Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00374 • Publication Date (Web): 18 Jan 2018

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

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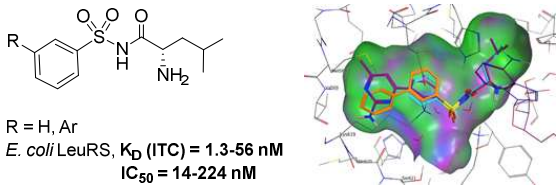
Keywords: leucyl-tRNA synthetase, inhibitors, antibacterial, sulfonamides, isothermal titration calorimetry



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 ug/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]

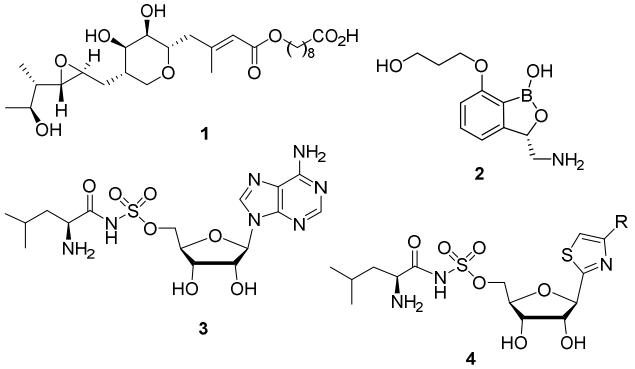


Figure 1. Representative class I aaRS inhibitors

A boron-containing molecule **2** (AN3365, GSK2251052, Figure 1)[9] 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.[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 also lack antibacterial activity, the latter owing to poor intracellular accumulation.[13,14]

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 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.[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 *N*-leucinyl benzenesulfonamides **7** as simplified bacterial LeuRS inhibitors (Table 1, see Supporting Information for the synthesis). The affinity of the inhibitors **7** for *Escherichia coli* LeuRS was determined using ITC. Enzymatic inhibition of compounds **7** against *E. coli*, *S. aureus* and human LeuRS was also determined.[19]

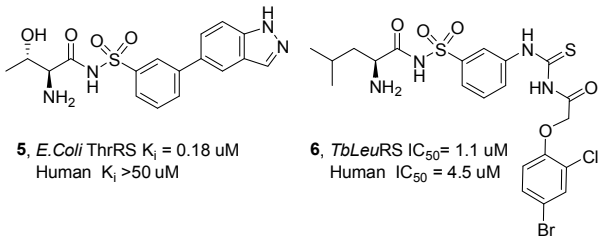


Figure 2. Known benzenesulfonamide-based aaRS inhibitors

Our initial hypothesis, guided by the known aaRS acylsulfonamide inhibitors and by analogy to inhibitor interactions at other ATP binding sites, for example in kinases, was that hydrogen bond interactions with Val569 and Met620, to mimic the interaction with adenine in the native substrate, would be beneficial. Inhibitor design was performed by docking of the benzenesulfonamide derivatives into a protein model based on an X-ray structure of *E. coli* LeuRS. These studies suggested that this could be achieved via amino pyridine or amino pyrimidine substituents at the meta position of of the benzenesulfonamide (Figure 3, compound **7c** shown as an example). Based on these considerations, several analogues **7a-c** were prepared and were found to exhibit binding affinity at nanomolar concentrations against *E. coli* LeuRS in ITC experiments. Notably, potent inhibition of *E. coli* LeuRS with high selectivity versus *S. aureus* and human LeuRS were also observed (Table 1, Entries 1-3). Interestingly, the installation of a methyl group in the pyrimidine ring (compound **7c**) led to an increased enthalpic contribution compared to analogues **7a,b**. However, this improvement is accompanied by a balancing decrease in entropic contribution to leave K_D practically unaltered. Replacement of the methyl with a phenyl group (compound **7d**, Table 1, entry 4) had a negative impact on binding due to reduced enthalpy compared to compound **7c**, which to some extent was counterbalanced with increased entropy. The net binding constant was unexpectedly strong given that compound **7d** does not fit into 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 **7c**, 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.

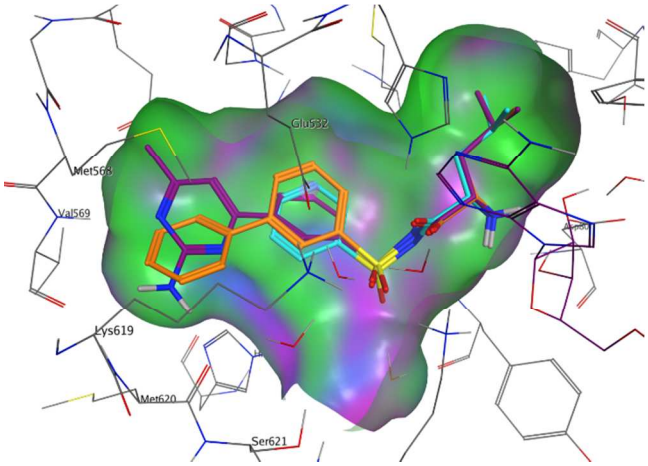
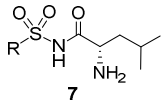


Figure 3. Docked poses of **7c** (purple), **7h** (orange) and **7j** (cyan) in active site of *E. coli* LeuRS (3zgz)

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-c,e,f**. 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.



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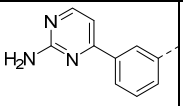
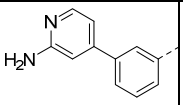
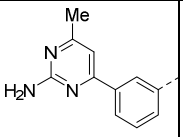
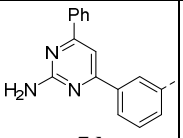
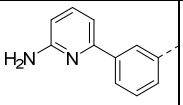
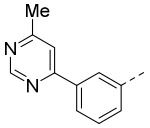
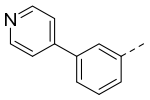
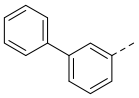
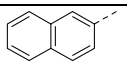
Entry	R, compound number	*E.coli* LeuRS ITC,a K_D, nM	ITC,a ΔH, kcal·mol^{-1}	ITC,a $-T\Delta S$, kcal·mol^{-1}	E.coli LeuRS IC_{50}, nM[b]	S. aureus LeuRS IC_{50}, nM[b]	Human LeuRS IC_{50}, nM[b]
1	 **7a**	14.5±0.3	-9.5±0.5	-1.2±0.5	54	9.2×10^3	3.9×10^3
2	 **7b**	12.8±1.2	-9.0±0.3	-1.7±0.3	14	650	490
3	 **7c**	10±3	-12.5±1.2	1.6±1.0	41	4.4×10^3	160
4	 **7d**	56±17	-7.6±0.3	-2.3±0.4	224	3.6×10^3	33
5	 **7e**	13.9±1.9	-9.10±0.11	-1.62±0.07	23	1.7×10^3	850
6	 **7f**	22±4	-7.05±0.05	-3.40±0.13	47	2.7×10^3	2.0×10^3
7	 **7g**	10.9±1.5	-8.27±0.11	-2.6±0.18	13	920	1.2×10^3
8	 **7h**	10.2±1.4	-8.1±0.5	-2.8±0.5	32	640	3.4×10^3
9	 **7i**	64±18	-6.5±0.9	-3.3±0.8	65	1.0×10^3	220
10	 **7j**	1.3±0.14	-13.1±0.3	1.1±0.2	35	5.4×10^3	1.1×10^3

[a]ITC data for binding to E. Coli LeuRS enzyme, [b]IC_{50} 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 benzenesulfonamide 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

Strains	Name	**7j** MIC ug/mL
E. coli	BW25113	32
E. coli	ATCC 25922	8
K. pneumoniae	clinical isolate	64
P. aeruginosa	PA01	64
Acinetobacter spp	ATCC 19606	64
E. cloacae	clinical isolate	128
S. aureus	JE2	>256

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.[20] 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 benzenesulfonamides **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.

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 coordinator of NABARSI 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.

REFERENCES

(1) Hurdle, J. G.; O'Neill, A. J.; Chopra, I. Prospects for Aminoacyl-tRNA Synthetase Inhibitors as New Antimicrobial Agents. *Antimicrob. Agents Chemother.* **2005**, *49* (12), 4821–4833.

(2) Cochrane, R. V. K.; Norquay, A. K.; Vederas, J. C. Natural Products and Their Derivatives as TRNA Synthetase Inhibitors and Antimicrobial Agents. *MedChemComm* **2016**, *7* (8), 1535–1545.

(3) Gadakh, B.; Van Aerschot, A. Aminoacyl-TRNA Synthetase Inhibitors as Antimicrobial Agents: A Patent Review from 2006 till Present. *Expert Opin. Ther. Pat.* **2012**, *22* (12), 1453–1465.

(4) Vondenhoff, G. H. M.; Van Aerschot, A. Aminoacyl-TRNA Synthetase Inhibitors as Potential Antibiotics. *Eur. J. Med. Chem.* **2011**, *46* (11), 5227–5236.

(5) Hughes, J.; Mellows, G. On the Mode of Action of Pseudomonic Acid: Inhibition of Protein Synthesis in Staphylococcus Aureus. *J. Antibiot. (Tokyo)* **1978**, *31* (4), 330–335.

(6) Pope, A. J.; Moore, K. J.; McVey, M.; Mensah, L.; Benson, N.; Osbourne, N.; Broom, N.; Brown, M. J. B.; O'Hanlon, P. Characterization of Isoleucyl-TRNA Synthetase from Staphylococcus Aureus: II. Mechanism of Inhibition by Reaction Intermediate and Pseudomonic Acid Analogues Studied Using Transient and Steady-State Kinetics. *J. Biol. Chem.* **1999**, *273* (48), 31691–31701.

(7) Silvian, L. F.; Wang, J.; Steitz, T. A. Insights into Editing from an Ile-TRNA Synthetase Structure with $TRNA^{Ile}$ and Mupirocin. *Science* **1999**, *285* (5430), 1074.

(8) Williamson, D. A.; Carter, G. P.; Howden, B. P. Current and Emerging Topical Antibacterials and Antiseptics: Agents, Action, and Resistance Patterns. *Clin. Microbiol. Rev.* **2017**, *30* (3), 827–860.

(9) Hernandez, V.; Crépin, T.; Palencia, A.; Cusack, S.; Akama, T.; Baker, S. J.; Bu, W.; Feng, L.; Freund, Y. R.; Liu, L.; Meewan, M.; Mohan, M.; Mao, W.; Rock, F. L.; Sexton, H.; Sheoran, A.; Zhang, Y.; Zhang, Y.-K.; Zhou, Y.; Nieman, J. A.; Anugula, M. R.; Keramane, E. M.; Savariraj, K.; Shekhar Reddy, D.; Sharma, R.; Subedi, R.; Singh, R.; O'Leary,

A.; Simon, N. L.; De Marsh, P. L.; Mushtaq, S.; Warner, M.; Livermore, D. M.; Alley, M. R. K.; Plattner, J. J. Discovery of a Novel Class of Boron-Based Antibacterials with Activity against Gram-Negative Bacteria. *Antimicrob. Agents Chemother.* **2013**, *57* (3), 1394–1403.

(10) O'Dwyer, K.; Spivak, A. T.; Ingraham, K.; Min, S.; Holmes, D. J.; Jakielaszek, C.; Rittenhouse, S.; Kwan, A. L.; Livi, G. P.; Sathe, G.; Thomas, E.; Van Horn, S.; Miller, L. A.; Twynholm, M.; Tomayko, J.; Dalessandro, M.; Caltabiano, M.; Scangarella-Oman, N. E.; Brown, J. R. Bacterial Resistance to Leucyl-TRNA Synthetase Inhibitor GSK2251052 Develops during Treatment of Complicated Urinary Tract Infections. *Antimicrob. Agents Chemother.* **2015**, *59* (1), 289–298.

(11) Gupta, A.; Monteferrante, C.; Rasina, D.; Leitis, G.; Randall, C. P.; Tomlinson, J. H.; Jirgensons, A.; Goessens, W. H. F.; Hays, J. P.; O'Neill, A. J. A Polymorphism in LeuS Confers Reduced Susceptibility to GSK2251052 in a Clinical Isolate of Staphylococcus Aureus. *Antimicrob. Agents Chemother.* **2016**, *60* (5), 3219–3221.

(12) Gadakh, B.; Smaers, S.; Rozenski, J.; Froeyen, M.; Van Aerschot, A. 5′-(N-Aminoacyl)-Sulfonamido-5′-Deoxyadenosine: Attempts for a Stable Alternative for Aminoacyl-Sulfamoyl Adenosines as AaRS Inhibitors. *Eur. J. Med. Chem.* **2015**, *93*, 227–236.

(13) Van de Vijver, P.; Vondenhoff, G. H. M.; Denivelle, S.; Rozenski, J.; Verhaegen, J.; Van Aerschot, A.; Herdewijn, P. Antibacterial 5′-O-(N-Dipeptidyl)-Sulfamoyladenosines. *Bioorg. Med. Chem.* **2009**, *17* (1), 260–269.

(14) Vondenhoff, G. H. M.; Dubiley, S.; Severinov, K.; Lescrinier, E.; Rozenski, J.; Van Aerschot, A. Extended Targeting Potential and Improved Synthesis of Microcin C Analogs as Antibacterials. *Bioorg. Med. Chem.* **2011**, *19* (18), 5462–5467.

(15) Yu, X. Y.; Hill, J. M.; Yu, G.; Wang, W.; Kluge, A. F.; Wendler, P.; Gallant, P. Synthesis and Structure-Activity Relationships of a Series of Novel Thiazoles as Inhibitors of Aminoacyl-TRNA Synthetases. *Bioorg. Med. Chem. Lett.* **1999**, *9* (3), 375–380.

(16) Schimmel, P.; Tao, J.; Hill, J. Aminoacyl TRNA Synthetases as Targets for New Anti-Infectives. *FASEB J.* **1998**, *12* (15), 1599–1609.

(17) Teng, M.; Hilgers, M. T.; Cunningham, M. L.; Borchardt, A.; Locke, J. B.; Abraham, S.; Haley, G.; Kwan, B. P.; Hall, C.; Hough, G. W.; Shaw, K. J.; Finn, J. Identification of Bacteria-Selective Threonyl-TRNA Synthetase Substrate Inhibitors by Structure-Based Design. *J. Med. Chem.* **2013**, *56* (4), 1748–1760.

(18) Zhang, F.; Du, J.; Wang, Q.; Hu, Q.; Zhang, J.; Ding, D.; Zhao, Y.; Yang, F.; Wang, E.; Zhou, H. Discovery of N-(4-Sulfamoylphenyl)Thioureas as Trypanosoma Brucei Leucyl-TRNA Synthetase Inhibitors. *Org. Biomol. Chem.* **2013**, *11* (32), 5310–5324.

(19) Saint-Léger, A.; Ribas de Pouplana, L. A New Set of Assays for the Discovery of Aminoacyl-TRNA Synthetase Inhibitors. *Aminoacyl-TRNA Synth.* **2017**, *113*, 34–45.

(20) Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H. Construction of Escherichia Coli K-12 in-Frame, Single-Gene Knockout Mutants: The Keio Collection. *Mol. Syst. Biol.* **2006**, *2*, 2006.0008-2006.0008.