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Research paper

An evaluation of Minor Groove Binders as anti-fungal and anti-mycobacterial therapeutics

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ABSTRACT

This study details the synthesis and biological evaluation of a collection of 19 structurally related Minor Groove Binders (MGBs), derived from the natural product distamycin, which were designed to probe antifungal and antimycobacterial activity. From this initial set, we report several MGBs that are worth more detailed investigation and optimisation. **MGB-4**, **MGB-317** and **MGB-325** have promising MIC_{80}s of 2, 4 and 0.25 μg/mL, respectively, against the fungus *C. neoformans*. **MGB-353** and **MGB-354** have MIC_{99}s of 3.1 μM against the mycobacterium *M. tuberculosis*. The selectivity and activity of these compounds is related to their physicochemical properties and the cell wall/membrane characteristics of the infective agents.

1. Introduction

Nearly every disease-causing microbe that infects humans has developed measurable resistance to its respective anti-infective therapies, and worryingly, this has happened much faster than anticipated. The resulting drug-resistant infections give rise to increased patient mortality, hospital stays, spread of infection and healthcare costs [1]. This paper specifically reports on the discovery of compounds with significant activities against *Cryptococcus neoformans* and *Mycobacterium tuberculosis*. Both of these organisms present difficulties for drug discovery due to the challenges of penetrating their respective cell walls [2].

The fungal pathogen *Cryptococcus neoformans* is capable of causing life-threatening cryptococcal meningitis in patients in an immunocompromised state. It is a particularly significant concern in patients with advanced AIDS, causing 15%–20% of AIDS-related mortality [3]; however, those on immunosuppressive therapies and those suffering from haematological malignancy are also at risk [4–6]. In general, susceptibility to cryptococcal meningitis is characterised by a failure in pro-inflammatory immune response to primary infection which is likely to occur in the lungs [7–9]. Alveolar macrophages are the first immune cells to encounter cryptococci; however, *C. neoformans* is able to parasitise these macrophages and proliferate within the phagosome [10,11].

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The World Health Organisation (WHO) guidelines recommend that cryptococcal meningitis be treated with a 2-week course of amphotericin B alongside flucytosine which is then followed by a consolidation therapy of fluconazole [12,13]. Person-to-person transmission of cryptococcal meningitis is rare and so resistance to these antifungal therapies is unlikely to spread rapidly; only a few reports of amphotericin B resistance, the key drug in the regimen, have been documented [3,14]. However, amphotericin B has a number of significant side-effects, such as anemia, hypokalemia, hypomagnesaemia and nephrotoxicity, making the discovery of alternative therapies particularly relevant [15].

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB) infections and this disease is now recognised as a global emergency. One third of the world's population, that is approximately 6 billion people, are said to be harbouring the tuberculosis bacillus, leading to 1.5 million deaths per year [16]. There is a growing incidence of both multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) which has led to difficult and lengthy first line treatments of the infection. A four drug cocktail of isoniazid, rifampin, pyrazinamide and ethambutol is given for the first two months followed by four months of only isoniazid and rifampin – the side effects of such a regimen are notably significant. Moreover, treatment of MDR-TB is characterised by relatively less effective, poorly tolerated, and expensive drugs that may need to be administered for years [17].

There are over 3 million instances of HIV and *Mycobacterium tuberculosis* co-infection which has problematic consequences for treatment options due to the drug-drug interactions between rifampin and many antiretrovirals. Specifically, the cytochrome P450 enzymes that metabolise many antiretrovirals are induced by treatment with rifampin making co-treatment of TB and HIV a significant challenge [18]. Clearly, more effective treatments, with shorter treatment times, are a necessity if the threat of MDR-TB and XDR-TB are to be managed.

At the University of Strathclyde, we have extensively investigated the Minor Groove Binder (MGB) class of compound, based on the natural product distamycin, as anti-infective agents. This distamycin template is built from *N*-methylpyrrole amino acid amides and has an amidine tail group (Fig. 1). Our approach has led to some divergence from this structure: less basic functional groups have been introduced to replace the amidine at the C-terminus (usually referred to as the tail group because of its flexibility); larger alkyl side chains have been substituted for methyl groups; thiazole rings have been introduced to the body of the MGB; and, aromatic rings have replaced the formyl group from distamycin [19,20].

Our extensive investigation of MGBs has resulted in the discovery of significantly active compounds against a range of diseases. We have recently identified MGBs with therapeutically interesting activities against: *Trypanosoma brucei brucei* for the treatment of human African trypanosomiasis [21]; *Trypanosoma vivax* and *T. congolense* for the treatment of animal African trypanosomiasis [unpublished results]; *Plasmodium falciparum* for the treatment of malaria [22]; and, lung cancer [23]. Moreover, our commercial partner, MGB Biopharma, has successfully progressed one compound through phase I clinical trials against the Gram-positive bacterium *Clostridium difficile* [24].

This study focuses on an investigation of a collection of structurally similar MGBs designed to target mycobacteria and fungi, although an examination of their biological activity profiles against a range of infectious organisms, including bacteria, fungi and parasites, is also presented. The compound collection under investigation is comprised of three series which differ in the heterocycle attached to the tail group, either *N*-methylpyrrole, *N*-isopentylpyrrole, or isopentylthiazole (Fig. 1). Within each series, the head group position is varied according to the structures indicated in Fig. 1; however, the isopentylthiazole series also included four additional head groups to further explore the interesting activity of this series discovered during the course of the study. The amidine-linked head group used in this study has been investigated in isolated cases in some of our previous work, but this present study is our first systematic study of MGBs containing it. This amidine head group link might have advantages in terms of solubility and intrinsic activity with pathogens with troublesome cell walls because it generates dicationic MGBs. The systematic structural variations in this study examine whether the inclusion of lipophilic moieties, such as thiazoles and alkyl side chains, are important for penetration of the waxy cell wall of mycobacteria. Similarly, the inclusion of amidine-linked head groups is to investigate their importance in providing an additional source of hydrogen bonding to assist in penetrating the polyglycan cell wall of fungi [2]. Structural features that allow cellular accumulation may be more important for the activity and selectivity of MGBs than their specific DNA binding properties.

2. Results and discussion

2.1. Synthesis

The synthesis of amidine-linked head group or thiazole containing MGBs has not been extensively described in our previous work. To prepare compounds containing isopentyl thiazole(Scheme 1), 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid was converted

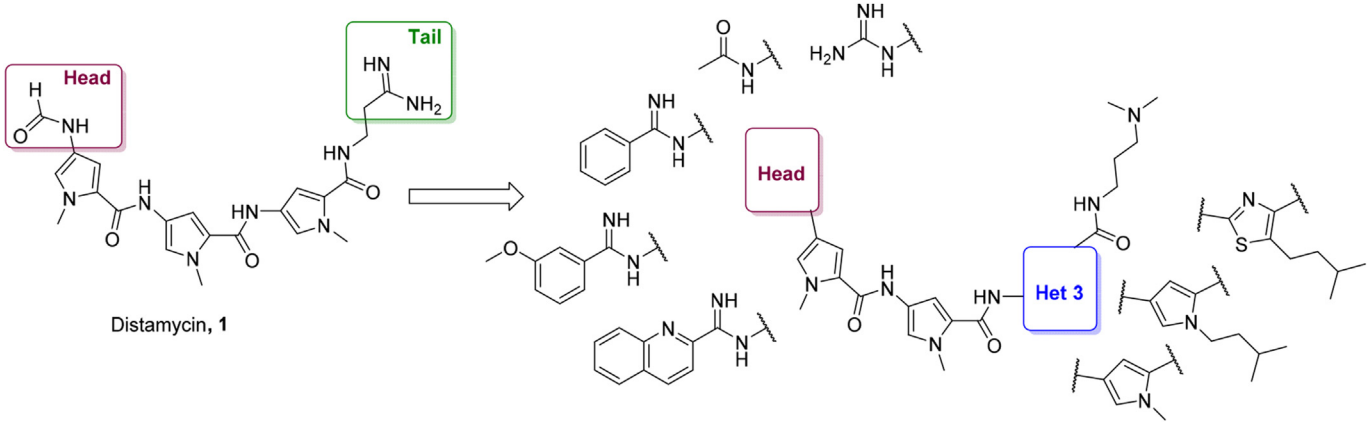

Fig. 1. Distamycin and examples of MGBs in this study.