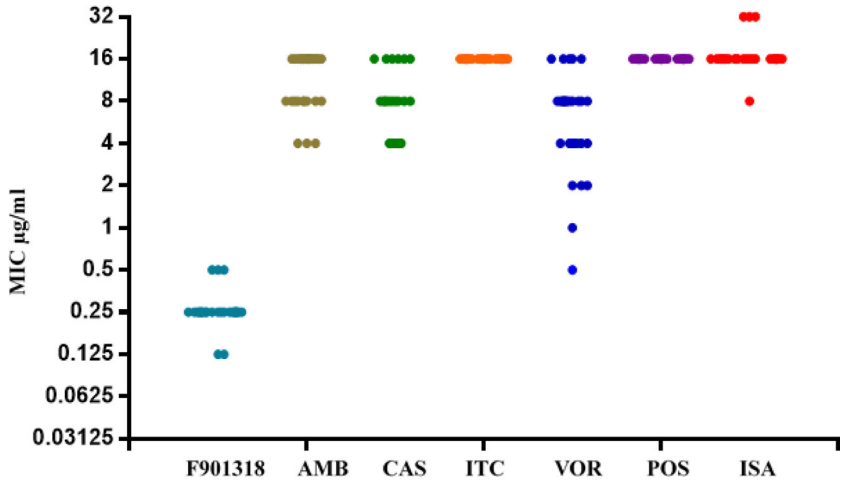


**Figure 1.** Minimum inhibitory concentration/ minimum effective concentration distributions (µg/ml) for antifungal agents against 30 isolates of *Lomentospora prolificans*. AMB, amphotericin B, CAS, caspofungin; ISA, isavuconazole; ITC, itraconazole; POS, posaconazole; VOR, voriconazole.

but the GM MIC was ~threefold less than that reported in the United States (5.66 µg/ml vs. >16 µg/ml).[7] VOR MICs varied widely, and others have observed MICs as low as 0.06 µg/ml.[15] Hence, susceptibility testing of all clinical isolates is recommended to guide therapy.[2,16] Current guidelines recommend using combination voriconazole and terbinafine to treat *L. prolificans* infections based on demonstration of synergy *in vitro*.[17,18] F901318 is a potentially effective alternative for use as a single agent to treat such infections. It also had good *in vitro* activity against the major *Scedosporium* species, with comparable activity to that of VOR and POS but up to 32-fold more active than ISA.

ISA demonstrated little *in vitro* activity against *L. prolificans* ($MIC_{90}$ 16 µg/ml) (Fig. 1), similar to results of one study.[19] However, as with VOR and POS, there may be synergistic interactions with terbinafine.[20] Among *Scedosporium* spp., *S. aurantiacum* was the least susceptible to ISA. As before,[7] neither AMB nor the echinocandins demonstrated good activity against *Scedosporium* or *Lomentospora* spp. However, in another study, micafungin was moderately active against *Scedosporium* spp.[16] Clinical trials are required to ascertain the role of echinocandins, including in combination with other drugs in the treatment of scedosporiosis.

In summary, the results of testing a large panel of Australian *Scedosporium* and *L. prolificans* isolates against F901318 support the conduct of clinical studies of this agent, especially for *L. prolificans* infection, where the activity of approved antifungals is poor.

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### Declaration of Interest

D.L., M.B., and J.R. are employees of F2G Limited and each has stock options in this company. T.C.S, M.S., and S.C.A.C. are on the advisory boards for MSD Australia, Gilead Sciences Inc., and have received untied research grants from MSD Australia, Pfizer Australia and Gilead Sciences. C.H. has received untied research grants from MSD Australia.

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