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Design, synthesis, and biological evaluation of aminothiazole derivatives against the fungal pathogens *Histoplasma capsulatum* and *Cryptococcus neoformans*

Ahmed Khalil [a,b], Jessica A. Edwards [c,d,e], Chad A. Rappleye [c,d,e], Werner Tjarks [a,e,*]

[a] Division of Medicinal Chemistry & Pharmacognosy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA
[b] Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt
[c] Department of Microbiology, The Ohio State University, USA
[d] Department of Microbial Infection and Immunity, The Ohio State University, USA
[e] The Center for Microbial Interface Biology, The Ohio State University, USA

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ABSTRACT

Invasive fungal disease constitutes a growing health burden and development of novel antifungal drugs with high potency and selectivity against new fungal molecular targets are urgently needed. Previously, an aminothiazole derivative, designated as 41F5, was identified in our laboratories as highly active against *Histoplasma* yeast (MIC_{50} 0.4–0.8 μM) through phenotypic high-throughput screening of a commercial library of 3600 purine mimicking compounds (*Antimicrob. Agents Chemother.* **2013**, *57*, 4349). Consequently, 68 analogues of 41F5 were designed and synthesized or obtained from commercial sources and their MIC_{50}s of growth inhibition were evaluated in *Histoplasma capsulatum* to establish a basic structure–activity-relationship (SAR) for this potentially new class of antifungals. The growth inhibiting potentials of smaller subsets of this library were also evaluated in *Cryptococcus neoformans* and human hepatocyte HepG2 cells, the latter to obtain selectivity indices (SIs). The results indicate that a thiazole core structure with a naphth-1-ylmethyl group at the 5-position and cyclohexylamide-, cyclohexylmethylamide-, or cyclohexylethylamide substituents at the 2-position caused the highest growth inhibition of *Histoplasma* yeast with MIC_{50}s of 0.4 μM. For these analogues, SIs of 92 to >100 indicated generally low host toxicity. Substitution at the 3- and 4-position decreased antifungal activity. Similarities and differences were observed between *Histoplasma* and *Cryptococcus* SARs. For *Cryptococcus*, the naphth-1-ylmethyl substituent at the 5-position and smaller cyclopentylamide- or cyclohexylamide groups at the 2-position were important for activity. In contrast, slightly larger cyclohexylmethyl- and cyclohexylethyl substituents markedly decreased activity.

1. Introduction

Over the past few decades, systemic and invasive fungal infections have emerged as a significant threat to public health. Invasive fungal infections cause more human deaths than tuberculosis, although the latter has gained more notoriety in the public eye.[1,2] *Cryptococcus* infections have been estimated to cause over 500,000 deaths annually among immunocompromised individuals.[2] Invasive fungal infections are not limited to individuals with compromised immune functions. For example, in the United States, infections with *Cryptococcus gattii* and *Histoplasma capsulatum* occur in immunocompetent as well as immunocompromised hosts, classifying these as primary, not just opportunistic, fungal pathogens.[3]

The shared eukaryotic nature of both the host and pathogen significantly complicates treatment options for fungal disease. Existing antifungals for systemic mycoses target either the fungal membrane sterol ergosterol or cell wall β-glucan.[4] Amphotericin B targets sterols directly and triazole-class antifungals impair

Abbreviations: ATCC, American type culture collection; DMAP, 4-dimethylaminopyridine; DMC, dichloromethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; FIC, fractional inhibitory concentration; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HMM, *Histoplasma*-macrophage media; HR-ESI, high resolution-electrospray ionization; MEM, minimum essential medium; MIC_{50}, minimal concentration that inhibits 50% of fungal growth; MIC, minimal inhibitory concentration; MW, molecular weight; PBS, phosphate-buffered saline; TEA, triethylamine; TFA, trifluoroacedic acid; TFAA, trifluoroacedic anhydride, THF, tetrahydrofuran; SI, selectivity index; SAR, structure–activity-relationship; YPD, yeast extract peptone dextrose.

* Corresponding author. Tel.: +1 614 292 7624; fax: +1 614 292 2435.
E-mail address: tjarks.1@osu.edu (W. Tjarks).

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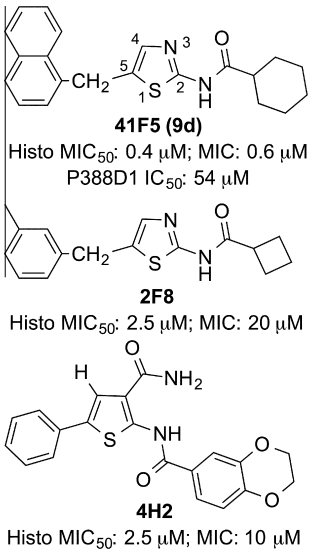


Figure 1. Structurally related thiazole/thiophene hit compounds 41F5, 2F8, and 4H2 identified in a phenotypic high-throughput screen of a purinome-focused library.[7]

sterol synthesis. However, both antifungal classes have significant host toxicity, which prohibits general prophylactic use of these antifungals.[5] The echinocandins are a third class of fungistatic antifungals recently developed, which target the synthesis of the essential fungal cell wall polysaccharide β-glucan. While better tolerated than amphotericin and the triazoles, the echinocandins lack efficacy against the more virulent fungal pathogens *Cryptococcus* and *Histoplasma*.[6] Further complicating antifungal treatment is the fact that *Cryptococcus* and *Histoplasma* yeasts invade immune cells (e.g., macrophages), and this intracellular location presents additional barriers to drug accessibility and efficacy. Thus, development of antifungal drugs with high potency and selectivity against new cellular targets are urgently needed to combat the growing health burden of invasive fungal disease.

Recently, our group performed a phenotypic high-throughput screen of a purinome-focused library of 3600 compounds with structural similarity to purines or any known purine analogue scaffold.[7] Inhibition of *Histoplasma* yeast growth was used as the screening phenotype. Concurrently, we measured mammalian cytotoxicity using a P388D1 macrophage cell line[8] since macrophages are the primary host cell for *Histoplasma* yeast. Among the 10 hits with the highest selectivity indices (SIs), a subgroup of three structurally related thiazole/thiophene derivatives (41F5, 2F8, 4H2, Fig. 1) were identified. The most active compound of this group was the aminothiazole 41F5, which had the lowest MIC_{50} (0.4–0.8 μM) and the highest SI (63–135) of all tested compounds relative to P388D1 macrophages. Preliminary studies also indicated selective toxicity of 41F5 against *Cryptococcus neoformans*.[7] Thus, the aminothiazole 41F5 has efficacy against *Histoplasma capsulatum* and *Cryptococcus neoformans*, two fungal pathogens that have natural resistance against the echinocandin class of antifungals.

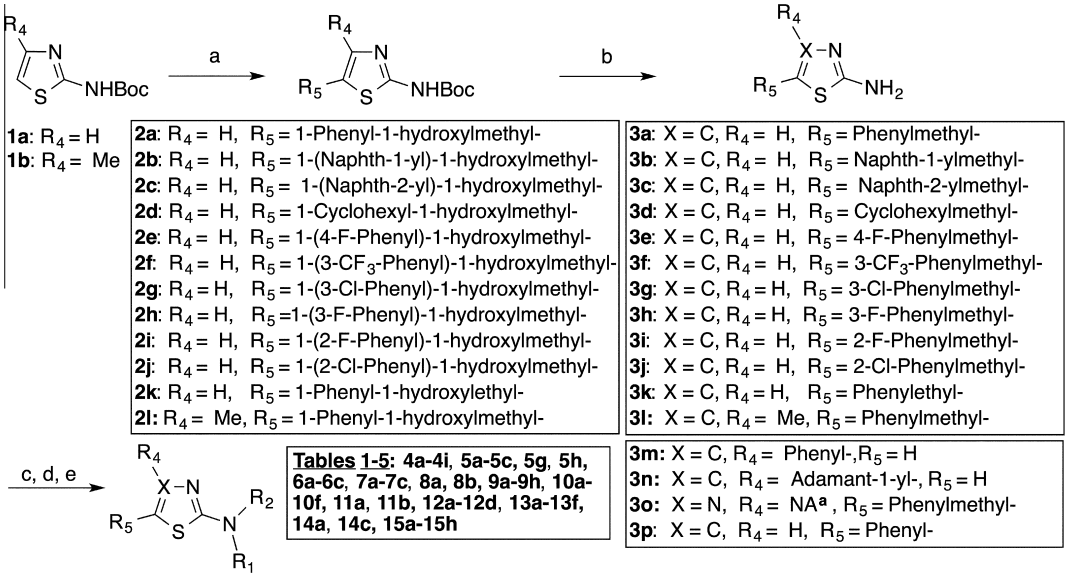
Compounds with aminothiazole scaffold display a wide range of biological activities,[9] including antiparasitic-,[10] antifungal-,[11] antibacterial-,[12] antitubucular-,[13] antiviral-,[14] anticancer-,[15,16] and antiprion[17] action. The study described here was carried out to establish the basic anti-*Histoplasma* and anti-*Cryptococcus* specific aminothiazole structure–activity relationships (SARs).

2. Results

2.1. Chemistry

The primary objective of our studies was to establish a *Histoplasma* SAR for aminothiazoles based on the 41F5 structure (Fig. 1). Other objectives were the development of a very basic *Cryptococcus* SAR for comparison and the evaluation of toxicity of promising novel compounds to hepatocyte (HepG2) cells. For this purpose we synthesized or purchased 68 compounds that are structurally related to 41F5. The thiazole core structure is easily amenable to modification. Due to its abundant use in drug design, numerous synthetic approaches have been developed and many synthetic precursor molecules for thiazole synthesis are commercially available or easily prepared.[15,16,18,19] Indeed, the design of this initial library was based to a significant extent on the synthetic feasibility and/or commercial availability of starting materials. Established synthetic procedures are shown in Scheme 1.

The reaction of compounds **1a** and **1b** with various aldehydes in presence of *n*-Buli at −78 °C afforded compounds **2a**–**2l** in yields



Scheme 1. Reagents and conditions: (a) R_5CHO, *n*-BuLi, THF, −78 °C, 2 h; (b) Et_3SiH, TFA, DCM, overnight, rt; (c) R_2COCl, Et_3N, THF, 15 min, rt or (d) R_2COOH, EDAC, DMAP, Et_3N, DMF/DCM (3:1, v/v), 2 h, rt or (e) TFAA, DCM, 30 min, rt. [a] Not applicable.