# Anti-fungal activity of PC1244, a novel azole, on azole sensitive and resistant *Aspergillus fumigatus* strains and other fungi

T. Colley[1], A. Chowdhary[2], A. Alanio[3], S. Kelly[4], D. Corbett[5], G. Sehra[1], A.G.S. Warrilow[4], J.E.Parker[4], D.E. Kelly[4], S. Birchall[5], A.Wise[5], M. Sunose[6], M. McConville[6], L. Payne[5], S. Bretagne[3], P.J. Murray[1], P. Strong[1], G. Rapeport[1] and K. Ito[1]

[1]Pulmocide Ltd, London, United Kingdom, [2] Department of Medical Mycology, V. P. Chest Institute, University of Delhi, Dehli, India, [3]St.Louis Hospital, Paris, France, [4]Centre for Cytochrome P450 Biodiversity, Swansea University, Swansea, United Kingdom, [5]Evotec (UK) Ltd, Manchester, United Kingdom, [6]Sygnature Ltd, Nottingham, United Kingdom

## Introduction

**PC1244 is a novel antifungal agent designed for inhalation treatment of invasive aspergillosis or difficult fungi. In this study, the *in vitro* profile of PC1244 was investigated against *Aspergillus fumigatus* (*A. fumigatus*) and a range of yeasts and moulds.**

## Methods

CYP51A and CYP51B binding affinity and enzyme inhibition were determined using recombinant *A. fumigatus* CYP51A/B. Anti-fungal potency was evaluated with using the EUCAST broth microdilution method by visual inspection and using optical density (OD) measurements to quantify growth. Anti-fungal potency against an extended range of fungus were evaluated using CLSI broth microdilution in Eurofins-Panlabs.

## Results

### PC1244 targets *A.fumigatus* CYP51

PC1244 has a high affinity for both *A. fumigatus* CYP51A and CYP51B proteins, and was a strong tight binding inhibitor of CYP51A/B enzyme activity. PC1244 also showed the depletion of ergosterol content in *A. fumigatus* membranes with the characteristic accumulation of 14-methylated sterols (lanosterol/obtusifoliol and eburicol).

**Figure 1. Inhibitory activity of PC1244 (❍) and posaconazole (●) against CYP51A/B**

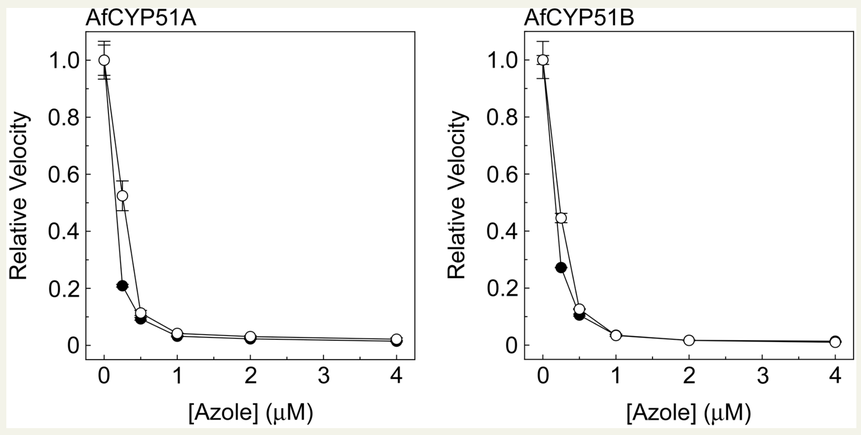


**Table 1. Inhibitory activities and binding properties of PC1244 and posaconazole**

| nM | Enzyme activity | | Enzyme binding | |
|---|---|---|---|---|
| | CYP51A | CYP51B | CYP51A | CYP51B |
| | $IC_{50}$ | $IC_{50}$ | $K_d$ | $K_d$ |
| PC1244 | 270 | 230 | 736 | 18.3 |
| Posaconazole | 160 | 170 | 961 | 11.6 |

**Table 2. Effects of PC1244 and posaconazole on sterol composition of *A.fumigatus***

| | Sterol compositions (%) | | | | | |
|---|---|---|---|---|---|---|
| | DMSO | 0.0001 μg/ml | 0.001 μg/ml | 0.01 μg/ml | 0.1 μg/ml | 1 μg/ml |
| **PC1244** | | | | | | |
| Ergosterol | 100 | 91.3 | 89.2 | 76.8 | 61.0 | 58.7 |
| Lanosterol/Obtusifoliol | 0 | 1.7 | 2.8 | 8.5 | 12.3 | 13.1 |
| Eburicol | 0 | 2.5 | 3.4 | 14.7 | 26.7 | 28.2 |
| **Posaconazole** | | | | | | |
| Ergosterol | 100 | 94.5 | 87.2 | 74.7 | 67.8 | 67.4 |
| Lanosterol/Obtusifoliol | 0 | 3.0 | 7.0 | 8.8 | 8.8 | 8.8 |
| Eburicol | 0 | 2.2 | 5.9 | 18.3 | 23.4 | 23.8 |

### PC1244 inhibits growth of azole sensitive and azole resistant *A.fumigatus*

In broth microdilution assays, PC1244 was a potent and highly effective inhibitor of growth of *A.fumigatus*-itraconazole susceptible strains (NCPF2010 and AF293) with $MIC_{90}$ (90% inhibition of growth determined by OD) of 0.0022 μg/mL and 0.012 μg/mL, respectively. PC1244 also showed more potent inhibition of growth of *A.fumigatus*-itraconazole resistant strains (AF91 (M220V), AF72 (G54E), TR34-L98H (Paris) and TR46-Y121/T289A (India)) than voriconazole or posaconazole, with $MIC_{90}$ of 0.024 μg/mL, 0.026 μg/mL, 0.024 μg/mL, 0.17 μg/mL, respectively.

**Table 3. Anti-fungal effects of PC1244 and reference compounds**

| $MIC_{90}$: μg/mL | *Aspergillus fumigatus* | | | | | |
|---|---|---|---|---|---|---|
| | NCPF2010 | AF293 | AF91 | AF72 | TR34-L98H | TR46-Y121/T289A |
| PC1244 | 0.0022 | 0.012 | 0.024 | 0.026 | 0.024 | 0.17 |
| Posaconazole | 0.0084 | 0.028 | 0.049 | 0.30 | 0.046 | 0.63 |
| Voriconazole | 0.21 | 0.74 | 0.28 | 0.065 | >1 | >1 |

### PC1244 inhibits growth of azole sensitive and azole resistant *A.fumigatus*

Against clinical strains, PC1244 also demonstrated potent inhibition of growth of 58 *A.fumigatus* isolates from the St Louis hospital including 8 TR34-L98H isolates (median visual MIC 0.016 μg/mL ($Min^m$ & $max^m$ 0.008 – 0.25) and 46 clinical isolates from North West England Mycology centre including 13 posaconazole resistant isolates based on EUCAST epidemiological cut-off (median visual MIC 0.25 μg/mL ($Min^m$ & $max^m$ 0.125 – 2 μg/mL).

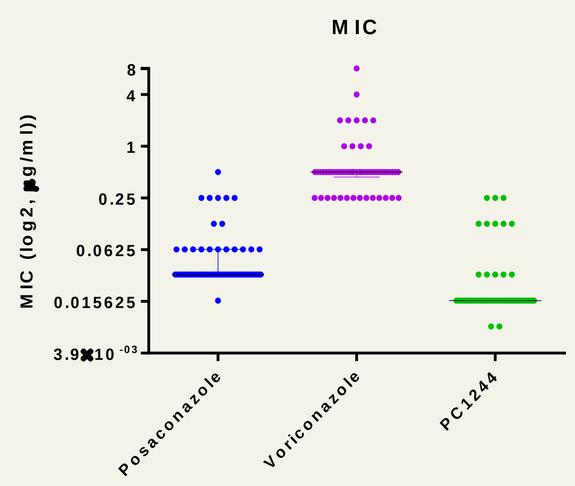


**Figure 2. MIC distribution of clinically isolated *A.fumigatus* from St Louis hospital.**

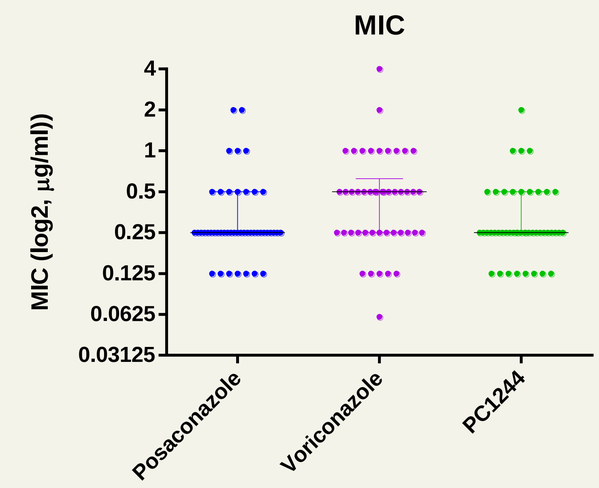


**Figure 3. MIC distribution of clinically isolated *A.fumigatus* from North West England mycology reference centre.**

### Anti-fungal effects of PC1244 and posaconazole in other fungal species

**Table 4. Anti-fungal effects against other fungal species**

| | MIC: μg/mL | |
|---|---|---|
| | PC1244 | Posaconazole |
| Aspergillus carbonarius | 0.063 | 0.063 |
| Aspergillus flavus | 0.13 | 0.13 |
| Aspergillus pullulans | 1 | 1 |
| Rhizopus oryzae | 0.5 | >8 |
| Cryptococcus neoformans | 0.063 | 0.25 |
| Chaetomium globosum | 0.13 | 0.25 |
| Cladosporium argillaceum | 0.25 | 0.25 |
| Penicillium chrysogenum | 0.13 | 0.13 |
| Penicillium citrinum | 1 | 0.5 |
| Fusarium graminearum | 0.5 | >8 |
| Trichophyton rubrum | 0.031 | 0.031 |
| Candida albicans ($MIC_{50}$) | 0.016 | 0.031 |
| Candida albicans ($MIC_{50}$) (azole resistant) | 0.13 | 0.25 |
| Candida glabrata ($MIC_{50}$) | 0.25 | 0.5 |
| Candida krusei | 0.25 | 0.25 |

In a panel against an extended range of fungi, PC1244 was found to be a potent inhibitor on other *Aspergillus spp. (flavus, carbonarius, pullulans), Rhizopus oryzae*, *Cryptococcus neoformans, Chaetomium globosum, Cladosporium argillaceum, Penicillium chrysogenum/ citrinum, Fusarium graminearum* and *Trichophyton rubrum* as well as *Candida* Spp. (MIC range: 0.0031 – 1 μg/mL)

## Conclusion

**In this study, PC1244 was shown to be a potent *A. fumigatus* CYP51 inhibitor and demonstrated more potent activity against several strains of *A. fumigatus,* including those with well characterised CYP51A mutations, and clinical isolates. We also found beneficial effects of PC1244 on several yeast and filamentous fungi. PC1244 therefore has the potential to be a novel therapy for the treatment of *A. fumigatus* and other difficult fungi infections in humans.**