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Charlton, MH, Aleksis, R, Saint-Leger, A et al. (15 more authors) (2018) N-Leucinyl Benzenesulfonamides as Structurally Simplified Leucyl-tRNA Synthetase Inhibitors. ACS Medicinal Chemistry Letters, 9 (2). pp. 84-88. ISSN 1948-5875

https://doi.org/10.1021/acsmedchemlett.7b00374

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

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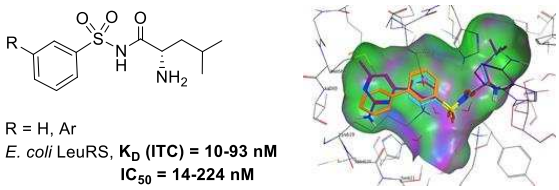
leucyl-tRNA synthetase, inhibitors, antibacterial, sulfonamides, isothermal titration calorimetry



ABSTRACT: N-Leucinyl benzenesulfonamides were 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.

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 the class I aaRS 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] A boron-containing molecule **2** (AN3365, GSK2251052, Figure 1)[9] that inhibits leucyl tRNA-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.[10,11] 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).[12-14] Potent inhibitors have been discovered, but in general these compounds lack selectivity for bacterial compared to human aaRSs and lack antibacterial activity, the latter owing to poor intracellular accumulation.[13,14]

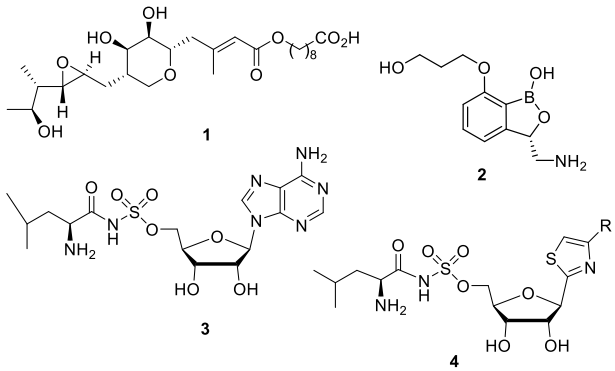


Figure 1. Representative class I aaRS inhibitors

Selectivity issues of bacterial LeuRS inhibition were overcome by researchers at Cubist who have developed acylsulphonamide-based LeuRS inhibitors **4** (Figure 1) in which the adenine ring is replaced with a substituted thiazole.[15] Moreover, selective homologous IleRS inhibitors 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.[16]

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 benzenesulfonamide motif. Teng et al. have developed selective nanomolar benzenesulfonamide based inhibitors **5** (Figure 2) of bacterial ThrRS including the enzyme from E. coli.[17] In these inhibitors, the meta-substituent at the benzenesulfonamide, such as indazole, was designed to pick up the H-bond interactions seen in the adenine of the native substrate. Zhang et al. have developed low micromolar benzenesulfonamide **6** (Figure 2) based T.brucei LeuRS inhibitors.[18] According to modeling studies, the acyl-thiourea group in these inhibitors provides additional H-bonding interactions with the enzyme. These findings motivated us to explore leucinyl benzenesulfonamides as a simplified scaffold for the design of bacterial LeuRS inhibitors.

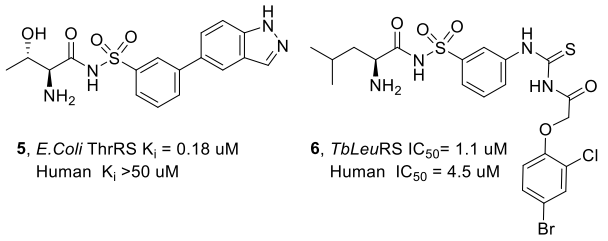


Figure 2. Known benzenesulfonamide-based aaRS inhibitors

A series of N-leucinyl benzenesulfonamides **7** were prepared (Table 1, see Supporting information for the synthesis). The affinity of the inhibitors **7** for Escherichia coli LeuRS was determined using ITC. Enzymatic activity of compounds **7** against E. coli, Staphylococcus aureus and human LeuRS was also determined.[19] It was pleasing to find that the simplest member of this compound class, inhibitor **7a,** exhibited binding affinity against E. coli LeuRS at nanomolar concentrations, with a K_D of c. 90 nM. Notably, this compound showed potent inhibition of E. coli LeuRS with high selectivity versus S. aureus and human LeuRS (Table 1, Entry 1). The ITC data imply that binding of compound **7a** with LeuRS is enthalpy driven and that entropy does not provide a favorable contribution to the Gibbs free energy. By increasing the aromatic ring size to naphthyl (compound **7b**), a small increase in binding affinity was achieved (Table 1, entry 2). The entropic contribution to binding was significantly improved; however, a considerable loss of binding enthalpy indicates that this compound does not adopt the optimal conformation in the active site of the enzyme. Moreover, lower selectivity in E. coli vs human LeuRS inhibition was observed. By replacing the napthyl group with biphenyl in inhibitor **7c**, about a 6-fold improvement in affinity was observed (Table 1, entry 3). The contribution of entropy to the binding is similar for both molecules **7b** and **7c**, however the latter has a more negative binding enthalpy. Similar results were obtained by replacing the metaphenyl substituent with pyridine (compound **7d**).

The hypothetical binding mode of compounds **7a,c** in the active site of the enzyme was predicted by docking of leucinyl benzenesulfonamides **7** in a protein model based on the X-ray structure of E. coli LeuRS (Figure 3). The docking studies suggested replacing the terminal phenyl group of compound **7c** with amino pyridine or amino pyrimidine (compound **7h** shown as an example) as these could mimic the adenine in the native substrate and provide hydrogen bond interactions with Val569 and Met620. Based on these considerations, several pyridine and pyrimidine analogues **7e-j** were prepared. Despite predictions, these molecules did not show improved binding to E. coli LeuRS, retaining the level of activity of inhibitor **7c**. According to ITC measurements, amino group-containing inhibitors **7e-h** showed slightly increased enthalpy of binding compared to compounds **7c,d**, which could be due to the additional H-bonding. This effect, however, is counterbalanced with decreased binding entropy for these compounds, possibly due to restricted C-C bond rotation between the two phenyl rings. The increased enthalpic contribution for inhibitor **7h** is notable compared to other analogues **7e-g**; however, the pattern that this improvement is accompanied by a balancing change in entropic contribution to leave K_D relatively unaltered, is maintained. Removing the amino group (compound **7i**) considerably decreased the binding enthalpy, implying an important role of this group for the binding of inhibitor **7h**.

Replacement of the methyl with a phenyl group (Compound **7j**) had a negative impact on binding due to reduced enthalpy compared to compound **7h**, which to some extent was counterbalanced with increased entropy. The net binding constant was unexpectedly high given that compound **7j** does not fit into known structure of E. coli LeuRS. This indicate further (as yet unobserved) conformational changes in the LeuRS structure on ligand binding which will require crystallography studies in the future to understand.

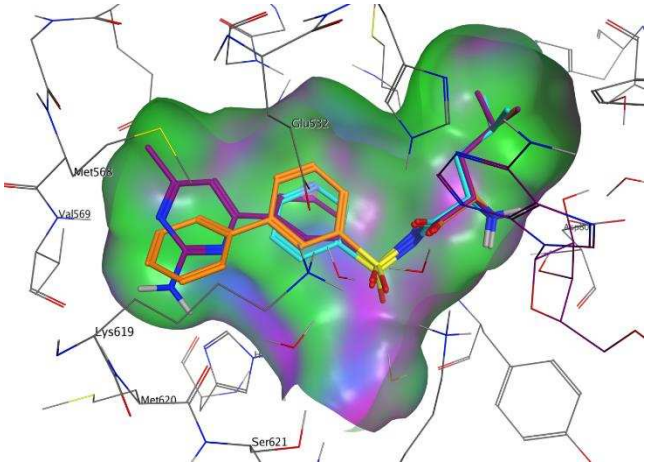
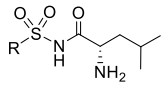


Figure 3. Docked poses of **7a** (cyan), **7c** (orange) and **7h** (purple) in active site of E. coli LeuRS (3zgz)

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.



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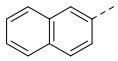
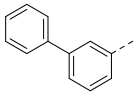
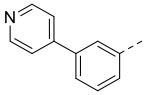
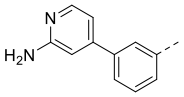
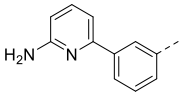
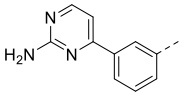
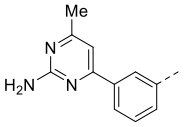
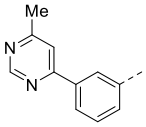
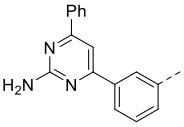
Entry	R, compound number	E.coli LeuRS ITC,[a] K_D, nM	ITC,[a] ΔH, kcal·mol^{-1}	ITC,[a] -TΔS, kcal·mol^{-1}	E.coli LeuRS IC_{50}, nM[b]	S. aureus LeuRS IC_{50}, nM[b]	Human LeuRS IC_{50}, nM[b]
1	 **7a**	92.9±1.0	-13.5±0.7	3.9±0.7	49	5.4 x 10^3	1.1 x 10^3
2	 **7b**	64±18	-6.5±0.9	-3.3±0.8	65	1.0 x 10^3	220
3	 **7c**	10.2±1.4	-8.1±0.5	-2.8±0.5	32	640	3.4 x 10^3
4	 **7d**	10.9±1.5	-8.27±0.11	-2.6±0.18	13	920	1.2 x 10^3
5	 **7e**	12.8±1.2	-9.0±0.3	-1.7±0.3	14	650	490
6	 **7f**	13.9±1.9	-9.10±0.11	-1.62±0.07	23	1.7 x 10^3	850
7	 **7g**	14.5±0.3	-9.5±0.5	-1.2±0.5	54	9.2 x 10^3	3.9 x 10^3
8	 **7h**	10±3	-12.5±1.2	1.6±1.0	41	4.4 x 10^3	160
9	 **7i**	22±4	-7.05±0.05	-3.40±0.13	47	2.7 x 10^3	2.0 x 10^3
10	 **7j**	56±17	-7.6±0.3	-2.3±0.4	224	3.6 x 10^3	33

[a]ITC data for binding to E. Coli LeuRS enzyme, [b] IC_{50} were within 10% of the error range.

Antibacterial susceptibility testing against a standard laboratory strain of E. coli (BW25113) was performed for inhibitors **7a-j**; none of these compounds showed detectable activity (minimum inhibitory concentrations >128 ug/mL, Table 2). However, antibacterial activity was observed for some of these inhibitors against derivatives of E. coli BW25113 lacking key components of multidrug efflux transporters[20] (Table 2), implying that the poor antibacterial activity of these compounds against the parental strain is at least in part attributable to efflux from the cell.

Table 2. Antibacterial activity of inhibitors **7a** and **7c** against E. coli strain BW25113 and derivatives deficient in components of multidrug efflux transporters

Strains	**7a** MIC ug/mL	**7c** MIC ug/mL
BW25113	>128	>128
BW25113 ΔtolC	16	>128
BW25113 ΔacrA	>128	128
BW25113 ΔacrB	>128	128

TolC is an outer membrane channel that functions in concert with AcrAB, but also works in association with other efflux pumps; given that the antibacterial activity of inhibitor **7a** was exclusively observed against the tolC deletion strain, this compound clearly represents a substrate for TolC-dependent efflux, but via a pump other than AcrAB. Inhibitor **7c** showed measurable activity against the AcrA and AcrB deletion strains; however, the activity was weak, implying that the activity of compound **7c** is limited by additional mechanisms other than TolC-dependent efflux. These results suggest that further work on this series should emphasize structural modifications to reduce the binding of the inhibitors to efflux transporters.

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. / ‡These authors contributed equally. (match statement to author names with a symbol)

Funding Sources

This work was supported by a grant from the European Union Framework 7 (FP7) 294 program - Health.2013.2.31-1-NABARSI, Grant agreement no: 601725.

ACKNOWLEDGEMENT

We thank coordinators of NABARSI Dr. W. Goessens and J.P Hays for the excellent management of the consortium. We thank Dr. J. Popelis and prof. E Liepins for assistance with NMR spectra. We thank E. Sarule for performing elemental analysis.

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.

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