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# In vitro activity of APX001A and comparators against clinical isolates of rare moulds

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## BACKGROUND

APX001A is a novel antifungal that inhibits Gwt1, a protein that plays an important role in fungal cell wall integrity. Previous studies have shown APX001A has broad activity against yeasts, *Aspergillus* and rare moulds, including, *Fusarium* and *Scedosporium*. The aim of this study was to further evaluate the activity of APX001A and other antifungal agents against rare moulds

## MATERIAL & METHODS

200 strains of *Alternaria, Fusarium, Scedorpoium/ Lomentospora*, cryptic species of *Aspergillus* and mucorales were tested for antifungal susceptibility following EUCAST and CLSI methodologies.

All strains were obtained from clinical samples and identified to species level by sequencing the Internal Transcribed Spacer (ITS) and part of the beta tubulin in *Aspergillus* spp. and *Scedosporium* spp. or elongation factor alpha in *Fusarium* spp.

The antifungals used were: amphotericin B (range 0.03-16 mg/L), posaconazole (0.015-8 mg/L), micafungin (0.004-2 mg/L) and APX001A (0.015-8 mg/L).

*Aspergillus flavus* ATCC204304 and *Aspergillus fumigatus* ATCC204305 were used as quality control strains.

Minimal Inhibitory Concentrations (MIC) for amphotericin B and posaconazole and Minimum Effective Concentrations (MEC) for micafungin and APX001A were read after 24 (mucorales) and 48 hours (*Alternaria, Aspergillus, Fusarium, Lomentospora and Scedosporium*) of incubation.

## RESULTS

APX001A was the most active drug against all isolates of *Scedosporium* and *Lomentospora* species and was the only compound with $MEC_{90}$ ≤2 mg/L (EUCAST). Cryptic species of *Aspergillus* were inhibited by APX001A and micafungin with $MEC_{90}$ values of ≤ 0.5 mg/L and ≤ 2 mg/L, respectively, except for *A. calidoustus* with a micafungin $MEC_{90}$ of 4 mg/L. APX001A showed variable activity against *Fusarium*, with some strains showing MEC ≤0.06 mg/L and others showing MEC >8 mg/L (EUCAST). APX001A had moderate activity against *A. alternata* ($MEC_{50}$ ≤0.5 mg/L and $MEC_{90}$ >2 mg/L), with micafungin being the most active drug for this species ($MEC_{90}$ <2mg/L). Amphotericin B was the most active compound for Mucorales with $MIC_{90}$ values (inhibition of 90% of the isolates) <2 mg/L for all species except for *C. bertholletiae* with $MIC_{90}$ >4 mg/L. APX001A was not active against any species of Mucorales with $MEC_{50}$ values for EUCAST at 24h of > 2mg/L for all species.

Table 1: $MIC_{50}$ and $MIC_{90}$ (inhibition of 50 and 90% of the isolates) by EUCAST and CLSI methods at 48h of incubation

| Table 1 | EUCAST mg/L at 48h of incubation | | | | | | | | CLSI mg/L at 48h of incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMB | | PCZ | | MCF | | APX | | AMB | | PCZ | | MCF | | APX | |
| Species (no. tested) | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ |
| *Alternaria alternata (10)* | 0.5 | 8 | 0.25 | 16 | 0.06 | 1 | 0.5 | 4 | 0.25 | 4 | 0.25 | 16 | 0.03 | 1 | 0.25 | 4 |
| *Aspergillus alliaceus (10)* | 32 | 32 | 0.06 | 0.25 | 0.03 | 0.06 | 0.06 | 0.12 | 32 | 32 | 0.06 | 0.12 | 0.004 | 0.007 | 0.015 | 0.03 |
| *Aspergillus calidoustus (10)* | 1 | 2 | 16 | 16 | 0.25 | 4 | 0.015 | 0.03 | 0.5 | 1 | 16 | 16 | 0.06 | 0.25 | 0.015 | 0.03 |
| *Aspergillus fumigatiaffinis (10)* | 16 | 32 | 0.25 | 0.5 | 0.004 | 0.015 | 0.015 | 0.03 | 2 | 4 | 0.25 | 0.5 | 0.004 | 0.007 | 0.03 | 0.03 |
| *Aspergillus lentulus (10)* | 4 | 4 | 0.25 | 0.5 | 0.004 | 0.007 | 0.015 | 0.015 | 0.5 | 1 | 0.5 | 1 | 0.004 | 0.007 | 0.015 | 0.03 |
| *Aspergillus pseudofischerii (10)* | 0.5 | 4 | 0.25 | 0.5 | 0.03 | 0.25 | 0.12 | 0.5 | 0.12 | 2 | 0.12 | 1 | 0.03 | 0.03 | 0.12 | 0.25 |
| *Aspergillus udagawae (10)* | 2 | 4 | 0.25 | 0.5 | 0.007 | 0.015 | 0.015 | 0.06 | 0.5 | 1 | 0.25 | 0.5 | 0.004 | 0.007 | 0.015 | 0.06 |
| *Fusarium oxysporum (10)* | 1 | 4 | 16 | 16 | 4 | 4 | 0.25 | 16 | 1 | 2 | 8 | 16 | 4 | 4 | 0.015 | 16 |
| *Fusarium verticilloides (10)* | 2 | 32 | 16 | 16 | 4 | 4 | 16 | 16 | 1 | 32 | 16 | 16 | 4 | 4 | 16 | 16 |
| *Lomentospora prolificans (10)* | 32 | 32 | 16 | 16 | 4 | 4 | 0.03 | 2 | 4 | 8 | 16 | 16 | 4 | 4 | 0.015 | 0.06 |
| *Scedosporium apiospermum (10)* | 2 | 32 | 2 | 4 | 0.5 | 4 | 0.06 | 2 | 0.5 | 32 | 2 | 8 | 0.12 | 4 | 0.03 | 16 |
| *Scedosporium aurantiacum (10)* | 32 | 32 | 2 | 16 | 4 | 4 | 0.03 | 0.06 | 4 | 16 | 2 | 16 | 4 | 4 | 0.03 | 0.03 |
| *Scedosporium boydii (10)* | 4 | 32 | 0.5 | 1 | 0.25 | 1 | 0.06 | 0.25 | 0.5 | 2 | 1 | 2 | 0.06 | 2 | 0.06 | 0.12 |

Table 2: $MIC_{50}$ and $MIC_{90}$ (inhibition of 50 and 90% of the isolates) by EUCAST and CLSI methods for Mucorales at 24 h

| Table 2 Mucorales | EUCAST mg/L at 24h of incubation | | | | | | | | CLSI mg/L at 24h of incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMB | | PCZ | | MCF | | APX | | AMB | | PCZ | | MCF | | APX | |
| Species (no. tested) | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ |
| *Cunninghamella bertholletiae (10)* | 8 | 32 | 1 | 4 | 4 | 4 | 16 | 16 | 2 | 4 | 0.5 | 2 | 4 | 4 | 16 | 16 |
| *Lichtheimia corymbifera (10)* | 0.06 | 0.25 | 0.25 | 4 | 4 | 4 | 4 | 16 | 0.03 | 0.12 | 0.5 | 2 | 4 | 4 | 16 | 16 |
| *Lichtheimia ramosa (10)* | 0.03 | 0.5 | 0.25 | 0.5 | 4 | 4 | 16 | 16 | 0.03 | 0.06 | 0.12 | 0.5 | 4 | 4 | 8 | 16 |
| *Mucor circinelloides (10)* | 0.06 | 0.12 | 16 | 16 | 4 | 4 | 8 | 16 | 0.03 | 0.03 | 1 | 1 | 4 | 4 | 4 | 8 |
| *Rhizomucor pusillus (10)* | 0.06 | 0.25 | 1 | 4 | 4 | 4 | 4 | 16 | 0.06 | 0.06 | 1 | 2 | 4 | 4 | 16 | 16 |
| *Rhizopus arrhizus (10)* | 0.12 | 0.25 | 1 | 2 | 4 | 4 | 16 | 16 | 0.12 | 0.12 | 0.5 | 0.5 | 4 | 4 | 16 | 16 |
| *Rhizopus microsporus (10)* | 0.25 | 1 | 1 | 2 | 4 | 4 | 16 | 16 | 0.03 | 0.12 | 0.5 | 2 | 4 | 4 | 4 | 16 |

## CONCLUSIONS

1. APX001A was active against *Scedosporium, Lomentospora,* all cryptic species of *Aspergillus* and some strains of *Fusarium* spp. It was inactive against the Mucorales species evaluated.
2. Amphotericin B was the most active compound for Mucorales and *Fusarium* spp.
3. APX001A showed good activity against species with intrinsic resistance to amphotericin B and/or azoles.
4. APX001A was the only drug active against the multiresistant species *L. prolificans* and *A. calidoustus*.

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