



EVALUATION OF NEW POTENTIAL COMPOUNDS FOR THE TREATMENT OF CHROMOBLASTOMYCOSIS

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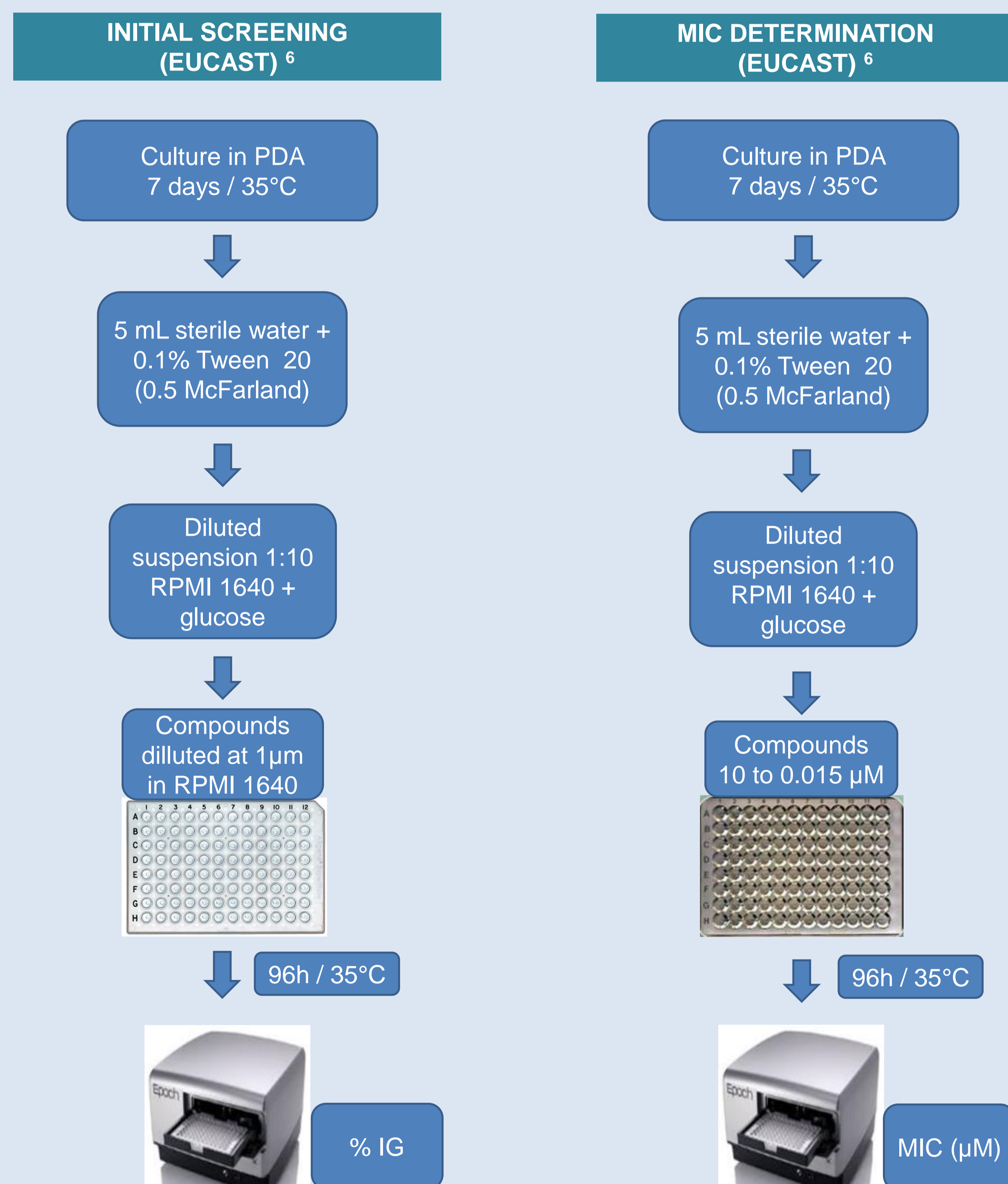
INTRODUCTION

Chromoblastomycosis (CBM) is a chronic fungal infection of the cutaneous and subcutaneous tissue caused by traumatic implantation of several species of black fungi into the host¹. The most common agents of CBM are *Fonsecaea pedrosoi*, *Fonsecaea monophora*, *Fonsecaea nubica*, *Cladophialophora carrionii*, *Phialophora verrucosa*, and *Exophiala dermatitidis*, among others^{2, 3}. There is no standard CBM treatment to follow. Therapy usually requires more than one drug to be administered and, sometimes, physical methods such as surgery or cryosurgery are necessary to improve the therapeutic response⁴. Itraconazole (ITZ) and terbinafine (TRB) are the most commonly antifungal drugs used to treat patients with CBM⁵. Due to the paucity of antifungal drugs that can be used in the treatment of this mycosis, the refractoriness of some cases, and potential recurrences of this mycosis, a preclinical study involving compounds already established for the treatment of other diseases and with known cytotoxicity will allow the discovery of potential new drugs that may be included in clinical trials for the treatment of CBM in the future.

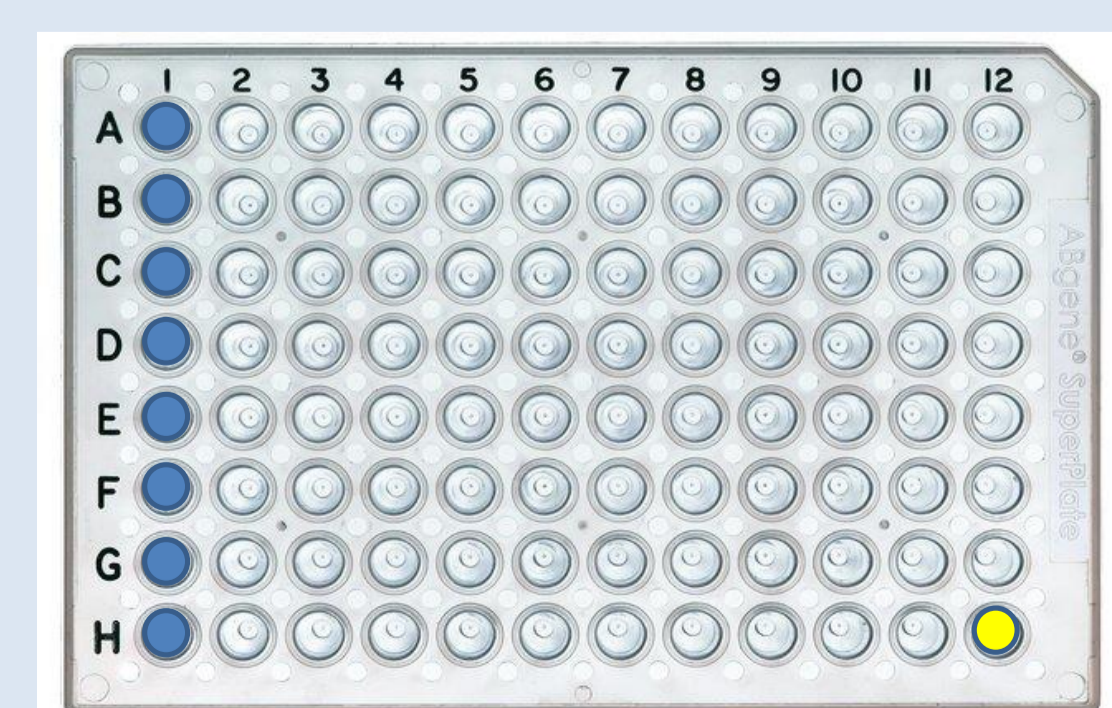
OBJECTIVE

The objective of this study was to evaluate the antifungal activity of 400 molecules present in the drug collection Pathogen box (Medicines for Malaria Venture) against CBM agents.

METHODS



COMBINATION WITH ITZ AND TRB (CHECKERBOARD)



Pathogen box compound (called drug A) was serially diluted (10 dilutions) in 96-well plates and each dilution applied to columns (2-11) of the plate.

- Negative Control (RPMI medium)
- Positive Control of fungal growth

The itraconazole or terbinafine antifungals (drugs called B) were serially diluted (7 dilutions) and each dilution applied to the plate lines (A-G).

The FICI was defined as:
$$\frac{MIC_{combined}}{MIC_{drug A alone}} + \frac{MIC_{combined}}{MIC_{drug B alone}}$$

- Interaction Rating^{7, 8}
- synergistic effect, FICI < 0,5;
 - no effect, 0,5 < FICI < 4;
 - antagonistic effect, FICI > 4

RESULTS

Two compounds were highlighted in the initial screening at 1µM: MMV021013 and MMV688978. When they were tested against different CBM agents, MIC values (100% inhibition) between 1.25 - 5µM and 1.25 - 2.5µM were found, respectively. The synergism was observed for MMV688978 in combination to ITZ for a *Cladophialophora carrionii* isolate. The combination with TRB was indifferent for both compounds.

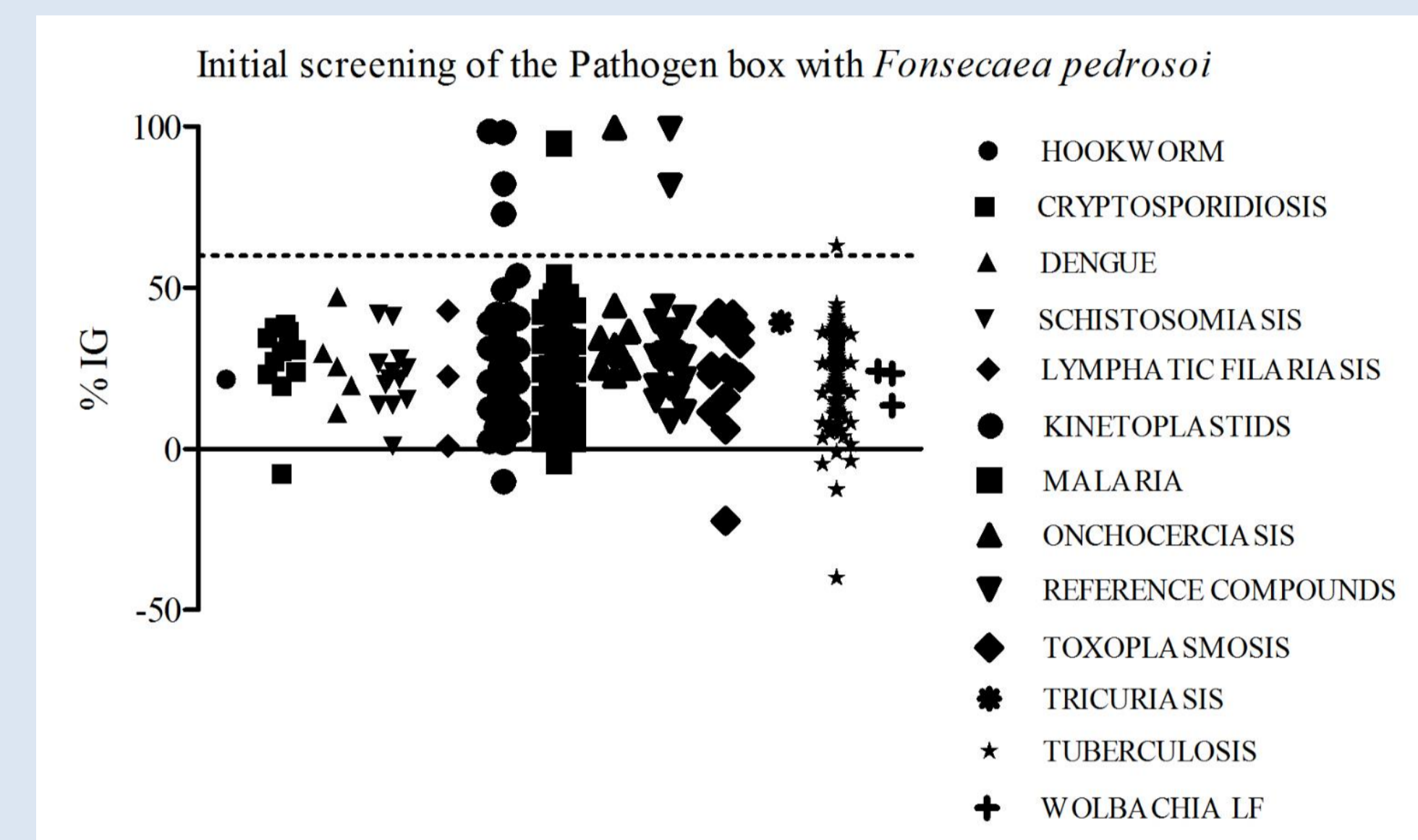


Table 1: Compounds of the Pathogen box selected on initial screening.

COMPOUNDS	ACTIVE AGAINST
MMV021013	Tuberculosis
MMV688978	Rheumatoid arthritis

Table 2: MIC values of the compounds MMV688978 and MMV021013.

STRAINS	MICs (µM)	
	MMV688978	MMV021013
RW01 - <i>Cladophialophora carrionii</i>	1,25	5
RW02 - <i>Phialophora verrucosa</i>	1,25	5
RW03 - <i>Exophiala dermatitidis</i>	2,5	5
RW04 - <i>Exophiala jeanselmei</i>	1,25	5
RW05 - <i>Fonsecaea pedrosoi</i>	1,25	2,5
RW06 - <i>Fonsecaea monophora</i>	1,25	2,5
RW07 - <i>Fonsecaea nubica</i>	1,25	1,25
RW08 - <i>Rhinocladiella similis</i>	2,5	2,5

Table 3: FICI values of the compounds MMV021013 and MMV688978 in combination with itraconazole.

Strains	MIC (µg/mL)			FICI	MIC (µg/mL)			FICI
	688978	ITZ	688978/ITZ		021013	ITZ	021013/ITZ	
RW01 - <i>Cladophialophora carrionii</i>	1.5	0.50	0.375/0.06	0.37	2.5	0.50	1.25/0.12	0.74
RW02 - <i>Phialophora verrucosa</i>	1.5	0.25	0.375/0.12	0.73	2.5	0.25	1.25/0.06	0.74
RW03 - <i>Exophiala dermatitidis</i>	0.75	0.25	0.375/0.06	0.74	2.5	0.50	0.003/1.0	2
RW04 - <i>Exophiala jeanselmei</i>	0.75	0.12	0.09/0.06	0.62	1.25	0.06	0.003/0.06	1
RW05 - <i>Fonsecaea pedrosoi</i>	0.75	0.12	0.01/0.12	1	1.25	0.12	0.625/0.06	1
RW06 - <i>Fonsecaea monophora</i>	0.75	0.06	0.01/0.06	1	2.5	0.06	0.003/0.06	1
RW07 - <i>Fonsecaea nubica</i>	0.375	0.12	0.01/0.12	1	0.625	0.12	0.15/0.06	0.74
RW08 - <i>Rhinocladiella similis</i>	0.75	0.25	0.75/0.06	1.24	2.5	0.25	0.15/0.12	0.54

Table 4: FICI values of the compounds MMV021013 and MMV688978 in combination with terbinafine.

Strains	MIC (µg/mL)			FICI	MIC (µg/mL)			FICI
	688978	TRB	688978/TRB		021013	TRB	021013/TRB	
RW01 - <i>Cladophialophora carrionii</i>	0.75	0.25	0.75/0.015	1.06	2.5	0.25	2.5/0.12	1.48
RW02 - <i>Phialophora verrucosa</i>	0.75	0.06	0.75/0.015	1.25	2.5	0.12	1.25/0.015	0.62
RW03 - <i>Exophiala dermatitidis</i>	0.75	0.25	0.75/0.015	1.06	5.0	0.25	0.625/0.25	1.12
RW04 - <i>Exophiala jeanselmei</i>	1.5	0.50	0.75/0.06	0.62	2.5	0.50	1.25/0.25	1
RW05 - <i>Fonsecaea pedrosoi</i>	0.75	0.06	0.375/0.03	1	2.5	0.06	0.003/0.06	1
RW06 - <i>Fonsecaea monophora</i>	1.5	0.12	0.375/0.12	1	2.5	0.12	2.5/0.015	1.12
RW07 - <i>Fonsecaea nubica</i>	0.375	0.12	0.005/0.12	1	1.25	0.12	0.625/0.015	0.62
RW08 - <i>Rhinocladiella similis</i>	0.75	1.0	0.75/0.50	1.50	2.5	1.0	2.5/0.50	1.50

CONCLUSIONS

The low MIC values of these compounds and the synergism of MMV688978 with ITZ against *C. carrionii* reveal promising new drugs to be tested in the treatment or prophylaxis of CBM in the future.

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Acknowledgements:

