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Original article

# Synthesis and cytotoxicity of novel (thiazol-2-yl)hydrazine derivatives as promising anti-*Candida* agents

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ABSTRACT

Thirty-eight new (4-(4-substituted-phenyl/2,4-disubstituted-phenyl)-thiazol-2-yl)hydrazine derivatives were synthesized in good yield and assayed for their *in vitro* anti-*Candida* activity, compared to topical and systemic antifungal drugs, against twenty-two clinical isolates of *Candida* spp. The concurrent presence of aliphatic chains or cycloaliphatic rings at N1-hydrazine and a 4-methyl/4-methoxyphenyl at C4 position of the thiazole nucleus exhibited an interesting anti-*Candida* inhibitory activity. Moreover, some of the most active compounds showed synergistic antifungal effects and lower cell toxicity when combined with clotrimazole.



## 1. Introduction

Opportunistic *Candida* species represent the most common fungal pathogens that affect humans and the mortality associated with invasive mycosis increased in the last decade. The pathogenesis of *Candida* spp. produces a wide spectrum of diseases, which depend primarily on the immune status of the host, ranging from superficial mucocutaneous diseases to invasive illnesses [1]. In healthy individuals, *Candida* infections are usually due to impaired epithelial barrier functions and statistically occur in all age groups, but are more frequent and harmful in the newborn and the elderly. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of risk factors (patients with cell-mediated immune deficiency and those receiving aggressive cancer therapies, excessive use of broad spectrum antibiotics, immunosuppression, or transplantation therapy). As it is often not possible to correct the underlying predisposing conditions, infections are usually more severe and generally do not respond correctly to topical/systemic treatment [2,3]. *Candida albicans*, *Candida glabrata*, and *Candida krusei* are the most frequent species isolated from clinical practice. Moreover, others species such as *Candida parapsilosis*, *Candida tropicalis*, and *Candida sakè* have also emerged as new opportunistic fungi threatening patients' life. Clinically, azoles are first-line agents in treating fungal infections, but their extensive use has led to the occurrence of resistant strains which greatly limited the therapeutic options. Antifungal resistance is a serious concern due to the limited number of available agents, therefore there is an emergent demand for the discovery of new antifungal agents to open the possibility of a novel therapeutic approach [4].

In the literature, it emerged that (4-aryl-thiazol-2-yl)hydrazines were endowed with potent anti-*Candida* activity against a large number of clinically relevant fungal strains [5,6]. In an attempt to improve the antifungal spectrum activity and drug-like properties of this scaffold, we have recently reported on new antifungal (thiazol-2-yl)hydrazine skeleton, with different substitutions at C2 and C4 positions of the thiazole nucleus [7–10], and their ability to inhibit growth of several fungal pathogens. As compared with the lead structures, the synthesized molecules showed promising antifungal activity, additive effects with clotrimazole, and low cell toxicity.

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Starting from the structure–activity relationships (SARs) extrapolated from biological results, we decided to design and evaluate a large number of such derivatives keeping constant the significant (thiazol-2-yl)hydrazine pharmacophore and introducing different moieties at N1-hydrazine (aliphatic chains and cycloaliphatic rings) and 4-substituted-phenyl at C4 position of thiazole nucleus (Table 1). According to the established guidelines of Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST, version 4.1 valid from 2012-03-14), we assayed the susceptibility of clinical *Candida* spp. and bacterial isolates to our novel compounds by determining their minimal inhibitory concentration (MIC) [11] thus providing a first indication of the potency and selectivity of such compounds.

## 2. Chemistry

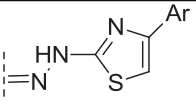
The chemical synthesis of **A**–**D** compounds is outlined in Scheme 1. Different carbonyl compounds were reacted directly with thiosemicarbazide with catalytic amounts of acetic acid in ethanol and the obtained thiosemicarbazones subsequently were condensed with newly synthesized α-bromo-4-substituted- or α-bromo-2,4-disubstituted-acetophenone (obtained by direct bromination of the corresponding 4-substituted or 2,4-disubstituted-acetophenone with $Br_2$ in $CHCl_3$) to 2,4-disubstituted-1,3-thiazoles (Hantzsch reaction). All synthesized compounds were fully characterized by analytical, spectral, and mass data (Experimental protocols and Supplementary material). In general in the IR spectrum, they showed strong bands at about 3220 and 1570 $cm^{-1}$ due to the presence of NH and C=N group, respectively.

## 3. Microbiology

Firstly, derivatives of **A**–**D** series, dissolved in dimethylsulfoxide (DMSO), were evaluated for their antibacterial activity (Table S1, Supplementary material). Organisms from routine clinical Gram-positive (*Staphylococcus aureus*, *Staphylococcus warneri*, *Streptococcus faecalis*, *Streptococcus α-hemolyticus*) and Gram-negative isolates (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter* spp., *Klebsiella oxytoca*) from the respiratory tract were collected from specimens of patients at the Hospital 'Azienda Policlinico Umberto I°' (Sapienza University of Rome). The isolates were subcultured on a qualified medium to ensure purity. The isolates were identified by conventional methodologies; all isolates were subcultured to ensure optimal growth. The *in vitro* antibacterial activities of the compounds were determined by the broth microdilution method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [12] with Mueller-Hinton II broth (BBL Microbiology Systems, Cockeysville, MD).

Then, the antifungal activity was evaluated against twenty-two clinical fungal isolates of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. sakè*) and compared with

**Table 1**
Minimal inhibitory concentration (MIC) of newly synthesized compounds (**A**–**D** series), clotrimazole (**C**), fluconazole (**F**), and griseofulvin (**G**) against 22 clinical strains of *Candida* species.




| Comp | Ar | Tested fungi (MIC values μg/mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *C. albicans* (8 strains) | *C. glabrata* (4 strains) | *C. tropicalis* (3 strains) | *C. krusei* (3 strains) | *C. parapsilopis* (2 strains) | *C. sakè* (2 strains) |
| **A1** | 4-$CH_3$-Ph | 0.50–2 | 0.50–2 | 2 | 2 | 2 | 2 |
| **A2** | 4-$CH_3$-Ph | 0.50–2 | 0.25–2 | 2 | 2 | 2 | 2 |
| **A3** | 4-$CH_3$-Ph | 0.50–2 | 2 | 4 | 8 | 8 | 8 |
| **B1** | 4-$OCH_3$-Ph | 0.25–2 | 0.50–2 | 2 | 16 | 16 | 16 |
| **B2** | 4-$OCH_3$-Ph | 0.25–2 | 2 | 2 | 2 | 2 | 2 |
| **B3** | 4-$OCH_3$-Ph | 0.25–2 | 2 | 2 | 2 | 2 | 2 |
| **B4** | 4-$OCH_3$-Ph | 16–64 | 32–64 | 64 | 256 | 256 | 256 |
| **B5** | 4-$OCH_3$-Ph | 4–16 | 8–16 | 16 | 16 | 16 | 16 |
| **B6** | 4-$OCH_3$-Ph | 0.50–2 | 0.50–2 | 2 | 2 | 2 | 2 |
| **B7** | 4-$OCH_3$-Ph | 2–8 | 4–8 | 8 | 32 | 32 | 32 |

(*continued on next page*)