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Phosphorylated hydroxyethylamines as novel inhibitors of the bacterial cell wall biosynthesis enzymes MurC to MurF

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ABSTRACT

Enzymes involved in the biosynthesis of bacterial peptidoglycan represent important targets for development of new antibacterial drugs. Among them, Mur ligases (MurC to MurF) catalyze the formation of the final cytoplasmic precursor UDP-*N*-acetylmuramyl-pentapeptide from UDP-*N*-acetylmuramic acid. We present the design, synthesis and biological evaluation of a series of phosphorylated hydroxyethylamines as new type of small-molecule inhibitors of Mur ligases. We show that the phosphate group attached to the hydroxyl moiety of the hydroxyethylamine core is essential for good inhibitory activity. The IC_{50} values of these inhibitors were in the micromolar range, which makes them a promising starting point for the development of multiple inhibitors of Mur ligases as potential antibacterial agents. In addition, 1-(4-methoxyphenylsulfonamido)-3-morpholinopropan-2-yl dihydrogen phosphate **7a** was discovered as one of the best inhibitors of MurE described so far.

1. Introduction

Treatment of infectious diseases is being compromised worldwide by the emergence of bacteria that are resistant to numerous antibiotics [1–3]. Increased morbidity and mortality are the most dramatic consequences of this resistance [4]. Therefore, there is an urgent need for the development of novel antibacterial agents. Some of the best known and most validated targets for antibacterial therapy are the enzymes involved in the biosynthesis of peptidoglycan [5,6]. β-Lactam and glycopeptide antibiotics are well known inhibitors of the late, extracellular stages of bacterial peptidoglycan biosynthesis. However, in the past few years, more attention has been focused on the early intracellular biosynthetic steps as potential drug targets [6–8].

Peptidoglycan is a major component of the cell wall of almost all eubacteria. Its main function is to provide the rigidity, flexibility and strength that are necessary for bacterial cells to grow and divide, while withstanding the high internal osmotic pressure [9]. Peptidoglycan is a complex heteropolymer that is composed of long glycan chains made up of alternating units of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). The D-lactoyl group of each MurNAc residue is substituted by a peptide unit with a composition that is generally L-alanyl-γ-D-glutamyl-*meso*-diaminopimeloyl(or L-lysyl)-D-alanine [9]. The biosynthesis of peptidoglycan involves a number of ATP-dependant ligases (MurC to MurF), which contribute to the formation of UDP-MurNAc-pentapeptide by successive additions to UDP-MurNAc of L-Ala (MurC), D-Glu (MurD), *meso*-diaminopimelic acid or L-Lys (MurE) and D-Ala-D-Ala (MurF) [6]. The MurC to MurF ligases have the same reaction mechanism (Fig. 1), which consists of the activation of the carboxyl group of the nucleotide precursor by ATP, generating an acyl phosphate intermediate and ADP. The acyl phosphate is then attacked by the amino group of the incoming amino acid (or dipeptide in the case of MurF), leading to the formation of a high-energy tetrahedral intermediate; this eventually breaks down into the product and P_i [6].

Over the last few years, a number of Mur ligase inhibitors have been developed [6,8,10]. Among these, transition-state analogues, like phosphonates, phosphinates and sulfonamides, have been described as inhibitors of MurC [11,12], MurD [13–20], MurE [20–22] and MurF [23]. Our aim was to design novel types of transition-state analogues as potential inhibitors of these Mur ligases. We focused our attention on hydroxyethylamines (HEAs, Fig. 1), which have been described as analogues of high-energy tetrahedral reaction intermediates of different proteases [24–29] and which represent the fundamental moiety used in inhibitors of HIV protease [28–30], cathepsin D [28,31], angiotensin-converting enzyme [32], malarial proteases [28,29,33,34] and β-amyloid cleaving enzyme (β-secretase) [35–39]. However, so far they have not been used in the design of Mur ligase inhibitors. In a previous report [40], we presented phosphorylated HEA derivatives (compounds **6a–b** and **7a–b** in Fig. 2) as inhibitors of the bacterial peptidoglycan biosynthesis enzymes D-alanine:D-alanine ligase (DdlB) and

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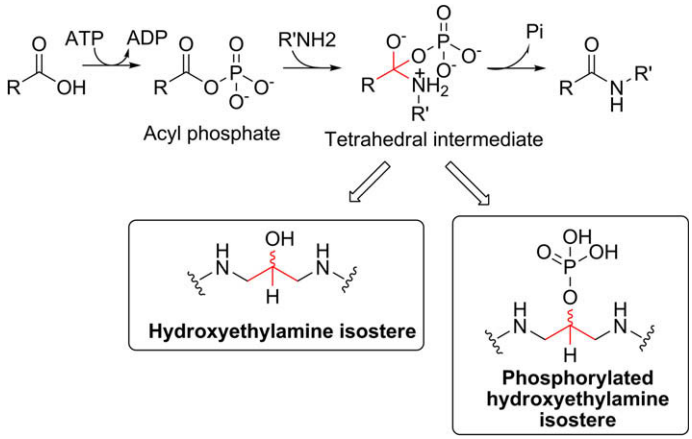

Fig. 1. Reaction mechanism of Mur ligases and design of hydroxyethylamine isosteres as transition-state analogues.

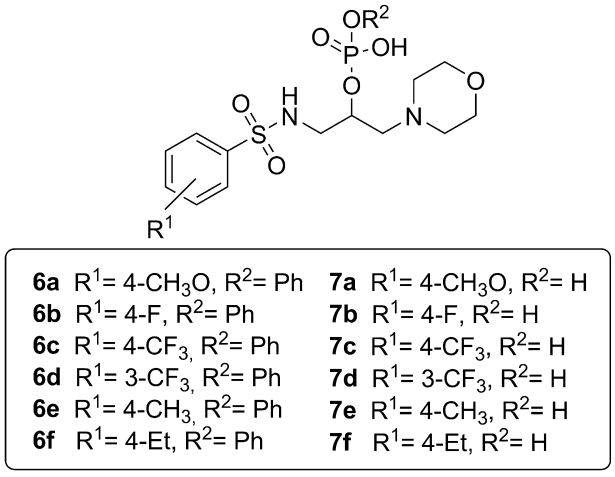

Fig. 2. General formulae of phosphorylated hydroxyethylamines as potential inhibitors of Mur ligases.

D-alanine:D-lactate ligase (VanA). These two enzymes are the ATP-dependent ligases. Although their three-dimensional structure is different, their reaction mechanism is similar to that of the Mur ligases [6]. In this paper we present the synthesis and initial structure–activity relationship of a series of phosphorylated HEAs as promising inhibitors of Mur ligases.

2. Methods and materials

2.1. Chemistry

All of the chemicals used were obtained from commercial sources (Acros, Aldrich, Fluka and Merck) and used without further purification. Biomol Green® reagent was purchased from Biomol® International, a brand of Enzo Life Sciences, Inc. Solvents were used without purification or drying, unless otherwise stated. Reactions were monitored using analytical TLC plates (Merck, silica gel 60 F_{254}, 0.25 mm), and compounds were visualized with ultraviolet light and ninhydrin or bromocresol green. Preparative thin-layer chromatography was carried out on PSC-Platten 20 × 20 cm Kieselgel 60 F_{254}, 2 mm (Merck). The microwave reactions were performed using a CEM Discover® microwave synthesis system. Circular chromatography was carried out on a Chromatotron® centrifugal thin-layer chromatograph (Harrison Research), using silica gel 60 GP_{254}-containing gypsum. Silica gel grade 60 (70–230 mesh, Merck) was used for column chromatography. NMR spectra were obtained on a Bruker Advance DPX 300 instrument. ^{1}H NMR were recorded at 300.13 MHz with tetramethylsilane as an internal standard. Mass spectra were obtained with a VG-Analytical Autospec Q mass spectrometer (Centre for Mass Spectrometry, Institute Jožef Stefan, Ljubljana). IR spectra were recorded on a Perkin–Elmer FTIR 1600 spectrometer. Microanalyses were carried out by the Department of Organic Chemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, on a 240 C Perkin Elmer elemental analyzer. Melting points were determined using a Reichert hot-stage microscope and are uncorrected. HPLC analyses were performed on a HP 1100 Agilent Technologies instrument with G1365B UV–vis detector (254 and 220 nm), using a Luna C18 column (4.6 mm × 250 mm) at a flow rate of 1 mL/min. The eluant was a mixture of 0.1% TFA in water (A) and acetonitrile (B). The gradient was from 5% B to 75% B in 15 min for compounds **6a–f** and **7a–f**, while for **6e** and **7e** the gradient was from 5% B to 95% B in 15 min.

2.1.1. General procedure for the synthesis of compounds *2a–f*

To a solution of **1a–f** (10.0 mmol) in anhydrous dichloromethane (50 mL), allylamine (21.0 mmol, 1.57 mL) was added slowly at 0 °C, with the resulting mixture stirred for 1 h at room temperature. The reaction mixture was filtered and washed with 10% citric acid (2 × 25 mL), water (25 mL) and brine (25 mL). The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to obtain **2a–f**.

2.1.2. General procedure for the synthesis of compounds *3a–f*

To a solution of **2a–f** (5.0 mmol) in dichloromethane (30 mL), *meta*-chloroperoxybenzoic acid (1.48 g, 70% wt., 6.0 mmol) was added at 0 °C and the resulting mixture was stirred for 48 h at room temperature. The reaction mixture was filtered and dichloromethane removed under reduced pressure. The residue was dissolved in diethylether (or ethyl acetate in case of low solubility in diethylether) (50 mL) and washed with 10% Na_2SO_3 (3 × 25 mL), 10% $NaHCO_3$ (4 × 25 mL), water (25 mL) and brine (25 mL). The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to obtain **3a–f**.

2.1.3. General procedure for the synthesis of compounds *4a–f*

To a solution of **3a–f** (3.0 mmol) in anhydrous dioxane (6 mL), morpholine (3.3 mmol, 0.29 mL) and calcium trifluoromethanesulfonate (1.0 mmol, 0.348 g) were added and the resulting mixture was stirred in a microwave reactor for 5 min at 120 °C. The reaction mixture was cooled to room temperature and filtered. Dioxane was