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**N-Leucinyl benzenesulfonamides as structurally simplified leucyl-tRNA synthetase inhibitors**

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leucyl-tRNA synthetase, inhibitors, antibacterial, sulfonamides, isothermal titration calorimetry

**ABSTRACT:** N-Leucinyl benzenesulfonamides have been discovered as a novel class of potent inhibitors of *E. coli* leucyl-tRNA synthetase. The binding of inhibitors to the enzyme was measured by using ITC. This provided information on enthalpy and entropy contributions to binding, which, together with docking studies, were used for SAR analysis. Enzymatic assays revealed that N-leucinyl benzenesulfonamides display remarkable selectivity for *E. coli* leucyl-tRNA synthetase compared to *S. aureus* and human orthologs. The simplest analog of the series – N-leucinyl benzenesulfonamide (R = H) showed the highest affinity against *E. coli* leucyl-tRNA synthetase and also exhibited antibacterial activity against gram-negative pathogens (the best MIC = 8 µg/mL, *E. coli* ATCC 25922) which renders it as a promising template for antibacterial drug discovery.

Aminoacyl-tRNA synthetase (aaRS) enzymes are conserved across bacteria and at the same time exhibit considerable evolutionary divergence with respect to the human enzymes. They have therefore attracted attention as therapeutic targets for the discovery of broad-spectrum antibacterials.1–4 The aaRS enzymes covalently link the cognate amino acid to its transfer RNA (tRNA) as part of protein biosynthesis. Aminoacylation of tRNA is a two-step process in which the enzymes initially form an aminoacyl adenylate intermediate from the amino acid and ATP. The amino acid is then transferred to the terminal adenosine residue of the tRNA. The enzymes are classified into two groups, depending upon whether the amino acid is transferred to the 2’- (Class I) or the 3’-hydroxyl group (Class II) of adenosine. Clinical validation of class I aaRS enzymes as druggable targets has been provided by the isoleucyl-tRNA synthetase (IleRS) inhibitor, mupirocin,5–7 also known as pseudomonic acid 1 (Figure 1), which is used to treat topical skin infections.8

![Figure 1. Representative class I aaRS inhibitors](image-url)
A boron-containing molecule 2 (AN3365, GSK2251052, Figure 1) that inhibits leucyltRNA synthetase (LeuRS) by binding at the enzyme’s editing site has also been evaluated clinically, although its development is encumbered due to the rapid emergence of resistance. Nevertheless, to date, no catalytic site LeuRS inhibitor has been advanced to clinical investigation.

Most of the precedent work to develop LeuRS catalytic site inhibitors has focused on non-hydrolyzable aminoacyl-AMP intermediate analogues such as LeuAMS 3 (Figure 1). Potent inhibitors have been discovered, but in general these compounds lack selectivity for bacterial compared to human aaRSs and also lack antibacterial activity, the latter owing to poor intracellular accumulation.

Selectivity issues of bacterial LeuRS inhibition were overcome by researchers at Cubist who have developed acyl-sulphonamide-based LeuRS inhibitors 4 (Figure 1) in which the adenine ring is replaced with a substituted thiazole. Moreover, selective inhibitors of the homologous IleRS enzyme, with activity against Gram-positive organisms have been achieved by replacing the adenine ring with substituted phenyltetrazoles linked to the sugar ring by a short alkyl group.

A drawback of adenosine analogues is their relatively complex structures. This limits rapid chemical modifications to obtain crucial requirements such as selectivity and antibacterial activity. There are some examples in the recent literature showing that the adenylate can be substituted with a benzenesulphonamide motif. Teng et al. have developed selective nonanomlar benzenesulphonamide based inhibitors 5 (Figure 2) of bacterial ThrRS, including the enzyme from E. coli. In these inhibitors, the meta-substituent at the benzenesulphonamide (such as indazole) was designed to pick up the H-bond interactions seen in the crystallographically-observed structures of E. coli LeuRS. This suggests further (as yet unobserved) conformational changes in the LeuRS structure on ligand binding which will require crystallography studies in the future to understand.

Amino pyridine 7e (Table 1, entry 5) showed similar ITC results to amino pyrimidine 7c which indicates no contribution of pyrimidine N1 to the binding. On the other hand, analogue 7f (Table 1, entry 6) without amino group showed decreased enthalpy of binding compared to inhibitor 7e, indicating that amino group provides additional H-bonding. However, compound 7f has comparable K_D which is again due to counterbalancing with decreased binding entropy for this compound.

![Figure 3. Docked poses of 7c (purple), 7h (orange) and 7j (cyan) in active site of E. coli LeuRS (3zgz)](Image)

The favourable decrease of binding entropy for compound 7f suggested the preparation and testing of more lipophilic compounds 7g-i (Table 1, entries 7-9). Pyridine and phenyl substituted analogues 7g,h showed K_D comparable to inhibitors 7a-e. However, by increasing the aromatic ring size to naphthyl (compound 7i), a considerable loss of binding enthalpy was observed which indicated that this compound does not adopt the optimal conformation in the active site of the enzyme.
Table 1. ITC binding constants of N-leucinyl benzenesulfonamides 7 to *E. coli* LeuRS and the enzymatic potency against *E. coli*, *S. aureus* and human LeuRS.

![Chemical structure of compound 7](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>R, compound number</th>
<th><em>E. coli</em> LeuRS ITC, a K_D, nM</th>
<th>ITC, a ΔH, kcal mol⁻¹</th>
<th>ITC, a -TΔS, kcal mol⁻¹</th>
<th><em>E. coli</em> LeuRS IC₅₀, nM</th>
<th><em>S. aureus</em> LeuRS IC₅₀, nM</th>
<th>Human LeuRS IC₅₀, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Structure 7a" /></td>
<td>14.5±0.3</td>
<td>-9.5±0.5</td>
<td>-1.2±0.5</td>
<td>54</td>
<td>9.2 x 10³</td>
<td>3.9 x 10³</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Structure 7b" /></td>
<td>12.8±1.2</td>
<td>-9.0±0.3</td>
<td>-1.7±0.3</td>
<td>14</td>
<td>650</td>
<td>490</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Structure 7c" /></td>
<td>10±3</td>
<td>-12.5±1.2</td>
<td>1.6±1.0</td>
<td>41</td>
<td>4.4 x 10³</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Structure 7d" /></td>
<td>56±17</td>
<td>-7.6±0.3</td>
<td>-2.3±0.4</td>
<td>224</td>
<td>3.6 x 10³</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 7e" /></td>
<td>13.9±1.9</td>
<td>-9.10±0.11</td>
<td>-1.62±0.07</td>
<td>23</td>
<td>1.7 x 10³</td>
<td>850</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Structure 7f" /></td>
<td>22±4</td>
<td>-7.05±0.05</td>
<td>-3.40±0.13</td>
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<td>2.7 x 10³</td>
<td>2.0 x 10³</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Structure 7g" /></td>
<td>10.9±1.5</td>
<td>-8.27±0.11</td>
<td>-2.6±0.18</td>
<td>13</td>
<td>920</td>
<td>1.2 x 10³</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Structure 7h" /></td>
<td>10.2±1.4</td>
<td>-8.1±0.5</td>
<td>-2.8±0.5</td>
<td>32</td>
<td>640</td>
<td>3.4 x 10³</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Structure 7i" /></td>
<td>64±18</td>
<td>-6.5±0.9</td>
<td>-3.3±0.8</td>
<td>65</td>
<td>1.0 x 10³</td>
<td>220</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Structure 7j" /></td>
<td>1.3±0.14</td>
<td>-13.1±0.3</td>
<td>1.1±0.2</td>
<td>35</td>
<td>5.4 x 10³</td>
<td>1.1 x 10³</td>
</tr>
</tbody>
</table>

*ITC data for binding to *E. Coli* LeuRS enzyme, IC₅₀ were within 10% of the error range.
At this point, our interpretation of the data indicated a limited contribution to $K_D$ from polar interactions and a variable contribution from more lipophilic compounds. Therefore, we decided to simplify the scaffold further. The simplest member of N-leucinyl benzencesulfonamide class, inhibitor 7j was prepared and it was found to exhibit the best binding affinity against *E. coli* LeuRS with a $K_D$ of 1.4 nM. The ITC data imply that binding of compound 7j with LeuRS is enthalpy driven and that entropy does not provide a favorable contribution to the Gibbs free energy of binding.

Antibacterial susceptibility testing against a standard laboratory strain of *E. coli* (BW25113) was performed for all inhibitors 7a-j. Only inhibitor 7j showed detectable activity (Table 2, entry 1) and was screened against wider panel of strains. The best antibacterial activity was observed against *E. coli* (ATCC 25922) strains while it was weaker for other gram-negative pathogens such as *K. pneumoniae*, *P. aeruginosa* and *E. cloacae*. No activity was detected against *S. aureus* as a representative Gram-positive pathogen. For comparison, compound 7c was also tested against the strains listed in Table 2, however it showed no detectable activity against any of the strains.

Table 2. Activity of inhibitor 7j against a panel of selected bacterial strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>Name</th>
<th>7j MIC ug/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>BW25113</td>
<td>32</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>ATCC 25922</td>
<td>8</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>clinical isolate</td>
<td>64</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>PA01</td>
<td>64</td>
</tr>
<tr>
<td><em>Acinetobacter spp</em></td>
<td>ATCC 19606</td>
<td>64</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>clinical isolate</td>
<td>128</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>JE2</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

Antibacterial activity of compounds 7c and 7j was tested against derivatives of *E. coli* (BW25113) lacking key components of multidrug efflux transporter components TolC and AcrAB. Nevertheless, neither tolC deletion strain nor AcrA and AcrB deletion strains showed improved susceptibility to compounds 7c,j. This indicates that the antibacterial activity of N-leucinyl benzencesulfonamides 7 is limited by additional mechanisms other than efflux.

In summary, we have identified N-leucinylbenzene sulfonamides as a novel class of *E. coli* LeuRS inhibitors. The simplicity and potency of this class offers significant potential for the development of much needed novel antibacterial agents.

ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of compounds 7; description of molecular modeling; ITC titration curves; description of enzymatic assay and antimicrobial susceptibility.

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Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS
ITC, isothermal titration calorimetry; aaRS, aminoacyl-tRNA synthetase; LeuRS, leucinyl-tRNA synthetase; IleRS, isoleucinyl-tRNA synthetase; ThrRS, threoninyl-tRNA synthetase; Tb, Trypanosoma brucei; MIC, minimum inhibitory concentration.

REFERENCES


