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4-Thiazolidinones: Novel Inhibitors of the Bacterial Enzyme MurB

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Abstract—4-Thiazolidinones were synthesized and evaluated for their ability to inhibit the bacterial enzyme MurB. Selected 4-thiazolidinones displayed activity against the enzyme in vitro. This activity, coupled with the design principles of the thiazolidinones, supports the postulate that 4-thiazolidinones may be recognized as diphosphate mimics by a biological selector.

Introduction

Peptidogylcan is an essential component of the cell wall of both Gram-positive and Gram-negative bacteria. During the biosynthesis of this polymer, the enzyme MurB carries out the reduction of enolpyruvyl uridine diphosphate *N*-acetylglucosamine (EP-UNAG) to uridine diphosphate *N*-acetylmuramic acid (UNAM),[1] an intermediate in the assembly of the UNAM-pentapeptide portion of its cell wall precursor. To date, there are no known small molecule inhibitors of MurB, an enzyme unique to prokaryotic cells. The emergence of both vancomycin and methicillin resistant bacteria in the last decade has greatly fueled the need for a novel class of antibiotics. Clearly, any molecule that would competitively inhibit this vital enzyme would possess the potential to be an attractive antibacterial agent.[2] This letter details the results of our efforts to identify a novel class of non-peptidic, small molecule inhibitors of MurB.

For our starting point, we relied on de novo structure based design using published[3] X-ray crystallographic data of MurB and the bound substrate EP-UNAG. Using the EP-UNAG substrate as a guide, we sought a suitable surrogate of the diphosphate moiety. Our evaluation criteria were 2-fold. First, the template should contain functionality that could potentially mimic key interactions of the diphosphate with the enzyme. Further, the template should be able to orient the resultant side chains in such a way that they would occupy space similar to the glucosamine and uridine moieties of the substrate.

Ultimately, this approach lead us to focus on substituted 4-thiazolidinones (Fig. 1) as potential diphosphate surrogates[4] and inhibitors of MurB.

Figure 2 depicts our overlay of the thiazolidinone core on the diphosphate region of the EP-UNAG substrate. The R_1 and R_2 side chains can be extended to occupy the relative position of the glucosamine. Upon careful analysis of the X-ray crystal structure of the EP-UNAG-MurB complex we noted that the only readily apparent ionic interaction is with the phosphate attached to the sugar and the protein lysine 217 side chain. The acid of R_1 (derived from the corresponding amino acid) nicely mimics this phosphate. We also noted that the uridine portion of the substrate made very little contact with MurB; thus, we hypothesized that this portion of the substrate may have little function in its binding to MurB and that the R_3 side chain may not be necessary for biological activity in our diphosphate surrogate. While the thiazolidinone does not specifically mimic all of the bonding interactions of the diphosphate, elimination of the corresponding rotatable bonds of the substrate was predicted to provide some entropic benefit to binding.

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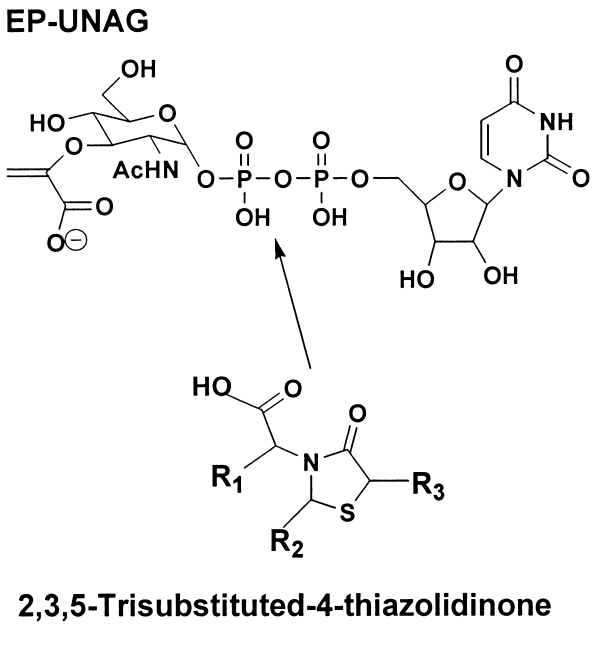


Figure 1. Structures of diphosphate containing EP-UNAG and the generalized 4-thiazolidinone.

Materials and Methods

A number of methods for the synthesis of 4-thiazolidinones have been reported.[5,6] Our general synthetic approach was based on the classical procedure of condensing thioglycolic acid, the requisite amino acid and aromatic aldehydes in a one-pot reaction.[6e] All compounds were characterized by LC-MS and select compounds further characterized by ^{1}H NMR.

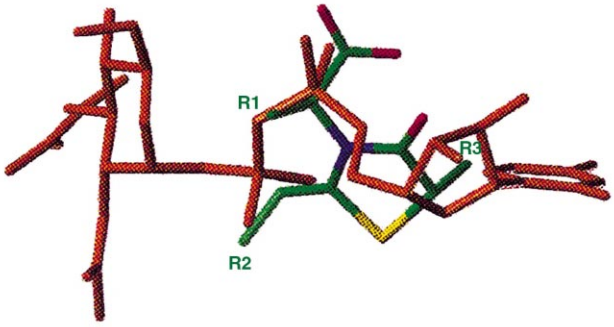
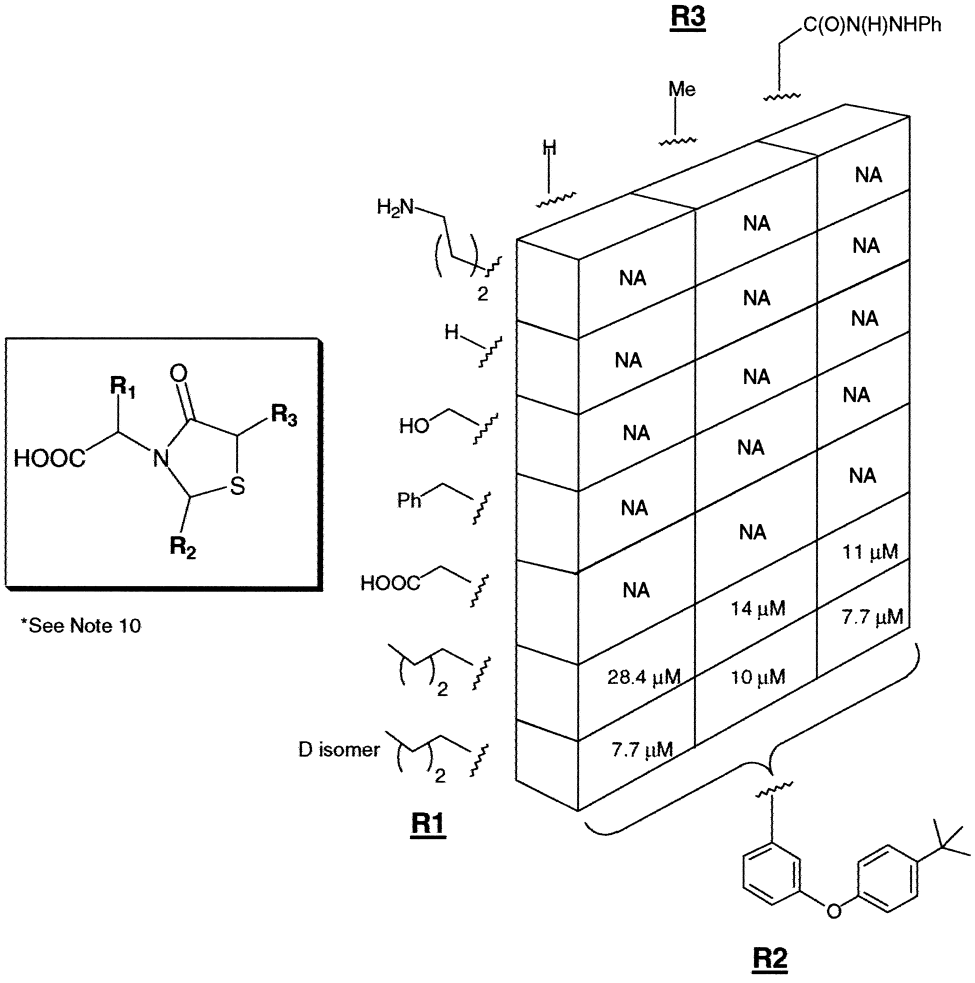


Figure 2. Overlay of 4-thiazolidinone core and diphosphate moiety.

Table 1. In vitro potency of 4-thiazolidinones



Enolpyruvyl uridine diphosphate *N*-acetylglucosamine (EP-UNAG) reductase (MurB) was over expressed and purified.[7] The assay for MurB activity in the presence or absence of an inhibitor was conducted with the aid of a Cetus Pro/Pette for ingredients dispensing and a Spectra Max 250 microplate spectrophotometer (by Molecular Device) for MurB activity determination. Each MurB assay mixture contained: 20 mM Tris–HCl, pH 7.4, 20 mM KCl, 0.5 mM DTT, 100 μM NADPH, 50 μM EP-UNAG. The compounds were individually dissolved in DMSO at 100-fold higher concentration than that used in the final assay. Three and a half microliters of compound solution was delivered to prelabeled flat bottom 96-well microtiter plates containing 320 μL MurB assay mixture. For the vehicle controls, 3.5 μL of DMSO was added. The reactions were started by the addition of 30 μL of MurB and the decrease of absorbency at 340 nm was monitored continuously on a Spectra Max 250 microplate spectrophotometer for 10 min. Each plate included eight vehicle control reactions and eight enzyme-free controls. The compounds that demonstrated greater than fifty percent inhibition of MurB activity in the primary assay were further evaluated for their relative potency by determination of the concentration (IC_{50}) that caused 50% inhibition of MurB activity. For IC_{50} determination, eight 2-fold serial diluted concentrations were tested for each compounds. All assays were run in duplicate.

Docking experiments of selected thiazolidinone substrates were run with the algorithm Dock[8] using published[3] MurB X-ray crystallographic data.

Results and Discussion

The effects of modifying the R_1, R_2, and R_3 side chains on inhibitory potency against the MurB enzyme are summarized in Table 1.[9]

Molecular modeling indicated that aromatic substituents would be tolerated at the R_2 position. The initial screening data obtained for the compounds with a simple phenyl group at R_2 demonstrated a lack of enzyme inhibition; however, the potency of these compounds was dramatically increased when *t*-butyl-*m*-phenoxy benzaldehyde was employed at R_2. This bulky side chain proved essential to enzyme inhibition and we postulate that it may fill a large hydrophobic pocket in the enzyme. Thiazolidinones that contain a *n*-butyl group at R_1 (synthesized from norleucine) constitute the most active compounds synthesized. In addition, stereochemistry seems to play a significant role in potency. Diastereomers synthesized from D-norleucine were nearly 4 times as potent as those synthesized from L-norleucine. Lastly, the lack of enhancement in activity when a methyl or hydrazide moiety is substituted at R_3 points to conformation of our postulate that the uridine portion of the EP-UNAG substrate may play only a minor role in the binding to MurB enzyme.

In summary, novel small molecule MurB inhibitors have been synthesized. The biological data obtained from these compounds supports our postulate that substituted 4-thiazolidinones may be acting as diphosphate mimics. Further studies aimed at unambiguously confirming the postulate that thiazolidinones are acting as diphosphate mimics are being performed; the results of these studies will be reported in due course.

References and Notes

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9. Although the stereochemistry at R_1 was fixed through the employment of chiral amino acids, no attempt was made to control the stereochemistry at R_2 . In addition, no attempt was made to separate the mixture of diasteriomers that resulted from the one-pot cyclization.
10. NA = not active. All amino acid side chains (R_1) were of the L configuration, except for D norleucine, which is designated D isomer in the table.