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Synthesis and antifungal activity of substituted 2,4,6-pyrimidinetrione carbaldehyde hydrazones

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ABSTRACT

Opportunistic fungal infections caused by the *Candida* spp. are the most common human fungal infections, often resulting in severe systemic infections—a significant cause of morbidity and mortality in at-risk populations. Azole antifungals remain the mainstay of antifungal treatment for candidiasis, however development of clinical resistance to azoles by *Candida* spp. limits the drugs' efficacy and highlights the need for discovery of novel therapeutics. Recently, it has been reported that simple hydrazone derivatives have the capability to potentiate antifungal activities in vitro. Similarly, pyrimidinetrione analogs have long been explored by medicinal chemists as potential therapeutics, with more recent focus being on the potential for pyrimidinetrione antimicrobial activity. In this work, we present the synthesis of a class of novel hydrazone-pyrimidinetrione analogs using novel synthetic procedures. In addition, structure–activity relationship studies focusing on fungal growth inhibition were also performed against two clinically significant fungal pathogens. A number of derivatives, including phenylhydrazones of 5-acylpyrimidinetrione exhibited potent growth inhibition at or below 10 μM with minimal mammalian cell toxicity. In addition, in vitro studies aimed at defining the mechanism of action of the most active analogs provide preliminary evidence that these compound decrease energy production and fungal cell respiration, making this class of analogs promising novel therapies, as they target pathways not targeted by currently available antifungals.

1. Introduction

Opportunistic fungal infections caused by the *Candida* spp. represent the most common fungal infections of humans.[1] *Candida* spp. infections can result in a broad spectrum of clinical manifestations, ranging from superficial mucocutaneous infections to the more severe invasive systemic fungal infections, and these invasive fungal infections (IFI's) are a significant cause of morbidity and mortality in at-risk populations, particularly transplant recipients, cancer patients and those infected with HIV and AIDS.[2,3] IFI's present further diagnostic and therapeutic challenges due to the fact that they are difficult to diagnose early and are associated with high resistance rates to currently marketed antifungal agents.[4] Finally, the incidence of candidiasis caused by non-albicans *Candida* spp. is increasing, with *Candida glabrata* and *Candida krusei* most frequently isolated in clinical settings, in addition to *Candida albicans*.[5,6] Currently available clinical therapies for both cutaneous and systemic candidiasis include the first-line treatments of azoles. However, the development of clinical resistance to azoles by the *Candida* spp. occurs through multiple mechanisms and limits azole efficacy. For example, resistance to azoles by *C. albicans* has been shown to be largely due to both the overexpression of efflux pumps[1] and point mutations in *ERG11* gene.[7] The opportunistic yeast pathogen *C. glabrata* is also recognized for its ability to acquire resistance during prolonged treatment with azole antifungals.[8] For these reasons, there is a continuous demand for the discovery of novel therapeutics to treat fungal infections, particularly *Candida* spp. infections.

Hydrazine derivatives have recently begun to emerge in the literature as novel classes of antifungal agents with therapeutic potential against numerous *Candida* spp., including species commonly resistant to azole antifungals. For example, it was shown that (4-aryl-thiazol-2-yl)hydrazines possessed potent antifungal activities against a number of clinically relevant *Candida* spp.[9] Analogs from derivatives of the C2 and C4 positions of this hydrazine skeleton also yielded a number of promising antifungal agents that had synergistic effects when used in combination with an azole, while maintaining low mammalian cell toxicity. Finally, the hydrazine pharmacophore with substitutions of N1, together with

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4-substituted phenyls at the C4 of a thiazole nucleus produced a number of potent and selective hydrazine derivatives that possessed antifungal activity in the μM range.[9]

Other literature reports have shown that hydrazone derivatives have also emerged as compounds with the ability to potentiate antifungal activities in vitro. For example, the ability of hydrazone derivatives to inhibit the growth of *Candida* spp. was recently explored by Altintop et al.[10] Hydrazone derivatives bearing 5-thio-1-methyl 1*H* tetrazole moiety were synthesized and evaluated for potential antifungal activity and mammalian cell toxicity, with a number of compounds showing potential for further development as antifungal agents.[10]

Finally, pyrimidinetrione analogs have long been explored by medicinal chemists as not only psychotropic compounds, but as anti-seizure, anticancer and antimicrobial compounds as well. For example, pyrazole and isoxazole derivatives have gained importance as potential chemotherapeutics that have applications as antimicrobials and are active against a number of different fungal species,[11] while other pyrimidinetrione derivatives, including bisoxadiazolyl and bisthiadiazolyl pyrimidinetriones have use as antibiotic and antifungal therapies.[12] In this manuscript, we present the synthesis of an extensive collection of substituted pyrimidinetrione derivatives using novel synthetic procedures. In addition, structure–activity relationship studies focusing on fungal growth inhibition were also performed against two clinically significant fungal pathogens, namely *C. albicans* and *C. glabrata* A number of derivatives, including phenylhydrazones of 5-acylpyrimidinetrione exhibited potent growth inhibition at or below 10 μM with minimal mammalian cell toxicity. In addition, in vitro studies aimed at defining the mechanism of action of the most active analogs provide preliminary evidence that these compound decrease energy production and fungal cell respiration, making this class of analogs promising novel therapies, as they target pathways not targeted by currently available antifungals.

2. Results

2.1. Synthesis

2.1.1. Preparation of 1,3-substituted 2,4,6-pyrimidinetriones

The pyrimidinetrione building blocks used for all subsequent syntheses reported here were first prepared by the condensation of diethyl malonate with substituted urea in the presence of sodium ethoxide and ethanol following the classic Dickey–Gray procedure.[13] If the appropriate substituted urea was not commercially available, then the desired substituted urea was prepared from the corresponding amines and phenyl chloroformate by following the procedure outlined in Scheme 1.[14] Using this method, a small library of 1,3-di and mono-substituted pyrimidinetrione derivatives were generated, and then used to further synthesize all substituted pyrimidinetrione analogs presented in this work.

2.1.2. Synthesis of 5-acyl-2,4,6-pyrimidinetriones

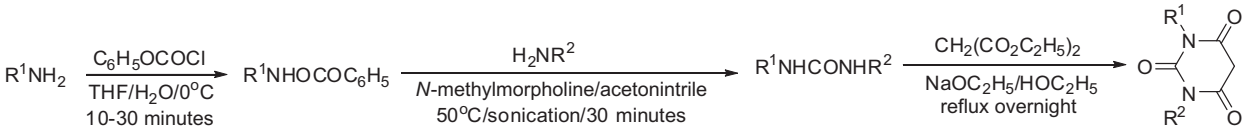
Selecting the optimal preparation method for 5-acyl pyrimidinetriones depends on both the nature of the substituted pyrimidinetrione moiety as well as the acyl moiety. Previously, we prepared a number of derivatives, including 5-formyl-1,3-dimethylpyrimidinetrione using a modified Reimer–Tiemann reaction.[15,16] However, the isolated yields using this method were <70% and furthermore, this method could not be used in the preparation of base sensitive 5-formylpyrimidinetriones. For these reasons, we developed a new and more efficient method for preparation of 5-formyl and 5-acetylpyrimidinetriones by using trimethyl orthoformate or triethyl orthoacetate as acylation reagents, respectively.[17] This method involved refluxing the corresponding pyrimidinetrione in trimethyl orthoformate with a catalytic amount of an acid catalyst (preferably with PTSA) for several hours (Scheme 2). For the preparation of acetyl derivatives, certain reaction precautions were taken. Namely, if the reaction was carried out with a temperature above 100 °C, a substantial amount of black tar was formed and isolation of the product was difficult. However, if the reaction was carried out at or below 80 °C overnight, the formation of black tar was minimal and the isolated yield was almost quantitative. New methods were also developed for the preparation of 5-aroylpyrimidinetriones (Scheme 2). The freshly prepared potassium salt of the corresponding pyrimidinetrione was condensed with aroyl chloride in THF–water as a reaction media. Although this method was excellent for pyrimidinetrione acylation with aromatic acid chlorides, dismal yields were obtained with aliphatic acid chlorides due to their fast hydrolysis in the reaction media. For this reason, other acyl pyrimidinetriones were prepared in pyridine as a reaction media by following our previously published procedures.[18]

2.1.3. Synthesis of 5-arylidene-2,4,6-pyrimidinetriones

The majority of condensation products between pyrimidinetriones and aromatic aldehydes (Knoevenagel condensation) were prepared by following our previously reported procedures.[19] However, when the condensation reaction was performed with electron-rich aromatic aldehydes, such as salicylaldehyde, 4-dimethylaminobenzaldehyde, or pyridinecarbaldehyde, special precautions were taken, due to fact that two rather than one pyrimidinetrione molecule can add easily to these aldehydes.[20] This is because the formed α,β-conjugates (Knoevenagel condensates) are very reactive Michael acceptors.[21] To control the second pyrimidinetrione addition, we eliminated the Knoevenagel condensate from the reaction mixture in the course of the reaction, in a manner similar to work presented by Deb and Bhuyan, where Knoevenagel condensation was done in aqueous media.[22] We applied the same approach to the electron rich aromatic aldehydes and pyrimidinetrione derivatives in water. Both pyrimidinetriones and the electron rich aromatic aldehydes were partially soluble in water, while the Knoevenagel condensation product was less soluble. Precipitation of the Knoevenagel product in aqueous media prevented the second pyrimidinetrione addition, and using this procedure, we were able to prepare a structurally diverse library of Knoevenagel condensates (Scheme 3).

2.1.4. Synthesis of 5-mono and 5,5-dialkylated-2,4,6-pyrimidinetriones

Previously, we prepared a several mono and di-alkylated pyrimidinetrione analogs using a different method of synthesis.[23] Here, we prepared both mono and di-alkylated pyrimidinetrione analogs



Scheme 1. Preparation of 1,3-substituted-2,4,6-pyrimidinetriones. Reagents and conditions: R^1 = C_6H_5, 2-HOC_6H_4, 3-HOC_6H_4, 4-HOC_6H_4, 4-$CH_3OC_6H_4$, 4-$(CH_3)_2NC_6H_4$, 4-$O_2NC_6H_4$, 4-$HO_2CC_6H_4$, 4-NCC_6H_4, 2-naphthyl, 2-phenanthrenyl, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, $(CH_2)_3CH_3$, $(CH_2)_2CO_2H$, $(CH_2)_5CO_2H$; R^2 = H, CH_3, C_6H_5, 4-HOC_6H_4, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl.