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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF NOVEL THIAZOLES AS INHIBITORS OF AMINOACYL-tRNA SYNTHETASES

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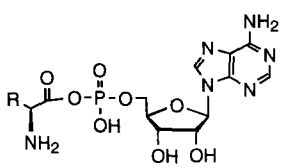
Abstract: A series of novel aminoacyl adenylate mimics has been prepared and evaluated for their inhibitory activity against aminoacyl-tRNA synthetases. Several of these thiazole derivatives displayed potent and selective enzyme activity against both Gram-positive and Gram-negative bacteria.

There is a critical need to develop new antimicrobial agents with novel modes of action due to the rise in drug resistant bacterial infections. Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes for biological cell growth.[1-5] The function of aaRS is to add one of the twenty different amino acids onto a tRNA forming a charged tRNA molecule. The charged tRNA molecules are necessary for incorporation of the respective amino acids into cellular proteins. AaRSs catalyze the charging of tRNA in a two-step reaction.[6-8] First, ATP reacts with the amino acid to form an enzyme-bound aminoacyl adenylate intermediate (AA-AMP) **1** and pyrophosphate (PPi). Second, The activated amino acid is transferred to the corresponding tRNA to form the aminoacyl-tRNA and AMP. Inhibition of this process leads to a halt in protein synthesis and subsequent inhibition of cell growth. Selective inhibition of bacterial aaRSs has been demonstrated to be an effective strategy for producing antibacterial agents.[9] Pseudomonic acid (A) is an isoleucyl-tRNA synthetase inhibitor[10-14] that has antibacterial activity against *S. aureus*, Staphylococcus species, and *Streptococcus pyogenes* and is effective in topical treatment of staphylococcal and streptococcal infections.[15] In addition, several other naturally produced and synthetic compounds have enzymatic activity against aminoacyl-tRNA synthetases.[16-25]



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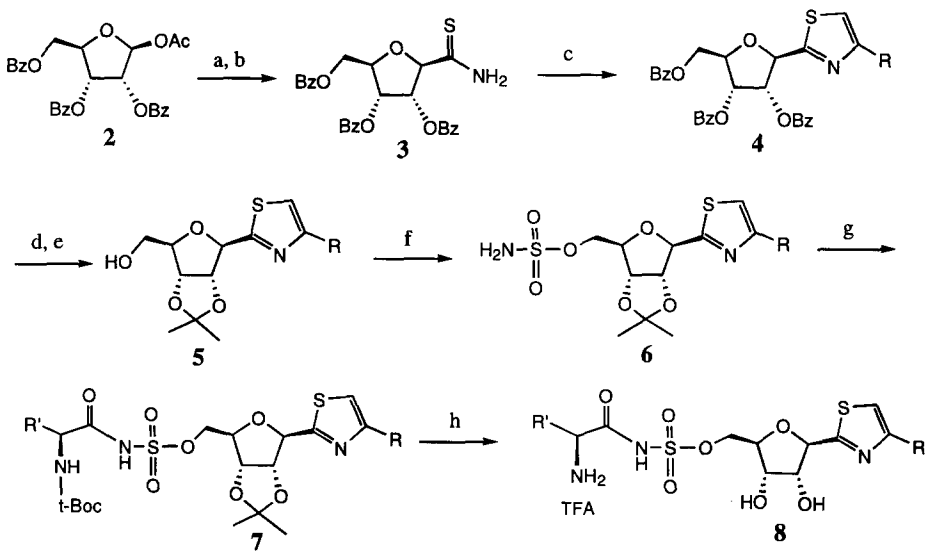
Recently, a series of novel tetrazole derivatives of aminoacyl adenylate mimics was discovered in our lab.[26] These compounds were found to have highly potent enzyme activity and selectivity against isoleucyl-tRNA synthetases. As part of our program to develop novel antibiotics targeting aminoacyl-tRNA synthetases, we have expanded our investigation into replacements of the adenine ring with a variety of other heterocycles. We report here studies on the synthesis and the inhibition of bacterial aminoacyl-tRNA synthetases of structurally novel thiazole aminoacyl adenylate mimics.



Scheme 1. Reagents and conditions: (a) TMSCN, BF_3.(Et_2O), CH_2Cl_2, rt, 24 h , 85%; (b) H_2S (l), DMAP, rt, 8 h , 88%; (c) $BrCH_2COR$, CH_3CN, 0 °C to rt, 24 h, 50-85%; (d) NaOMe, MeOH, rt, 8 h, 70-85%; (e) Acetone, HCl (cat), $CH(OEt)_3$, 0 °C to rt, 24 h , 75-85%; (f) NH_2SO_2Cl, Et_3N, CH_2Cl_2, rt, 5 h, 80-90%; (g) *N*-*t*-Boc-L-amino-acid, EDAC.HCl, DMAP, CH_2Cl_2, rt, 24 h, 85-90%; (h) TFA, H_2O, rt, 3 h, >95%;

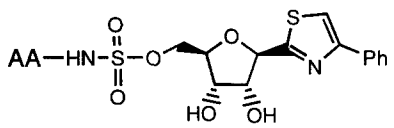
Enzymatic intermediate **1** containing an acyl phosphate is chemically unstable and can be replaced with acylsulfamate, which is known to be a good acyl phosphate mimic.[27] The synthesis of aminoacyl sulfamoyl thiazole analogs **8** is shown in Scheme 1. Reaction of 1-*O*-acetyl-2,3,5-*O*-tribenzoyl-β-D-ribofuranose (**2**) with trimethylsilyl cyanide in the presence of boron trifluoride followed by treatment with liquid hydrogen sulfide generated 3,4,6-tri-*O*-benzoyl-β-D-allonthioamide **3**.[28,29] Reaction of thioamides **3** with a variety of aryl and alkenyl α-bromo ketones in acetonitrile afforded the corresponding 2,4-disubstitued thiazole derivatives **4** in yields ranging from 50-85%.[29-31] The required aryl α-bromo methyl ketones[32,33] and alkenyl α-bromo methyl ketones[34] were prepared by known procedures. Hydrolysis of tribenzoyl groups with sodium methoxide in methanol, followed by protection of diol, provided 2',3'-*O*-isopropylidene thiazoles **5**. Treatment of **5** with sulfamoyl chloride gave rise to sulfamoyl thiazole intermediates **6**. Reaction of **6** with a variety of *N*-*t*-Boc-L-

amino acids afforded **7**. Deprotection of **7** with trifluoroacetic acid yielded the target products **8** in nearly quantitative yields.[35]

The extent of aminoacylation of tRNA with amino acid catalyzed by aminoacyl-tRNA synthetase enzyme was measured by the amount of incorporation of [^{3}H] amino acid in the trichloroacetic acid precipitate of tRNA in the presence of a compound.[26] The IC_{50} value is the compound concentration that inhibits 50% of the indicated aminoacyl-tRNA synthetase activity of the particular species in the tRNA charging assay.

The results of inhibition of *E.coli* aminoacyl-tRNA synthetases of various amino acid phenyl thiazole analogs are reported in Table 1. Isoleucine thiazole analog **9** exhibited IleRS inhibition. Replacement of isoleucine with valine, which is similar in structure to isoleucine, resulted in much less IleRS inhibition while replacement with alanine produced a greater loss in the IleRS inhibition. Both histidine and proline analogs **12, 13** led to complete loss of the IleRS inhibition. Studies on the mechanism of action for isoleucine analog **9** shows that it is a competitive inhibitor[36] with respect to isoleucine (K_i = 0.031 μM) and ATP (K_i = 0.048 μM). These results indicate that the binding site for the amino acid is very specific and is consistent with an interpretation that the aminoacyl sulfamoyl group fits well into the binding pocket of the corresponding aminoacyl-tRNA synthetase. This result is further substantiated by the observation that the alanine analog **11** exhibited inhibition of the corresponding AlaRS.

Table 1. Inhibition of *E. coli* Isoleucyl-tRNA Synthetase

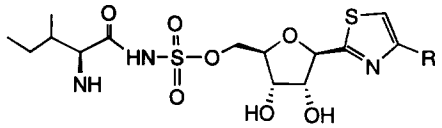


Compd	AA	IC_{50} (μM)/IleRS	IC_{50} (μM)/LeuRS	IC_{50} (μM)/AlaRS
9	Ile	0.1	1.0	>100
10	Val	15	14	>100
11	Ala	100	>100	0.5
12	His	>100	>100	>100
13	Phe	>100	>100	>100

A wide variety of substituted thiazole analogs were investigated. The inhibition of Gram-positive and Gram-negative bacterial and human isoleucyl-tRNA synthetases is shown in Table 2. These analogs have the isoleucyl group on the left side chain. Replacement of the phenyl group with the furan ring (**14**) increased the inihbition of *S.aureus* IleRS relative to phenyl analog **9**, but with no selectivity versus the human enzyme. The introduction of a methoxy group on the phenyl ring (**15-17**) increased the inhibition of *S. aureus* IleRS. Alkenyl biphenyl ether analog **20**, however, did have moderate selectivity versus the human enzyme. Replacement of the phenyl group with the alkenyl phenyl group (**21**) increased the inhibition of the two bacterial IleRS but did

not change that of the human enzyme, therefore increasing the selectivity. Overall, the selectivity for bacterial isoleucyl-tRNA synthetases versus the human enzyme is relatively low.

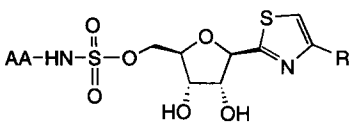
Table 2. Inhibition of Isoleucyl-tRNA Synthetases



Compd	R	IC_{50} (μM) (*S.aureus* IleRS)	IC_{50} (μM) (*E.coli* IleRS)	IC_{50} (μM) (Human IleRS)	Selectivity (Hu/*E.coli*)
9	Ph	8.0	0.10	0.33	3.3
14	4'-cyano-furanyl	0.24	0.12	0.12	1.0
15	2'-OMe-Ph	0.30	0.03	0.10	3.3
16	3'-OMe-Ph	0.05	0.015	0.034	2.3
17	4'-OMe-Ph	0.10	0.10	0.20	2.0
18	6'-OMe-2-naphthyl	0.13	0.02	0.40	4.0
19	PhOPh	0.27	0.045	0.37	8.2
20	CH_2CH_2PhOPh	0.12	0.11	1.02	9.3
21	CH_2CH_2Ph	0.04	0.03	0.30	10

The selectivity of bacterial leucyl-tRNA synthetase versus the human enzyme is shown in Table 3. Isoleucine analog **9** with less potent inhibition of *E. coli* LeuRS showed good selectivity. Leucine analogs **22-24**, which possessed the excellent inhibition of *E. coli* LeuRS, also demonstrated the high selectivity of *E. coli* LeuRS versus the human enzyme.

Table 3. Selectivity of Bacterial Leucyl-tRNA Synthetases versus the Human Enzyme



Compd	AA	R	IC_{50} (μM) (*S.aureus* LeuRS)	IC_{50} (μM) (*E.coli* LeuRS)	IC_{50} (μM) (Human LeuRS)	Selectivity (Hu/*E.coli*)
9	Ile	Ph	>20	1.0	200	200
22	Leu	PhOPh	0.054	0.0016	0.60	375
23	Leu	CH_2CH_2PhOPh	0.1	0.006	1.15	192
24	Leu	CH_2CH_2Ph	0.09	<0.002	0.73	>365

In summary, we have developed a series of potent, selective and structurally novel aminoacyl-tRNA synthetase inhibitors in which a thiazole ring system replaces the adenine ring in the aminoacyl adenylate intermediate.

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References and Notes

1. Carter, C. W. Jr. *Annu. Rev. Biochem.* **1993**, *62*, 715.
2. Delarue, M.; Moras, D. *BioEssays* **1993**, *15*, 675.
3. McClain, W. H. M. *J. Mol. Biol.* **1993**, *234*, 257.
4. Ribas, D. P.; Buechter, D. D.; Davis, M. W.; Schimmel, P. *Protein Sci.* **1993**, *2*, 2259.
5. Davis, M. W.; Buechter, D. D.; Schimmel P. *Biochemistry* **1994**, *33*, 9904.
6. Friest, W. *Biochemistry* **1989**, *28*, 6787.
7. Cramer, F.; Friest, W. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 190.
8. Delarue, M. *Current Opinion in Structural Biology* **1995**, *5*, 48.
9. van der Haar, F.; Gabius, H. J.; Cramer, F. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 217.
10. Hughes, J.; Mellows, G. *Biochem. J.* **1978**, *176*, 305.
11. Hughes, J.; Mellows, G. *J. Antibiotics* **1978**, *31*, 330.
12. Hughes, J.; Mellows, G. *Biochem. J.* **1980**, *191*, 209.
13. Class, Y. J.; DeShong, P. *Chem. Review* **1995**, *95*, 1843.
14. Brown, P.; Davies, D. T.; O'Hanlon, P. J.; Wilson, J. M. *J. Med. Chem.* **1996**, *39*, 446.
15. White, A. R.; Beale, A. S.; Boon, R. J.; Griffin, K. E.; Masters, P. J.; Sutherland, R. In *Bactroban; Proceedings of an International Symposium;* Dobson, R. L.; Leyden, J. J.; Noble, W. C.; Price, J. D., Eds.; Excerpta Medica: Amsterdam, 1985; pp 19-36.
16. Cassio, D.; Lemoine, F.; Waller, J. P.; Sandrin, E.; Boissonas, R. A. *Biochemistry* **1967**, *6*, 827.
17. Göring, G.; Cramer, F. *Chem. Ber.* **1973**, *106*, 2460.
18. Werner, R. G.; Thorpe, L. F.; Reuter, W.; Nierhaus, K. H. *Eur. J. Biochem.* **1976**, *68*,1.
19. Konrad, I.; Roschenthaler, R. *FEBS Lett.* **1977**, *83*, 341.
20. Walter, R. D.; Ossikovsk, E. *Molecular and Biochemical Parasitology* **1985**, *14*, 23.
21. Biryukov, A. I.; Zhukov, Y. N.; Lavrik, O. I.; Khomutov, R. M. *FEBS* **1990**, *273*, 208.
22. Dorion, C.; Chênevert, R.; Lacoste, L.; Lapointe, J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2699.
23. Cochereau, C.; Sanchez. D.; Bourhaoui, A.; Creppy, E. E. *Toxical. Appl. Pharmacol.* **1996**, *141*, 133.
24. Heacock, D.; Forsyth, C. J.; Shiba, K.; Musier-Forsyth, K. *Bioorg. Chem.* **1996**, *24*, 273.
25. Desjardins, M.; Desgagnés, J.; Lacoste, L.; Yang, F.; Morin, M.; Lapointe, J.; Chênevert, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2363.
26. Hill, J. M.; Yu, G.; Shue, Y.; Zydowsky, T. M.; Rebek, J. Jr. U. S. patent, 5,726,195.
27. Belrhali, H.; Yaremchuk, A.; Tukalo, M.; Larsen, K.; Berthet-Colominas, C.; Leberman, R.; Beijer, B.; Sproat, B.; Als-Nielsen, J.; Grubel, G.; Legrand, J.; Lehmann, M.; Cusack, S. *Science* **1994**, *263*, 1432.

28. Bobek, M.; Farkas, J. *Collect. Czech. Chem. Commun.* **1969**, *34*, 247.
29. Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256.
30. Fuertes, M.; Garcia-Lopez, T.; Garcia-Munoz, G.; Stud, M. *J. Org. Chem.* **1976**, *41*, 4075.
31. Clingerman, M. C.; Secrist, J. A. *J. Org. Chem.* **1983**, *48*, 3141.
32. Marquet, A.; Jacques, J. *Bull. Soc. Chim. France* **1962**, 90.
33. Johnson, W. S.; Bass, J. D.; Williamson, K. L. *Tetrahedron* **1963**, *19*, 861.
34. Armstrong, V. W.; Chishti, N. H.; Ramage, R. *Tetrahedron Lett.* **1975**, *6*, 373.
35. All new compounds displayed spectra consistent with assigned structure and analytical data.
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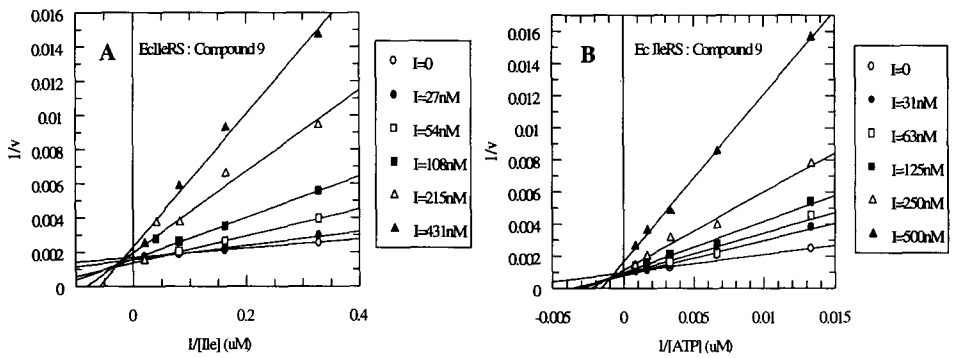

Figure 1 : Lineweaver-Burke analysis on the inhibition of Compound **9** on *E.coli* isoleucyl-tRNA synthetase. A : Ki determination against isoleucine at saturating ATP (2 mM) and tRNA (90 μM) concentrations. B : Ki determination against ATP at saturating isoleucine (50 μM) and tRNA (90 μM) concentrations.