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Synthesis and biological evaluation of aminothiazoles against *Histoplasma capsulatum* and *Cryptococcus neoformans*

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ABSTRACT

The design and synthesis of a library of forty novel 2-aminoazole analogues as well as their evaluation as antifungal compounds against *Histoplasma capsulatum* and *Cryptococcus neoformans* is described. These structures were derived from *N*-[5-(1-naphthalenylmethyl)-2-thiazolyl]cyclohexanecarboxamide (41F5), a fungistatic agent previously identified through phenotypic screening (*Antimicrob Agents Chemother.* 2013;57:4349). Modifications to improve potency and water-solubility of 41F5 focused primarily on the 5-naphthalenyl group, the thiazole core, and the methylene linker between these two structural elements. In general, compounds with lipophilic [5+6] bicyclic ring systems, such as the 7-benzothiophenyl- and 4-indanyl groups, at the 5-position were 2–3 times more active against both fungal species as compared to 41F5. Also, introduction of a carbonyl group at the methylene linker of 41F5 resulted in a 2–3-fold increase in potency. These highly active compounds also showed generally low toxicities against murine P388D1 macrophages resulting in selectivity indices ranging from 63 to >200. Compounds that were highly active against fluconazole-sensitive *C. neoformans* strains had almost identical activity against fluconazole-resistant variants of this fungus indicating that 14α-demethylase is not their molecular target. Highly active compounds also retained activity against *H. capsulatum* phagocytosed into P388D1 macrophages.

1. Introduction

Histoplasma capsulatum, the fungal pathogen causing histoplasmosis, is endemic to the Ohio- and Mississippi River valleys of North America as well as parts of Latin American and Africa, where up to 90% of the residents display signs of prior infection.[1] Up to 50 million people have had contracted *H. capsulatum* and an estimated 500,000 cases of new infections occur annually in the US.[2,3] Fortunately, most infections are subclinical but over 3000 hospitalizations occur per year.[4] Histoplasmosis is the most common cause for hospitalization among endemic fungal disease.[4] The cost for each hospitalization is estimated to be $20,300 for children and $17,000 for adults.[4] *Cryptococcus neoformans*, the culprit of cryptococcosis, affects primarily the immunocompromised population in sub-Saharan Africa, where the human immunodeficiency virus (HIV) burden is the highest in the world. In sub-Saharan Africa, the approximate number of cases of cryptococcal meningitis is 720,000 and the 90-day case fatality is 70%.[5–8]

Major classes of current antifungal drugs include allylamines, azoles, pyrimidine analogues, polyenes, echinocandins, and oxaboroles.[9,10] These agents have different molecular targets found in the fungal cell membrane or in fungal biochemical processes.[11] All clinically utilized antifungals suffer from side effects and limited activity spectra.[9,10,12–20] In particular, echinocandins, which have the least host toxicity potential, are ineffective against

Abbreviations: Boc, *tert*-butyloxycarbonyl; DMAP, 4-dimethylaminopyridine; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HIV, human immunodeficiency virus; HOBt, hydroxybenzotriazole; HR-ESI, high resolution–electrospray ionization; LDA, lithium diisopropyl amide; MIC, minimal inhibitory concentration; rxn, reaction; SAR, structure-activity-relationship; SD, standard deviation; SM, supplementary material; SI, selectivity index; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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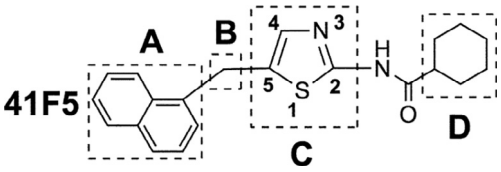
Table 1
Alignment of research objectives with areas of structural modifications at the 41F5 structure and the numbering system for all target compounds.



Objective	Areas of chemical modifications	Target compounds
1 (Increased potency against *H. capsulatum* and *C. neoformans*)	A, B, C, and D	All the target compounds (**14**–**33**, **36**, **38**, **40**)
2 (Increased water solubility)	A and C	**28** (quinoline-substituted); **29**, **31**, **32** (pyridine-substituted); **40** (imidazole-substituted)
3 (Molecular target identification)	A, B and D	**14**, **15**, **33** (ester-modified at 2- or 5-substituents)

H. capsulatum and *C. neoformans*.[12–14] Therefore, it is important to explore novel structural classes for the development of antifungal agents.

Edwards et al. performed a phenotypic screening of a purinome-focused library of 3600 commercially available compounds against *H. capsulatum* to find novel antifungal candidates and identified 41F5, which has a thiazole core structure, as the most active compound of this library (Table 1).[21] This agent displayed fungistatic activity with an IC_{50} value of 0.87 μM against *H. capsulatum* and a selectivity index of ~63 over macrophages. It was also found that 41F5 is active against *C. neoformans* with an IC_{50} of 1.25 μM but 41F5 was inactive against the fungi *Candida albicans*, *Aspergillus fumigatus* and *Blastomyces dermatitidis*.[21] 41F5 did not enhance sensitivity of *H. capsulatum* to fluconazole suggesting the target of 41F5 differs from the cytochrome P450 sterol 14α-demethylase inhibited by azole-class antifungals.[22]

The thiazole core structure has been utilized as a pharmacophore in a number of biologically active agents.[23] Examples are the anticancer agents tiazofurin and dasatinib, the anti-HIV agent ritonavir, the antiparasitic drug nitazoxanide, the anti-inflammatory agents fanetizole, meloxicam, and fentiazac, the antiulcer agent nizatidine, and the insecticide thiamethoxam.[24–26] Abafungin is an antifungal drug that contains a thiazole ring systems but is otherwise structurally different from 41F5. This compound has inhibitory activity against sterol-C24-methyltransferase and can directly damage fungal cell membrane.[27] Abafungin is fungicidal rather than fungistatic, and it is active against *Candida albicans* and *Aspergillus fumigatus*.[24] Therefore, it was suggested that abafungin and 41F5 have different mechanisms of action.[21]

Khalil et al. synthesized 68 analogues of 41F5 to improve potency against *H. capsulatum* and *C. neoformans* and to develop a structure-activity relationship (SAR) for 41F5-derived antifungal compounds.[22] Unfortunately, compounds with higher potency than 41F5 were not identified. Therefore, we synthesized and evaluated further analogues of 41F5 with the intention to improve potency against *H. capsulatum* and *C. neoformans* and to enhance water solubility. Another objective was the synthesis of 41F5 analogues that have the potential to be used for molecular target identification by unbiased affinity chromatography techniques. The results of these studies are presented in this paper.

2. Results

2.1. Design strategies

Table 1 summarizes the primary objectives of our studies and aligns these with areas of structural modifications of the 41F5 structure and the numbering system for all target compounds. Analogues of 41F5 were synthesized to (1) improve potency against *H. capsulatum* and *C. neoformans* yeasts, (2) enhance water solubility, and (3) identify the molecular target(s) of 41F5-derived antifungal compounds, which is(are) currently unknown. Additional related objectives were the evaluation of compound toxicities against fluconazole-resistant *C. neoformans* yeast, murine macrophages, and *H. capsulatum* phagocytosed into murine macrophages.

The "A" group was extensively modified to achieve all the three objectives (Table 1). In order to investigate the effect of the size on antifungal activity, the naphthalenyl group at the 5-position was replaced with tricyclic rings (fluorenyl), bicyclic rings (benzothiophenyl, benzofuranyl, 2,1,3-benzothiadiazolyl, indanyl, quinolinyl and tetralinyl), monocyclic rings (pyridinyl, cyclopentyl, and ester-modified phenyl) or the methyl group. Bioisosteric considerations played an important role in modifying this group (objective 1).[28–30] To potentially improve water solubility (objective 2), pyridine- (pKa = 5.2) (**29a**/**b**, **31**, **32a**/**b**)[31] and quinoline substituents (pKa = 4.85) (**28a**/**b**)[32] were introduced at the 5-position. The phenyl ring at the 5-position of the thiazole core was substituted with an ester group either at the 3- or 4-position (objective 3). Using standard chemical methodology, such an ester function could be converted to an alkyne-containing amide group, which could be coupled to an azide-containing solid support matrix via click chemistry. A stationary phase modified in this way could potentially be used in an unbiased affinity chromatography approach for molecular target identification,[33] which may have the potential to facilitate the structure-based design of 41F5-derived antifungal compounds. A crucial milestone in pursuing this methodology would be to initially evaluate the toxicity of ester containing compounds against *H. capsulatum* and *C. neoformans*. If such compounds would have significantly reduced activity as compared to 41F5, the fairly bulky ester group must interfere with binding to the target protein. In such a case, it would be reasonable to assume that further increased steric hindrance by subsequent chemical modifications would be even more detrimental to binding, thus, rendering this affinity chromatography approach futile. In the "B" area, the methylene linker at the 5-position was replaced with a carbonyl group in order to explore the nature of the linker on potency (objective 1). In the "C" area, other heteroaromatic ring systems, such as oxazole (**38a** and **38b**) and imidazole (**40**) were introduced to address objective 1. In addition, imidazole (pKa = 6.95)[34] was also chosen as a core replacement to explore objective 2. Similar to the attachment of an ester group to a phenyl group at the 5-position ("A" area), modifications of the "D" area encompassed introduction of ester groups to a cyclohexane ring at the 2-position (**33a**, **33b** and **33c**) to potentially explore affinity chromatography approaches for molecular target identification (objective 3).