Benzothioxalone derivatives as novel inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferases (MurA and MurZ)

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Objectives: We sought to identify and characterize new inhibitors of MurA and MurZ, which are enzymes involved in the early stages of bacterial peptidoglycan synthesis.

Methods: A library of ~650000 compounds was screened for inhibitors of Escherichia coli MurA in an endpoint assay measuring release of inorganic phosphate from phosphoenolpyruvate. Hits were validated by determining the concentrations required for 50% inhibition (IC50) of MurA from E. coli and MurA/MurZ from Staphylococcus aureus. The mode of action of selected inhibitors was explored by examining the reversibility of MurA inhibition, the binding of a radiolabelled inhibitor to MurA proteins and through docking studies. Inhibitors were further characterized by determining their antibacterial activity against E. coli and S. aureus.

Results: Benzothioxalone derivatives were identified that inhibited MurA from E. coli and MurA/MurZ from S. aureus with IC50 values between 0.25 and 51 μM. Several inhibitors also exhibited activity against S. aureus with MICs in the range 4–128 mg/L. Inhibition of MurA was irreversible and a radiolabelled inhibitor from this compound class displayed stoichiometric binding to the enzyme, which was displaced by dithiothreitol. Binding was undetectable with a C115D mutant MurA protein.

Conclusions: The results suggest a mode of action for the benzothioxalones that involves the formation of a disulfide bond with MurA/MurZ, via attack from an active site cysteine on the thioxalone ring carbonyl group, followed by ring opening to yield an S-acylated protein. The proposed covalent mode of action may prove useful in the design of new antibacterial agents.

Keywords: peptidoglycan synthesis, Mur enzymes, drug targets

Introduction

The widespread emergence of bacteria resistant to clinically important antibiotics continues to underline the urgent need to discover novel antibacterial agents that are potent against these resistant strains. Interference with bacterial peptidoglycan synthesis is firmly established as an excellent basis for the development of potent antibacterials, e.g. the β-lactams and vancomycin specifically inhibit different stages in the synthesis of this essential polymer.1

The enzyme MurA (UDP-N-acetylglucosamine enolpyruvyl transferase), in the first committed step of peptidoglycan biosynthesis, catalyses the transfer of enolpyruvate from phosphoenolpyruvate (PEP) to the 3′-hydroxyl group of UDP-N-acetylglucosamine (UDP-GlcNAc) yielding enolpyruvyl UDP-N-acetylglucosamine (EP-UDP-GlcNAc) and inorganic phosphate (Pi) (Figure 1).2 MurA
is conserved across Gram-positive and Gram-negative bacterial species and is an essential enzyme with no mammalian counterpart. Furthermore it is clinically validated by the antibiotic fosfomycin. However, apart from fosfomycin, no other inhibitors of MurA have been developed for clinical use. Indeed there are only a few reports in the literature that describe compounds that inhibit this enzyme and many of these inhibitors lack selectivity for MurA.3–10 

Gram-positive bacteria with a low guanosine–cytosine content also possess the gene murZ that encodes a second class of transferase enzyme. In Streptococcus pneumoniae and Staphylococcus aureus this enzyme is able to compensate for MurA in the synthesis of EP-UDP-GlcNAc and catalyses the same reaction as MurA (Figure 1).3

Since MurA and MurZ have not been exploited for the development of new antibacterials they remain attractive as drug targets. Here we report the identification of a novel series of Escherichia coli and S. aureus MurA inhibitors that are also active against MurZ from S. aureus, but lack activity against two unrelated mammalian enzymes. Evidence is provided to support a covalent mode of action against MurA and MurZ involving attack of the thioxalone carbonyl group by an active site cysteine.

Materials and methods

Reagents

Malate dehydrogenase (from porcine heart), α-chymotrypsin type II (from bovine pancreas) and 14C-labelled methyl iodide (6.1 mCi/mmol) were purchased from Sigma-Aldrich (Poole, UK). Emulsifier-safe scintillation cocktail was purchased from Perkin-Elmer (Little Chalfont, UK) and 7-methyl-6-thioguanosine (MESG) from Berry and Associates (Dexter, MI, USA).

Chemical library for screening

The compound collection was provided by Novartis Institute for Biomedical Research (Cambridge, MA, USA). Approximately 650000 natural and synthetic compounds were screened. The identities and purities of hit compounds were confirmed using 1H NMR spectroscopy (Bruker DPX300, 300 MHz spectrometer) and high-resolution electrospray mass spectrometry (Micromass GCT Premier).

Preparation of MurA and MurZ enzymes

The E. coli JM109 murA gene and the murA and murZ genes from S. aureus SH1000 were cloned and expressed according to previously published methods.3,6,11

High-throughput screening

An endpoint assay measuring the MurA-dependent release of Pi from PEP using the PiPer™ Phosphorus Assay Kit (Molecular Probes) was employed. The assay is composed of two steps. In the first step, an enol-pyruvyl group is transferred from PEP to UDP-GlcNAc by the enzymatic activity of MurA. In this process, Pi is released. In the second step, Pi is detected by the components of the PiPer™ Phosphorylase Assay Kit. In the presence of Pi, maltose phosphorylase converts maltose to glucose-1-phosphate and glucose. Then, glucose oxidase converts glucose into glucono-δ-lactone and hydrogen peroxide (H₂O₂). Finally, with horseradish peroxidase as a catalyst, the H₂O₂ reacts with Amplex Red (10-acetyl-3,7-dihydroxyphenoxazone) to generate the fluorescent product, resorufin. The observed relative fluorescence unit values are proportional to the amount of Pi present in the reaction.

Hit validation via orthogonal screening

E. coli MurA and S. aureus MurA and MurZ activities were monitored by the detection of Pi generated during the reaction based on a colorimetric malachite green method.11 Inhibition of MurA and MurZ activity was measured as previously described.5 All assays were carried out at 25°C. To measure inhibition of E. coli MurA, a 2-fold dilution series of the inhibitor (15 μL) was added to 33.3 mg/L MurA and preincubated for 10 min in 50 mM Tris–HCl buffer (pH 7.8) containing 187.5 μM UDP-GlcNAc (20 μL). The reaction was started with the addition of 15 μL of 66.7 μM PEP (final concentration 20 μM) followed by incubation for 30 min. Malachite green–molybdate (0.045% w/v malachite green hydrochloride and 4.2% w/v ammonium molybdate in 4 N HCl) was then added (50 μL) and incubated for 10 min. Absorbance at 660 nm was then measured with a Molecular Devices SpectraMax 250 plate reader. An accompanying series of inorganic phosphate standards was run for each plate. Under these conditions, the production of phosphate was linear as a function of assay time.

To determine the IC₅₀ values of inhibitors, absorbance values at 660 nm were measured and plotted against log₁₀ inhibitor concentration using GraphPad Prism Version 4.02. Non-linear regression, sigmoidal dose–response (variable slope) analysis was used to calculate the IC₅₀ values.

The MurA and MurZ enzymes from S. aureus were assayed in a similar fashion with the following exceptions. The staphylococcal enzymes were each added to final concentrations of 12.5 mg/L. UDP-GlcNAc concentrations were 620 μM (MurA) and 845 μM (MurZ), and the final concentration of PEP was 58 μM for both enzymes. The substrate concentrations used for the staphylococcal enzymes are based upon the full kinetic characterizations of these enzymes.3

Preparation of [O¹⁴CH₃]-5-methoxybenzo-[d][1,3]oxathiol-2-one (¹⁴C-labelled version of compound 18)

A scheme for the synthesis of compound 18 was devised using methyl iodide, thereby permitting substitution with ¹⁴C-labelled methyl iodide to produce a ¹⁴C-labelled version of compound 18.

Preparation of non-radioactive compound 18 as was follows: 5-hydroxybenzo[d][1,3]oxathiol-2-one 1 (0.10 g, 0.59 mmol) was
dissolved in anhydrous dimethylformamide (4 mL) and anhydrous potassium carbonate (0.12 g, 0.89 mmol) added. The mixture was stirred at room temperature under dry N₂ for 1 h at which time a blue colour had developed. Methyl iodide (55 µL, 0.89 mmol) was added via a syringe (colour change from blue to yellow) and the mixture stirred at room temperature overnight. The mixture was then poured into ice/water (20 mL) and extracted with EtOAc (2 × 10 mL). The organic phase was washed successively with water (3 × 10 mL), 1 M NaOH solution (10 mL) and brine (10 mL), dried (MgSO₄) and evaporated in vacuo to give an orange oil. Purification using flash column chromatography on silica gel, eluting with petroleum ether (40–60 °C)/EtOAc 4:1 afforded compound 18 as a colourless crystalline solid (0.70 g, 65 %): melting point 73–74.5 °C (reported 75–76 °C); ¹H NMR (300 MHz, CDCl₃): 3.82 (3H, s, OCH₃), 6.85 (1H, dd, J = 8.9, 2.7, Ar-H), 6.93 (1H, d, J = 2.7, Ar-H), 7.20 (1H, d, J = 8.9, Ar-H); ¹³C NMR (75 MHz, CDCl₃): 55.9, 107.3, 112.6, 113.5, 123.9, 142.2, 157.1, 169.0; found C (52.8%), H (3.4%), S (17.7%), C₈H₆O₃S requires C (52.7 %), H (3.3%), S (17.6%). Compound 5-[methoxybenzo[d][1,3]oxathiol-2-one], T a b l e 1) was prepared by methylation of 5-hydroxybenzo[d][1,3]oxathiol-2-one as a colourless crystalline solid. The product was purified by flash column chromatography (as above) to give 63.7 mg (chemical yield 59%) of ¹⁴C-labelled 5-hydroxybenzo[d][1,3]oxathiol-2-one (compound 18, Table 1) using ¹⁴C-labelled methyl iodide. In the reaction, ¹⁴C-labelled methyl iodide (250 µCi ¹⁴C) was mixed with 126 mg of unlabelled methyl iodide and used with 100 mg of 5-hydroxybenzo[d][1,3]oxathiol-2-one. The product was purified by flash column chromatography (as above) to give 63.7 mg (chemical yield 59%) of ¹⁴C-labelled 5-methoxybenzo[d][1,3]oxathiol-2-one as a colourless crystalline solid. The solid had a ³¹C specific activity of 322 Ci mmol⁻¹, which was achieved with a radiochemical yield of 45%.

Selectivity assays
Molate dehydrogenase and chymotrypsin assays were performed as described previously. Reversibility of inhibition
A dilution assay was used to determine whether the binding of inhibitors was reversible. Enzyme activity was monitored by coupling the reaction with a continuous spectrophotometric assay for inorganic phosphate. Reactions were performed at final volumes of 200 µL (50 mM Tris–HCl, pH 7.85) in 96-well plates using the following optimized conditions.

Table 1. Inhibition of MurA and MurZ by benzothioxalones and their antibacterial activity against E. coli JM109 and S. aureus SH1000

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC₅₀ (E. coli MurA), µM</th>
<th>IC₅₀ (S. aureus MurA), µM</th>
<th>IC₅₀ (S. aureus MurZ), µM</th>
<th>MIC (E. coli), mg/L</th>
<th>MIC (S. aureus), mg/L</th>
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<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Structure 1" /></td>
<td>0.53 (+0.06)</td>
<td>27.07 (+2.42)</td>
<td>27.66 (+0.63)</td>
<td>256</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Structure 2" /></td>
<td>8.01 (+1.28)</td>
<td>7.46 (+0.18)</td>
<td>9.89 (+0.66)</td>
<td>256</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Structure 3" /></td>
<td>0.46 (+0.05)</td>
<td>2.62 (+0.16)</td>
<td>3.26 (+0.18)</td>
<td>&gt;256</td>
<td>256</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Structure 4" /></td>
<td>0.58 (+0.30)</td>
<td>8.42 (+0.29)</td>
<td>8.58 (+0.34)</td>
<td>&gt;256</td>
<td>128</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 5" /></td>
<td>2.86 (+0.02)</td>
<td>41.79 (+1.41)</td>
<td>25.81 (+1.08)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
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</table>

Continued
Novel inhibitors of MurA and MurZ

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC$_{50}$ (E. coli MurA), µM</th>
<th>IC$_{50}$ (S. aureus MurA), µM</th>
<th>IC$_{50}$ (S. aureus MurZ), µM</th>
<th>MIC (E. coli), mg/L</th>
<th>MIC (S. aureus), mg/L</th>
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<tr>
<td>6</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>9.51 (±0.16)</td>
<td>slight inhibition at 120</td>
<td>slight inhibition at 120</td>
<td>&gt;256</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>18.54 (±1.48)</td>
<td>slight inhibition at 120</td>
<td>slight inhibition at 120</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>7.27 (±0.72)</td>
<td>slight inhibition at 120</td>
<td>slight inhibition at 120</td>
<td>&gt;256</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>0.28 (±0.24)</td>
<td>1.09 (±0.05)</td>
<td>2.89 (±0.04)</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>10</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>1.55 (±0.24)</td>
<td>26.09 (±2.56)</td>
<td>51.46 (±2.50)</td>
<td>128</td>
<td>16</td>
</tr>
<tr>
<td>11</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>0.76 (±0.09)</td>
<td>1.13 (±0.07)</td>
<td>1.33 (±0.12)</td>
<td>&gt;256</td>
<td>32</td>
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<tr>
<td>12</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>0.25 (±0.08)</td>
<td>17.94 (±0.42)</td>
<td>7.74 (±0.68)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>13</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>2.43 (±0.40)</td>
<td>2.39 (±0.05)</td>
<td>3.54 (±0.48)</td>
<td>&gt;256</td>
<td>128</td>
</tr>
<tr>
<td>14</td>
<td><img src="image9.png" alt="Structure" /></td>
<td>1.96 (±0.51)</td>
<td>6.68 (±0.30)</td>
<td>6.68 (±0.79)</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>15</td>
<td><img src="image10.png" alt="Structure" /></td>
<td>5.15 (±0.22)</td>
<td>10.79 (±0.52)</td>
<td>14.58 (±0.00)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>16</td>
<td><img src="image11.png" alt="Structure" /></td>
<td>0.95 (±0.23)</td>
<td>3.49 (±0.34)</td>
<td>5.86 (±0.59)</td>
<td>&gt;256</td>
<td>128</td>
</tr>
<tr>
<td>17</td>
<td><img src="image12.png" alt="Structure" /></td>
<td>3.10 (±1.73)</td>
<td>24.31 (±0.35)</td>
<td>47.10 (±1.96)</td>
<td>128</td>
<td>16</td>
</tr>
<tr>
<td>18</td>
<td><img src="image13.png" alt="Structure" /></td>
<td>6.45 (±0.24)</td>
<td>24.79 (±1.20)</td>
<td>35.99 (±3.61)</td>
<td>&gt;256</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td><img src="image14.png" alt="Structure" /></td>
<td>14.28 (±5.64)</td>
<td>slight inhibition at 120</td>
<td>slight inhibition at 120</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td><img src="image15.png" alt="Structure" /></td>
<td>0.60 (±0.04)</td>
<td>1.25 (±0.05)</td>
<td>1.11 (±0.01)</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

ND, not determined.
screening hits were subsequently shown to possess IC$_{50}$ values between 0.25 and 18.54 µM for E. coli MurA (Table 1). Compounds 1–5 and 9–18 were also identified as inhibitors of MurA and MurZ from S. aureus with IC$_{50}$ values in the range 1.09–51.46 µM (Table 1). Since the mode of action of fosfomycin involves attack by cysteine-115 (C115) of MurA and MurZ, we investigated whether this residue was also required for inhibition by the identified MurA/Z hits using an E. coli MurA mutant containing a C115D substitution.$^{18}$ The C115D mutant enzyme was not inhibited by these compounds (data not shown).

Some structure–activity trends were evident. The presence of an oxygen atom at either C-5 or C-6 of the thiazolone ring was essential and thiazolones lacking these features were generally poor inhibitors of MurA and MurZ (data not shown). However, the nature of this substituent could be varied and included free hydroxy groups (compounds 1, 6, 7, 8, 9, 10, 16 and 17), and alkoxy (2 and 18). In general, the thiazolones displayed greatest inhibitory activity towards E. coli MurA, with 6 of the 18 active thiazolones (compounds 1, 3, 4, 9, 11, 12 and 16) displaying sub-micromolar activity. Interestingly, all three thiazolones containing anyl substitution at C-7 (compounds 6, 7 and 8) were poor inhibitors of both S. aureus enzymes.

Also of interest was the observation that thiazine 19, which lacks the carbonyl group present in compounds 1–18 showed inhibition of E. coli MurA, but was essentially inactive against the S. aureus enzymes.

Thiazolones 1–18 generally showed poor antibacterial activity against E. coli, with the most active compounds having an MIC of only 128 µg/mL (Table 1). Against S. aureus, however, there was whole-cell activity for several inhibitors with MICs from 4 to >256 µg/mL.

To establish the selectivity of compounds 1–18 as Mur ligase inhibitors, their inhibitory activity against malate dehydrogenase and chymotrypsin, two unrelated mammalian enzymes, was determined. None of the compounds inhibited the reaction catalysed by the mammalian enzymes (data not shown).

**Reversibility of inhibition**

The mode of inhibition of the benzothioxalones derivatives against the enzymes was probed by first determining whether MurA activity was recoverable following dilution of the inhibitor from the enzyme:inhibitor mixture. Prior to measuring MurA activity, the enzyme was treated with the benzothioxalones 1, alongside a positive control sample treated with the covalent modifier fosfomycin and a non-inhibited sample, and then subjected to a 50-fold dilution to remove excess inhibitor. Monitoring the reaction over a period of 1 h resulted in no recovery of enzyme activity for the samples treated with 1 and fosfomycin (Figure 2), a pattern consistent with irreversible inhibition and therefore suggestive of very tight non-covalent binding, or covalent modification. However, we failed to observe covalent binding of 1 to E. coli MurA by mass spectrometry analysis, even though the formation of a fosfomycin–MurA adduct was readily detected (data not shown).

**Radiolabelling studies**

To test the hypothesis of a covalent mechanism of inhibition by the benzothioxalones on the MurA/Z enzymes, a $^{14}$C-labelled version of the methoxy derivative 18 was prepared by reaction of 1 with $[^{14}$C]$\text{methyl iodide and then used to monitor the mechanism of binding to E. coli MurA. Up to a concentration of 1 mM, }$\[^{14}$C]$\text{ showed an approximately stoichiometric binding to the wild-type protein, which was not detected with the C115D mutant form of the protein (Figure 3a). Binding of }$\[^{14}$C]$\text{ to the wild-type protein was completely displaced by treating the enzyme with dithiothreitol (DTT) (Figure 3b). Since mass spectrometry demonstrated that 1 was stable in the presence of 0.1 M DTT for several days (data not shown), these results are consistent with the benzothioxalones inhibiting the enzymes via a covalent mechanism involving the position corresponding to C115 in E. coli MurA. The binding of }$\[^{14}$C]$\text{ to wild-type MurA was essentially blocked by prior incubation of the enzyme with the benzothioxalone 1, confirming that both inhibitors bind at the same site(s) (Figure 3c). Interestingly, however, this binding was not blocked by fosfomycin (Figure 3d).}

**Discussion**

Despite the relatively modest binding affinity displayed by fosfomycin for MurA, the drug is a highly effective inhibitor of the enzyme. The reason is apparent upon inspection of the mechanism of inhibition of MurA by the antibiotic. This mechanism involves selective and irreversible covalent bonding via nucleophilic attack of the thiol from C115, with concomitant ring-opening of the epoxide moiety within fosfomycin.

Although all of the thiazolone-based inhibitors have poor Gram-negative antibacterial activity, five of the inhibitors have comparable Gram-positive antibacterial activity compared with fosfomycin, with one (compound 18) displaying improved potency. Additionally, 5 of the 18 inhibitors showed in vitro MurA inhibition activity comparable or better than that of fosfomycin, with the activity of compounds 9 and 12 in particular, being ~2-fold more potent than fosfomycin against E. coli MurA.
Figure 3. Radiolabelling of *E. coli* MurA by \[^{14}\text{C}]18\text{.} The data are presented as nmol \(^{14}\text{C}\)-labelled 18/nmol MurA. (a) Binding of \[^{14}\text{C}]18\text{ to *E. coli* wild-type MurA and the mutant C115D. (b) Displacement of \[^{14}\text{C}]18\ binding to wild-type MurA by DTT. (c) Blockage of \[^{14}\text{C}]18\ binding to wild-type MurA by the benzothioxalone 1\text{. (d) Binding of \[^{14}\text{C}]18\ text{ to wild-type MurA in the presence of fosfomycin. All data points are the average of duplicate measurements. All data points for samples with MurA present are background subtracted; i.e. background control values [open circles in graph (a)] have been subtracted.}
Mechanism of inhibition

Attack of the thiol moiety from C115 could occur at the carbonyl of thiazolone 1 to yield the S-acylated covalent adduct 1b (Figure 4). However, we failed to observe covalent binding of 1 to E. coli MurA by mass spectrometry analysis, even though the formation of a fosfomycin–MurA adduct was readily detected. Nevertheless, the formation of a covalent adduct between 1 and MurA is consistent with the experimental observations derived from the use of the radiolabelled inhibitor and the fact that inhibition was not observed in the presence of DTT. Since no evidence was obtained for reaction of compound 1 with DTT, these results suggest deacylation of adduct 1b by DTT and release of the free enzyme. In light of this, however, it is interesting that thiazine 19, which lacks the carbonyl function present in compounds 1–18, was found to inhibit E. coli MurA, but did not appreciably inhibit either of the S. aureus enzymes. Additionally, the fact that radiolabelling of MurA by [14C]18 was not prevented by fosfomycin suggests a more complex binding mode.

To refine the hypothesis on the mode of binding of these inhibitors, molecular docking studies were performed using inhibitor 1 and the X-ray crystal structure of E. coli MurA19 (PDB code 1UAE). Docking of 1 into the MurA–UDP-GlcNAc co-crystal structure indicates that 1 binds into a pocket that locates the carbonyl group of the thioxalone close to the thiol of C115, consistent with the requirements for covalent adduct formation (Figure 5a) and loss of inhibitor activity against the mutant C115D MurA enzyme. Docking of 1 into the MurA–UDP-GlcNAc crystal structure that contains a fosfomycin fragment covalently linked to C115 was also studied. This revealed that although the original binding pocket was now occupied by the C115–linked fosfomycin fragment, binding of inhibitor 1 occurred within a second pocket, located on the opposite side of UDP-GlcNAc (Figure 5b). This predicted alternative binding pocket may account for the observation that [14C]18 is capable of binding to MurA that had been pre-treated with fosfomycin.

Conclusions

We have discovered a new series of inhibitors of MurA and MurZ based around a benzothioxalone motif. The mechanism of binding of these inhibitors is consistent with a covalent binding mode involving C115 although some observations, including modelling studies, suggest the existence of an additional binding pocket in fosfomycin-inhibited enzymes. These results continue to provide insight into the design of new antibacterial agents.

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Transparency declarations

J. A. L. and M. S. are employees of Novartis and J. D. A. is a former employee of Novartis. These authors own stock in the company. Other authors have no conflicts of interest to declare.
References


