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Research paper

Pyrrolidine nucleoside bisphosphonates as antituberculosis agents targeting hypoxanthine-guanine phosphoribosyltransferase

Wai Soon Eng [a], Dominik Rejman [b, **], Radek Pohl [b], Nicholas P. West [a], Kyra Woods [a], Lieve M.J. Naesens [c], Dianne T. Keough [a], Luke W. Guddat [a, *]

[a] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072, QLD, Australia
[b] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i. Flemingovo nam. 2, CZ-166 10 Prague 6, Czech Republic
[c] Rega Institute for Medical Research, KU Leuven – University of Leuven, Herestraat 49, B-3000, Leuven, Belgium

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ABSTRACT

Therapeutic treatment of tuberculosis (TB) is becoming increasingly problematic due to the emergence of drug resistant *Mycobacterium tuberculosis* (*Mt*). Thus, new targets for anti-TB drug discovery need to be identified to combat and eradicate this disease. One such target is hypoxanthine-guanine phosphoribosyltransferase (HGPRT) which synthesises the 6-oxopurine nucleoside monophosphates essential for DNA/RNA production. [3R,4R]-4-Hypoxanthin-9-yl-3-((*S*)-2-hydroxy-2-phosphonoethyl)oxy-1-N-(phosphonopropionyl)pyrrolidine and [3R,4R]-4-guanin-9-yl-3-((*S*)-2-hydroxy-2-phosphonoethyl)oxy-1-N-(phosphonopropionyl)pyrrolidine (compound **6**) are the most potent inhibitors of *Mt*HGPRT yet discovered having K_i values of 60 nM. The crystal structure of the *Mt*HGPRT.**6** complex was obtained and compared with that of human HGPRT in complex with the same inhibitor. These structures provide explanations for the 60-fold difference in the inhibition constants between these two enzymes and a foundation for the design of next generation inhibitors. In addition, crystal structures of *Mt*HGPRT in complex with two pyrrolidine nucleoside phosphosphonate inhibitors plus pyrophosphate provide insights into the final stage of the catalytic reaction. As the first step in ascertaining if such compounds have the potential to be developed as anti-TB therapeutics, the tetra-(ethyl L-phenylalanine) tetraamide prodrug of **6** was tested in cell based assays. This compound arrested the growth of virulent *Mt* not only in its replicating phase (IC_{50} of 14 μM) but also in its latent phase (IC_{50} of 29 μM). Furthermore, it arrested the growth of *Mt* in infected macrophages (MIC_{50} of 85 μM) and has a low cytotoxicity in mammalian cells (CC_{50} of 132 ± 20 μM). These inhibitors are therefore viewed as forerunners of new anti-TB chemotherapeutics.

1. Introduction

Mycobacterium tuberculosis (*Mt*) is the predominant etiological agent for human tuberculosis (TB) [1]. TB remains a global public health threat, with 10 million cases of active disease per annum resulting in 1.8 million deaths [2]. Current treatment for TB is a standard six-month regimen of rifampicin and isoniazid, supplemented with pyrazinamide and ethambutol in the first two months [3]. However, the emergence of multidrug-resistant *Mt* (MDR-TB) [4] and extensively drug-resistant *Mt* (XDR-TB) [5] has limited the capacity to eradicate this disease. A major obstacle in eradication is the development of drugs that efficiently target both the replicating and non-replicating/dormant stages of *Mt* [6]. For example, when in dormancy, *Mt* is insensitive to the frontline drug, isoniazid [7]. Thus, there is an urgent need for new cost-effective TB therapeutics directed against not only the replicating stage of this pathogen but also its dormant (latent/persistent) stage. Crucially, apart from bedaquiline, which has associated toxicity concerns [8] and is currently the subject of clinical investigation [9,10], it has been

Abbreviations: TB, Tuberculosis; *Mt*, Mycobacterium tuberculosis; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; MDR, multidrug resistant; XDR, extensively drug-resistant; PP_i, pyrophosphate; *P*Rib-*PP*, 5-phospho-α-D-ribosyl-1-pyrophosphate; ANP, acyclic nucleoside phosphonate; PNP, pyrrolidine nucleoside phosphonates; PNBP, pyrrolidine nucleoside bisphosphonates; AIP, acyclic immucillin phosphonate.

* Corresponding author.
** Corresponding author.
E-mail addresses: dominik.rejman@uochb.cas.cz (D. Rejman), luke.guddat@uq.edu.au (L.W. Guddat).

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more than 50 years since a new therapeutically viable anti-TB drug was commercially introduced onto the market [11].

Evidence that hypoxanthine-guanine phosphoribosyltransferase is a target for the development of anti-TB therapeutics comes from two sources. The first is a random transposon mutagenesis study which showed that the expression of this enzyme is essential for the survival of *Mt* [12]. The second is a recent study which showed that prodrugs of inhibitors of *Mt* hypoxanthine-guanine phosphoribosyltransferase (*Mt*HGPRT) activity arrested the growth of a virulent strain of *Mt* in cell culture [13]. Thus, began the search for the design of new and more potent inhibitors which could also posses anti-TB activity.

*Mt*HGPRT catalyses the formation of the nucleoside monophosphates, IMP or GMP, and pyrophosphate (PP_i). The substrates are 5-phospho-α-D-ribosyl-1-pyrophosphate (*P*Rib-*PP*) and hypoxanthine (Hx) or guanine (G); xanthine is not a substrate [13,14]. For catalysis to occur, a divalent metal ion, usually magnesium, is required (Fig. 1). Literature reports suggest that the mechanism of action of *Mt*HGPRT is ordered and sequential [15], similar to that of human HGPRT [16]. However, the kinetic parameters for the two enzymes are very different. *P*Rib-*PP*, for example, has a much higher K_m for *Mt*HGPRT than for human HGPRT with values of 465 μM with guanine as the base and 1443 μM with hypoxanthine as the base [13,14] compared with 60 μM and 30 μM, respectively, for human HGPRT [16,17]. The values for the purine bases, however, differ by only two-fold with the K_m for guanine and hypoxanthine being 4.4 and 8.3 μM for *Mt*HGPRT [13,17] while, for human HGPRT, these values are 1.9 and 3.4 μM [17], respectively. The k_{cat} values for these two enzymes in the forward reaction are also widely different. *Mt*HGPRT has a k_{cat} values of 0.6 s^{-1} (guanine as base) and 0.5 s^{-1} (hypoxanthine as base) [13] while, for human HGPRT, the k_{cat} values are 10-fold higher, 8.2 s^{-1} (guanine) and 5.2 s^{-1} (hypoxanthine) [17]. The tetrameric structure of both enzymes also differ in the arrangement of their subunits. Thus, in the quaternary structure of the human enzyme, the large mobile loop which moves to cover the active site during catalysis is located on the outside of the structure where, in its open position, it is exposed to solvent. In contrast, this loop is buried at the interface of the dimer pairs in *Mt*HGPRT [13]. Whether this structural arrangement has an effect on the kinetic constants is, however, unknown at present.

The only reported inhibitors of *Mt*HGPRT to date are the acyclic nucleoside phosphonates (ANPs) [13]. Their structure consists of a phosphonate moiety connected to a nucleobase via an acyclic linker. The advantage of using these compounds as a template for therapeutics is the presence of the carbon phosphorus bond within the phosphonate moiety which makes them enzymatically and chemically stable within the cell [18]. The genesis for these potential anti-TB drugs is based on structure of the successful antiviral agent tenofovir that was developed by Antonin Holý and colleagues [18]. Prodrugs of the ANPs arrest the growth of *Pf* in cell culture [19–21] highlighting the possibility that selective design could lead to the development of chemotherapeutics against *Mt* where the activity of this enzyme also appears to be essential for the survival and reproduction of this organism.

Pyrrolidine nucleoside monophosphonates (PNPs) inhibit the two 6-oxopurine PRTases from *Escherichia coli,* XGPRT and HPRT [22], *Plasmodium falciparum* HGXPRT [19], *Plasmodium vivax* HGPRT [19] and human HGPRT [19,22]. In these compounds, a purine base is attached to a phosphonate moiety via a pyrrolidine ring in the linker which connects the two functional groups. The five membered pyrrolidine ring is connected to the N [9] atom of the purine base (Fig. 1), as is the ribose moiety of the products of the catalytic reaction, GMP and IMP. The basic structure of this class of inhibitor is shown in Fig. 2 indicating their structural diversity and how and where chemical modifications can be made to increase their potency for the 6-oxopurine phosphoribosyltransferases from different organisms. For example, different chemical attachments can be made at the carbon (R^1) and/or the nitrogen (R^2) position of the pyrrolidine ring (Fig. 2).

In this report, eight compounds containing a pyrrolidine group in the acyclic linker were trialled as inhibitors of *Mt*HGPRT (Fig. 3). Two of these belong to the purine nucleoside monophosphonate (PNP) class of compounds while six belong to the pyrrolidine nucleoside bisphosphonates (PNBPs) class. The first class contains a single phosphonate group while the second contains two phosphonate groups. X-ray crystal structures of the two PNPs, ([3R,4R]-(4-(hypoxanthin-9-yl)pyrrolidin-3-yl)-oxymethanephosphonic and [3R,4R]-(4-(guanin-9-yl)pyrrolidin-3-yl)oxymethanephosphonic acid were obtained in complex *Mt*HGPRT in the presence of pyrophosphate. The X-ray structure of one of the PNBPs, ([3R,4R]-4-guanin-9-yl-3-((*S*)-2-hydroxy-2-phosphonoethyl)oxy-1-N-(phosphonopropionyl) pyrrolidine) (**6**) in complex with *Mt*HGPRT was also obtained. This structure was then compared with that of the human counterpart in complex with the same inhibitor to explain the 60-fold difference in the K_i values and to provide the necessary tools to improve their design aimed at increasing potency for *Mt*HGPRT. In order to determine if such coumpounds have the potential for further development as anti-TB agents, a tetra-(ethyl L-phenylalanine) tetraamide prodrug of **6** was tested against TB in cell culture. These studies were

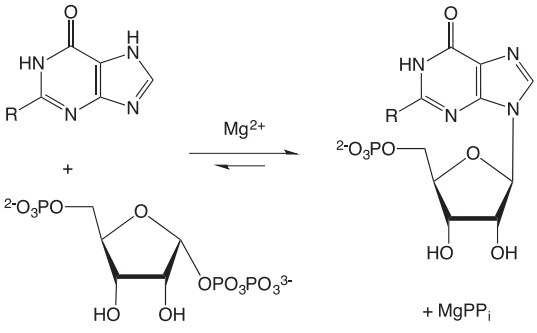

Fig. 1. The reaction catalysed by *Mt*HGPRT. R = -H (hypoxanthine); $-NH_2$ (guanine). The other naturally-occurring purine base, xanthine (R = -OH), is not a substrate [13,14].

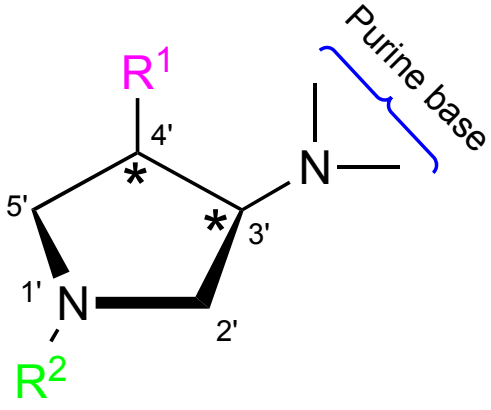

Fig. 2. The chemical structure of the phosphonates which contain a pyrrolidine ring in the linker. The pyrrolidine group can be attached to any purine base at the 9-position in the imidazole ring. R^1 and R^2 denote where different chemical moities can be attached to the pyrrolidine ring. In the two pyrrolidine nucleoside monophosphonates (PNPs) described here, a phosphonate group is attached at the R^1 position and, in the pyrrolidine nucleoside bisphosphonates (PNBPs), a second phosphonate group is attached at R [2]. The (*) shows the stereocenters.