TIMM Trends in Medical Mycology

P391

Antifungal Activity of a New Derivative of 5-Aminoimidazole-4-Carbohydrazonamide

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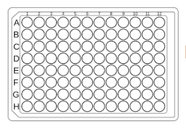
Introduction

Fungal infections, as recognized by the World Health Organization (WHO), are among the most worrying challenges for the medical community due to their high **incidence**, **recurrence**, and the **emergence of resistance to the few available drugs and therapies**. The discovery of **new molecules with antifungal activity**, exhibiting new mechanisms of action and less side effects, thus represents an important step forward in the development of alternative treatments [1].

The compound **2h** (5-amino-1-methyl-*N*'-phenyl-1*H*-imidazole-4-carbohydrazonamide) has already been validated as an effective **antifungal agent** against *Candida krusei*, *Candida albicans* and *Cryptococcus neoformans* [2]. A **trimeric derivative** compound was now prepared from spontaneous oxidation of 2h in contact with the air and the **main objective of this work** was to evaluate its **fungicidal activity** against *Candida krusei* and *Candida albicans*. The effects of both compound 2h and the trimeric derivative on yeast metabolic activity and mitochondrial function was also evaluated. Furthermore, the cytotoxicity of these compounds was also evaluated in immortalized human keratinocytes (HaCaT cells), 24 hours after exposure, and using three distinct cytotoxicity assays.

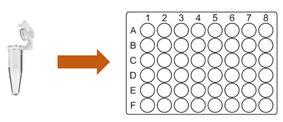
Methodology

1. MIC determination



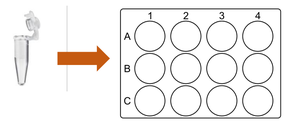
Candida krusei ATCC 6258 and *Candida albicans* ATCC 10231 → Select of Minimal inhibitory concentration

2. Yeast metabolic activity – REZ reduction assay



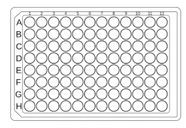
Candida krusei ATCC 6258 and *Candida albicans* ATCC 10231 → 2MIC, MIC and MIC/2 effects of metabolic activity

3. Mitochondrial function – JC-1 assay



Candida krusei ATCC 6258 and *Candida albicans* ATCC 10231 → 2MIC, MIC and MIC/2 effects of mitochondrial function

4. Cytotoxicity evaluation towards HaCaT cells



HaCaT cells → NR uptake, REZ reduction and SRB binding assays

Results are presented as Mean ± SD from 4 or 5 independent experiments, performed in duplicate. Statistical comparisons were made using One-way ANOVA followed by the Dunnett's multiple comparisons test. MIC, minimal inhibitory concentration.

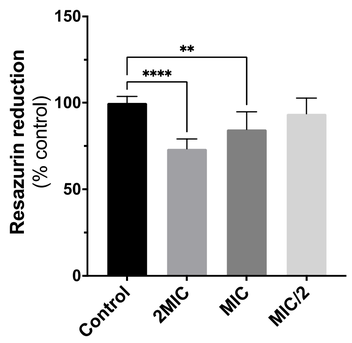
Results

1. The trimeric derivative exhibited equivalent or higher fungicidal activity against the yeasts tested, with Minimal Fungicidal Concentration of 2-64 µg/mL.

2. Effects on yeast's metabolic activity – Resazurin reduction assay

2h

Candida krusei: MIC = 4 µg/mL



Candida albicans: MIC = 32 µg/mL

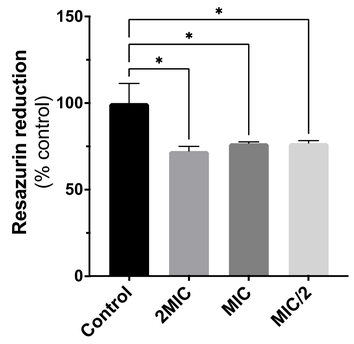
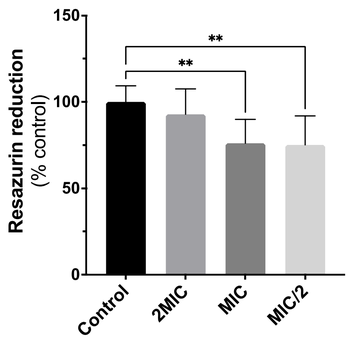


Figure 1. Metabolic activity of *Candida krusei* ATCC 6258 and *Candida albicans* ATCC 10231 cells treated with different concentrations of 5-amino-N'-phenyl-1H-imidazole-4-carbohydrazonamide (2h). (** $p < 0.01$; **** $p < 0.0001$)

Trimeric derivative

Candida krusei: MIC = 4 µg/mL



Candida albicans: MIC = 32 µg/mL

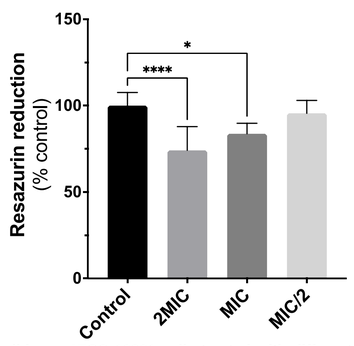


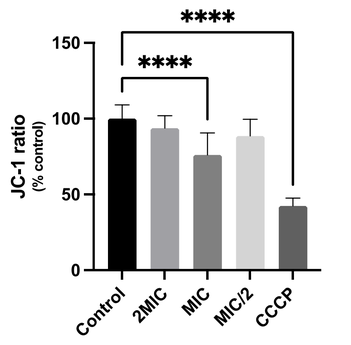
Figure 2. Metabolic activity of *Candida krusei* ATCC 6258 and *Candida albicans* ATCC 10231 cells treated with different concentrations of Trimeric derivative. (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$)

Significant decrease in resazurin reduction indicating a decrease in yeasts' metabolic activity

3. Effects on yeasts' mitochondrial function – JC-1 assay

2h

Candida krusei: MIC = 4 µg/mL



Candida albicans: MIC = 32 µg/mL

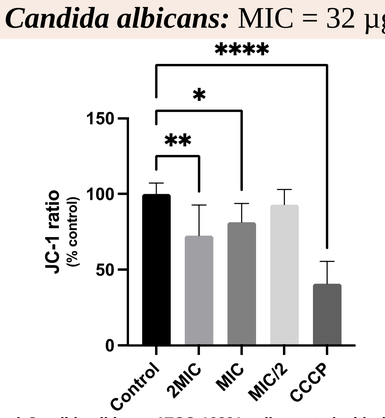
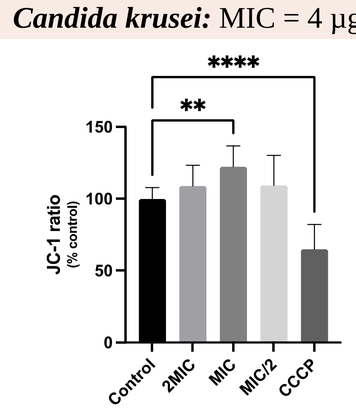


Figure 3. Mitochondrial function of *Candida krusei* ATCC 6258 and *Candida albicans* ATCC 10231 cells treated with different concentrations of 5-amino-N'-phenyl-1H-imidazole-4-carbohydrazonamide (2h). (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$). Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was used as a positive control.

Trimeric derivative

Candida krusei: MIC = 4 µg/mL



Candida albicans: MIC = 32 µg/mL

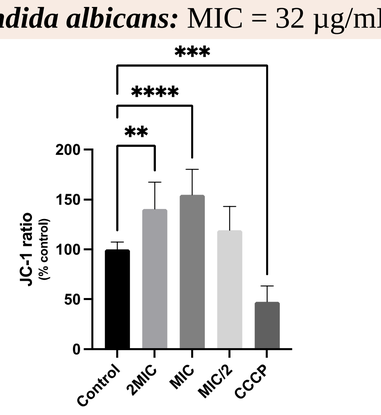


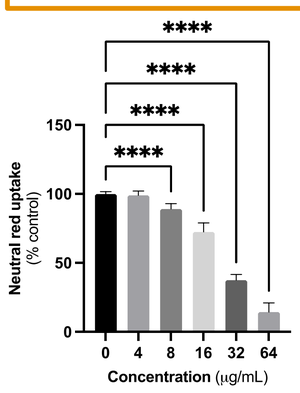
Figure 4. Mitochondrial function of *Candida krusei* ATCC 6258 and *Candida albicans* ATCC 10231 cells treated with different concentrations of Trimeric derivative. (** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was used as a positive control.

2h promoted a depolarization of mitochondrial membrane, while the trimeric derivative promoted a significant hyperpolarization of the mitochondrial membrane

4. Compounds' cytotoxicity towards HaCaT cells – NR uptake, REZ reduction and SRB binding assays

Neutral Red uptake

2h



Trimeric derivative

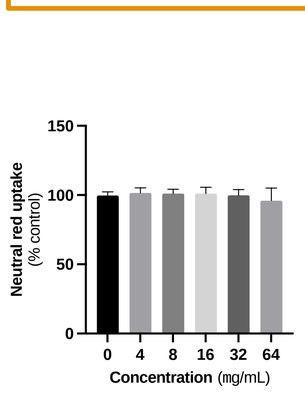
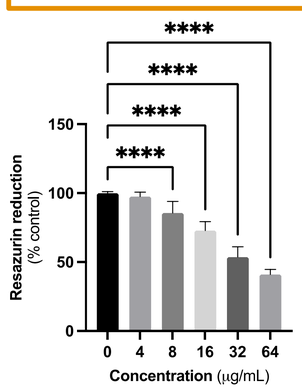


Figure 5. Compounds (0-64 µg/mL) cytotoxicity evaluated by the Neutral red uptake assay 24 hours after exposure. (**** $p < 0.0001$)

REZ reduction

2h



Trimeric derivative

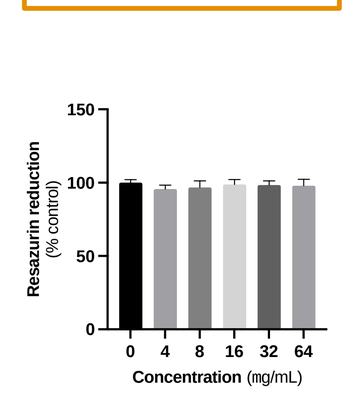
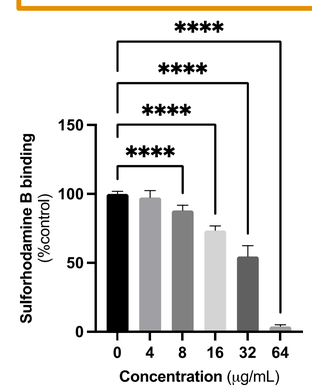


Figure 6. Compounds (0-64 µg/mL) cytotoxicity evaluated by the Resazurin reduction assay 24 hours after exposure. (**** $p < 0.0001$)

SRB binding

2h



Trimeric derivative

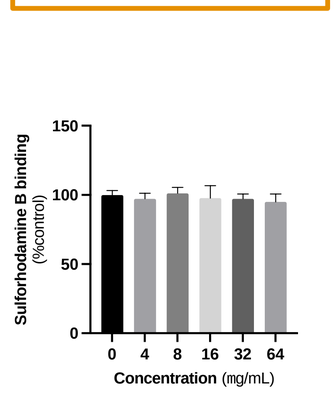


Figure 7. Compounds (0-64 µg/mL) cytotoxicity evaluated by the Sulforhodamine B binding assay 24 hours after exposure. (**** $p < 0.0001$)

2h → Significant and concentration-dependent cytotoxic effect was detected
Trimeric derivative → did not significantly affect cell viability for all the tested concentrations

Conclusion

The **high activity** of **TRIMERIC DERIVATIVE** observed against *C. krusei* and *C. albicans* and the **low toxicity towards HaCaT cells** are remarkably important and **reinforce the usefulness of this compound as an antifungal agent**. The application of these compounds in **biomedical materials**, namely in the **development of smart and functional textiles with antimicrobial properties**, is currently being tested, opening new perspectives in this emergent field of research.

REFERENCES

[1] Cerqueira F.,Maia M, *Gabriel C, et al. Antibiotics, 10 (2021), 183.; [2] Ribeiro AI, Gabriel C, Cerqueira F, et al. Bioorganic* & Medicinal Chemistry Letters, 24 (**2014**), 4699-4702.

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