mitoxantrone dropped from 2.29 to 1.26 μM and from 6.41 to 2.92 nM (Figure 1b), and the GI_{50} values of SN-38 and doxorubicin dropped from 0.34 to 0.19 μM and from 35.6 to 16.68 nM (Figure 1c), respectively.

Next, the general cytotoxicity of the three drug combinations that act additively on trypanosomes was assayed with human myeloid leukaemia HL-60 cells. Each drug combination was tested at the concentration that inhibited the growth of trypanosomes by 50%. The combinations topotecan/mitoxantrone (1.26 μM/2.92 nM) and SN-38/doxorubicin (0.19 μM/16.68 nM) were much more toxic to HL-60 cells than to trypanosomes. They inhibited the growth of the human cells by 97% and 99%, respectively. Encouragingly, the combination eflornithine/mitoxantrone (34.23 μM/2.89 nM) was less toxic to HL-60 cells than to the parasite cells. The eflornithine/mitoxantrone combination inhibited the growth of HL-60 cells by only 20%.

In this study, we tested 44 drug combinations of which 3 showed an additive effect on the growth inhibition of bloodstream forms of *T. brucei*. Of the three drug combinations with additive action, only the eflornithine/mitoxantrone combination was found to be less toxic to human HL-60 cells than to trypanosomes, indicating some degree of selectivity. This finding suggests that the combination of eflornithine and mitoxantrone could be a potential alternative treatment for HAT. As this was an *in vitro* study, further investigation should be carried out in order to establish the *in vivo* efficacy of the eflornithine/mitoxantrone combination.

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In vitro activities of eight antifungal drugs against 70 clinical and environmental isolates of *Alternaria* species

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Sir,

Melanized fungi of the order Pleosporales are emerging as causing opportunistic, cutaneous infections in immunocompromised patients mostly resulting from traumatic inoculation of contaminated plant debris. Among the most common aetiological agents of skin disease in the latter disorder are species of the genus *Alternaria*.[1] Disseminated infection is uncommon but increasing, particularly among severely immunocompromised individuals.[2] Recommendations for therapy are based on case reports and small *in vitro* studies. New antifungal agents with a better activity may help to improve the management of these infections. We aimed at evaluating the *in vitro* susceptibility of a collection of *Alternaria* spp. to amphotericin B, five triazoles and two echinocandins.

A total of 70 strains of *Alternaria* (CBS Fungal Biodiversity Centre, The Netherlands) consisted of the following isolates: *Alternaria infectoria* ($n=50$), *Alternaria alternata* ($n=7$) and *Alternaria malorum* ($n=13$). Correct identification of isolates was done by sequence analysis of ribosomal DNA. Except for enhancement of sporulation (1 week of incubation at 23°C under an alternating light/dark cycle consisting of 8 h of cool-white fluorescent daylight and 16 h of darkness), antifungal susceptibility testing was performed according to CLSI M38-A2.[3] Amphotericin B (Bristol–Myers Squibb, Woerden, The Netherlands),

fluconazole, voriconazole, anidulafungin (Pfizer Central Research, Sandwich, UK), itraconazole (Janssen Research Foundation, Beerse, Belgium), posaconazole (Schering–Plough, Kenilworth, NJ, USA), isavuconazole (Basilea Pharmaceutica Ltd, Basel, Switzerland) and caspofungin (Merck Sharp & Dohme BV, Haarlem, The Netherlands) were obtained as reagent-grade powders.[3] The antifungal agents were dispensed into microdilution trays at a final concentration of 0.016–16 mg/L for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin, 0.063–64 mg/L for fluconazole and 0.008–8 mg/L for

Table 1. *In vitro* susceptibility of *Alternaria* species to eight antifungal drugs

Species (no. and origin) and drug	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
All *Alternaria* isolates ($n=70$, E and C)			
amphotericin B	0.125–1	0.25	0.5
fluconazole	4–32	32	32
itraconazole	0.125–2	0.5	1
voriconazole	0.25–16	2	4
posaconazole	0.031–0.5	0.125	0.25
isavuconazole	0.5–4	4	4
caspofungin	0.5–16	1	2
anidulafungin	0.008–16	0.008	0.031
A. infectoria ($n=40$, E)			
amphotericin B	0.125–1	0.25	0.5
fluconazole	16–32	32	32
itraconazole	0.125–2	0.5	1
voriconazole	1–16	2	8
posaconazole	0.063–0.5	0.125	0.25
isavuconazole	2–4	4	4
caspofungin	0.5–2	1	1
anidulafungin	0.008–0.031	0.008	0.016
A. infectoria ($n=10$, C)			
amphotericin B	0.25–1	0.25	1
fluconazole	16–32	32	32
itraconazole	0.25–1	0.5	1
voriconazole	1–4	2	4
posaconazole	0.063–0.125	0.125	0.125
isavuconazole	2–4	4	4
caspofungin	1	1	1
anidulafungin	0.008–0.016	0.008	0.008
A. alternata ($n=7$, E and C)			
amphotericin B	0.25–1	0.5	1
fluconazole	8–32	32	32
itraconazole	0.25–1	1	1
voriconazole	1–4	2	4
posaconazole	0.125–0.5	0.125	0.5
isavuconazole	2–4	4	4
caspofungin	0.5–1	1	1
anidulafungin	0.008	0.008	0.008
A. malorum ($n=13$, E)			
amphotericin B	0.125–1	0.25	1
fluconazole	4–32	32	32
itraconazole	0.125–1	0.25	0.5
voriconazole	0.25–2	1	2
posaconazole	0.063–0.125	0.063	0.125
isavuconazole	0.5–4	4	4
caspofungin	0.5–16	1	16
anidulafungin	0.008–16	0.016	>8

E, environmental; C, clinical.
For caspofungin and anidulafungin, MICs are read as MECs.

isavuconazole and anidulafungin. Inoculum suspensions were prepared from 7 to 14 day cultures on potato carrot agar, by slightly scraping the surface of mature colonies with a sterile cotton swab wetted with sterile saline including Tween 40 (0.05%). The supernatants were adjusted spectrophotometrically at a wavelength of 530 nm to an optical density (OD) that ranged from 0.25 to 0.3 (2×10^4–7×10^4 cfu/mL).[3] Microdilution plates were incubated at 30°C for 48 h (plates with insufficient growth were incubated for 72 h) and read visually after agitation.

Table 1 summarizes the results of all *in vitro* activities of the eight antifungal drugs. Both environmental and clinical *Alternaria* isolates showed an MIC range from 0.125 to 1 mg/L for amphotericin B. We found the widest range and the highest MICs for fluconazole (range 4–32 mg/L). Surprisingly, most isolates of *A. infectoria* and *A. alternata* had high MICs of voriconazole, although the environmental isolates of *A. malorum* (a non-human pathogenic species) had lower MICs. Isavuconazole had high MICs similar to voriconazole with complete inhibition endpoints exhibiting MIC_{50} and MIC_{90} values of 4 mg/L against all *Alternaria* isolates. Itraconazole demonstrated better *in vitro* activity than voriconazole and isavuconazole with an MIC_{90} of 1 mg/L. Posaconazole had the lowest MIC_{90} (0.25 mg/L) of all the azoles. The echinocandin drugs caspofungin and anidulafungin both demonstrated *in vitro* activity against *A. infectoria* and *A. alternata*, with anidulafungin exhibiting the lowest MEC_{90} (0.008 mg/L) compared with caspofungin (MEC_{90} 1 mg/L); MEC stands for minimal effective concentration. Both echinocandins had no activity against *A. malorum* ($MEC_{90}>8$–16 mg/L). More than 150 cases of infection have been described in the world literature, with the skin being the most involved organ.[1] Severe deep infections are rare complications and optimal treatment is not known.[2] Various antifungal drugs have been used in the treatment of *Alternaria* infection, such as amphotericin B, flucytosine, fluconazole, miconazole and nystatin.[1] There are few published data available concerning the *in vitro* antifungal susceptibility with microdilution techniques of *Alternaria* species;[4,5] the largest collection included just 13 strains.[4] Reported MIC_{50} and MIC_{90} values of posaconazole, itraconazole and amphotericin B are comparable to our results. Among the azoles, fluconazole showed uniform low activities against *Alternaria* species in this and previous studies.[4,5] Except one report involving 11 isolates,[5] itraconazole, voriconazole and posaconazole had good *in vitro* activity. Recently, the new azole isavuconazole was tested and generated MIC_{50} and MIC_{90} values of 1 mg/L.[6] We describe lower *in vitro* activities (MIC_{90} 4 mg/L) of voriconazole and isavuconazole. There was no isolate with an MIC of isavuconazole >4 mg/L, suggesting a potential role in therapy and prophylaxis if adequate drug levels are achieved at the site of infection. In contrast, posaconazole demonstrated potent activity (MIC_{90} 0.25 mg/L) against all clinical and environmental isolates of *Alternaria*, but it remains to be confirmed that high enough drug levels can be reached at a remote site of infection. Isavuconazole has not been used clinically for *Alternaria* infections, but posaconazole was the only clinically active antifungal drug in the treatment of a severe invasive *Alternaria* infection in an immunocompetent patient.[1] In the present study, the antifungal activities of posaconazole and anidulafungin were superior against clinical isolates of *A. infectoria*. Furthermore, we demonstrated better activity of anidulafungin compared with that of caspofungin, but it is not known whether these *in vitro* differences are clinically significant. In conclusion, posaconazole and anidulafungin demonstrated the highest *in vitro* antifungal activity against *Alternaria* species, including those clinical and environmental isolates that had higher MICs of voriconazole and isavuconazole; their clinical effectiveness in the treatment of *Alternaria* infection remains to be determined.

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Successful treatment with doripenem and tobramycin of ventriculitis due to imipenem- and meropenem-resistant *Pseudomonas aeruginosa*

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