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Synthesis of sulfamide analogues of deoxythymidine monophosphate as potential inhibitors of mycobacterial cell wall biosynthesis

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ABSTRACT

The recently discovered enzyme *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPK*mt*), which catalyses the phosphorylation of deoxythymidine monophosphate (dTMP) to give deoxythymidine diphosphate (dTDP), is indispensable for the growth and survival of *M. tuberculosis* as it plays an essential role in DNA synthesis. Inhibition of TMPK*mt* is an attractive avenue for the development of novel anti-tuberculosis agents. Based on the premise that sulfamide may be a suitable isostere of phosphate, deoxythymidine analogues comprising various substituted sulfamides at C5′ were modelled *in silico* into the active site of TMPK*mt* (PDB accession code: 1N5K) using induced-fit docking methods. A selection of modelled compounds was synthesized, and their activity as inhibitors of TMPK*mt* was evaluated. Three compounds showed competitive inhibition of TMPK*mt* in the micromolar range (10 −50 μM). Compounds were tested *in vitro* for anti-mycobacterial activity against *M. smegmatis*: three compounds showed weak anti-mycobacterial activity (MIC 250 μg/mL).

1. Introduction

Tuberculosis (TB) is one of the primary infectious diseases worldwide, especially in developing countries. *Mycobacterium tuberculosis* is recognised as the causative agent of TB [1]. TB has affected humans since antiquity, and remains as a major threat to human health. Alarmingly up to one third of the world's population is currently latently infected, and 10.4 million new cases and 1.4 million deaths were reported in 2016 [2]. People infected by Human Immunodeficiency Virus (HIV) are at an especially high risk of contracting TB due to their compromised immune system. In many countries, the rate of increase of infection is exacerbated by both poor public health and apparent synergism with HIV [2,3]. The resurgence of the disease has prompted interest in increasing understanding of TB and the development of new anti-mycobacterial agents against drug-resistant tuberculosis.

The current requirement for long duration treatments and the emergence of multi- (MDR-TB) and extensively drug resistant (XDR-TB) forms of *M. tuberculosis* represent significant challenges in the effective treatment of tuberculosis [4]. The discovery of new mycobacterial targets and the development of agents that require shorter treatment durations are essential to combat the rise of MDR-TB and XDR-TB.

The recently discovered enzyme *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPK*mt*) is indispensable for the growth and survival of *M. tuberculosis*, as it plays an essential role in DNA synthesis [5]. TMPK*mt* catalyses the phosphorylation of deoxythymidine monophosphate (dTMP **1a**) to give deoxythymidine diphosphate (dTDP **1b**) using ATP as the phosphoryl donor (Fig. 1), and is essential for maintaining the deoxythymidine triphosphate pool that is required for DNA synthesis and bacterial replication. This phosphorylation step occurs at the junction of the *de novo* and salvage pathways in the biosynthesis of deoxythymidine triphosphate (dTTP). Importantly TMPK*mt* has low (22%) sequence identity with the human isozyme, and therefore represents a promising target for the development of selective inhibitors [6].

The synthesis of potential inhibitors of TMPK*mt* has previously been reported by Van Calenbergh [7–10] and several others

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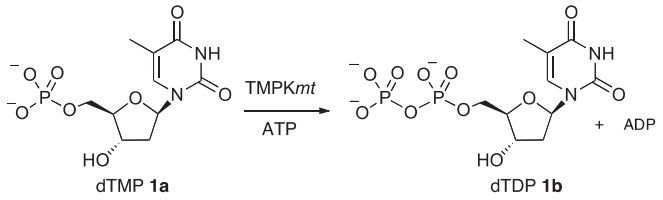

Fig. 1. Action of TMPK*mt*.

[11–15], including a series of 5′-thiourea-substitued α-thymidine derivatives [10] which displayed promising inhibitory activity; these had K_is vs. TMPK*mt* in the 0.17–260 μM range, and also inhibited *M. bovis* in Alamar Blue assays with minimum inhibitory concentrations (MICs) in the range from 20 to 100 μg/mL [7,10].

Although the synthesis and biological activities of various sulfonamides and sulfamates have been widely reported, there are only a limited number of investigations into sulfamides as possible phosphate isosteres. Amongst these limited studies, Aldrich [16,17] reported the use of sulfamide as an isosteric replacement for phosphate in the search for anti-mycobacterial agents by way of their inhibition of siderophore biosynthesis; in this case salicyl sulfamoyl adenosine and its derivatives displayed promising inhibitory activity, with MICs ranging from 0.19 to 6.25 μg/mL against *M. tuberculosis*.

Previously we reported the synthesis and biological activity [18] of a series of *arabino*-glycosyl sulfamides as potential mimics of decaprenolphosphoarabinose (DPA), and thus as novel anti-mycobacterial agents. Crystallographic studies performed during that work showing the clear tetrahedral geometry at sulphur gave further credence to the idea that this interesting and under-explored motif may have value in the design of new pharmacological agents comprising sulfamide as a phosphate isostere. In line with our on-going interest in the development of new anti-mycobacterial agents, the design and synthesis of sulfamides as analogues of deoxythymidine monophosphate was selected as an avenue for study. We report herein the *in silico* modelling of a variety of sulfamide analogues of dTMP **1a** into the active site of TMPK*mt* using induced-fit docking methods [19], the synthesis of a selection of these compounds and their evaluation *in vitro* as inhibitors of TMPK*mt*, and finally a study of their anti-mycobacterial activity against *M. smegmatis*.

2. Results and discussion

2.1. Docking studies

A small library of sulfamide analogues of dTMP **1a** was designed and screened with the published three-dimensional structure of TMPK*mt* (PDB accession code: 1N5K) [20], using an induced fit docking method with Schrödinger Suite 2014 [21,22]. Firstly the natural substrate dTMP was modelled back into the active site (Fig. 2) with a rigid docking protocol in Glide [23], and it was confirmed that the binding mode of the substrate could be successfully reproduced by docking calculations [20].

Next, in order to investigate the binding affinity of a variety of sulfamide structures, a number of target compounds was selected (Fig. 3). Besides simple alkylated (**2a-c, 2f**) and un-substituted (**2g**) sulfamides a variety of other compounds comprising substituted aromatic rings was also selected in order to study any importance of aryl substituents. In particular since Van Calenbergh had previously shown that a variety of thiourea derivatives of thymidine [8,10] possessing 3-trifluoromethyl-4-chloro- and halogenated phenyl rings had displayed promising inhibitory activity (with K_is

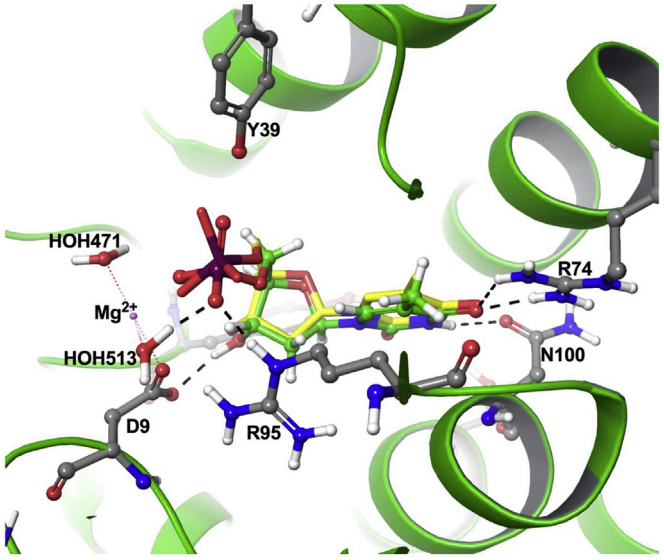

Fig. 2. Superimposition of the modelled dTMP **1a** conformation (green carbon atoms) with that found in the reported crystal structure (yellow carbon atoms) in the active site of TMPK*mt* (PDB: 1N5K, displayed with grey carbon atoms and green ribbon). Hydrogen bonds formed between the modelled conformation of dTMP and the active site are shown as black dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vs. TMPK*mt* in the 0.17–3.2 μM range), a variety of sulfamides containing these structural motifs was selected. Additionally since these studies had also highlighted the potential for α-thymidine derivatives to inhibit TMPK*mt*, the corresponding α-anomers **2p-r** were also considered. Compounds **2a-r** were then modelled into the TMPK*mt* active site using the induced fit docking protocol [22], and the docking scores are listed in Table 1.

Non-substituted sulfamide **2g** is the structurally most similar analogue to dTMP, and gave a docking score of −12.153 kcal/mol, suggesting that the binding affinity of compound **2g** should actually be less than that of the natural substrate dTMP **1a** (−14.611 kcal/mol). As shown in Fig. 4, the sulfamide group of sulfamide **2g** is located in close proximity to the phosphate-binding site. The phosphate group of the natural substrate dTMP **1a** forms H-bonds with a water molecule and Arg95 (R95: Fig. 2). In the case of the sulfamide **2g**, there is no strong interaction with Arg95 due to the lack of a full negative charge on the sulfamide. However the N-H groups of the sulfamide **2g** are seen to form H-bonds with the OH group of Tyr39 (Y39: Fig. 4), providing an example of an additional interaction that may increase compound binding affinity.

The docking scores of the compounds screened ranged from −10.9 to −12.6 kcal/mol. In fact surprisingly all sulfamides, except the trifluoroethyl substituted sulfamide **2f**, gave docking scores which were similar to sulfamide **2g**, i.e. in the range from −11 to −12 kcal/mol, suggesting that adding extra functional groups to the sulfamide may not improve binding. Furthermore the binding poses predicted by the induced-fit docking suggested that additional groups would be mostly solvent exposed, as there is not sufficient space in the active site to accommodate extra groups. However, in a few cases the docking studies predicted extra binding interactions between amino acids of the protein and additional functional groups incorporated into the substituted sulfamides. For example, docking of azidophenyl substituted sulfamide **2k** predicted the formation of a pi-cation interaction between Arg95 (R95) and the aromatic ring, and a salt bridge between Asp94 (D94) and the azide (Fig. 5A). Furthermore, docking of the trifluoromethyl substituted phenyl sulfamide **2h** also revealed a pi-cation interaction between the substituted aromatic