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A series of spirocyclic analogues as potent inhibitors of bacterial phenylalanyl-*t*RNA synthetases

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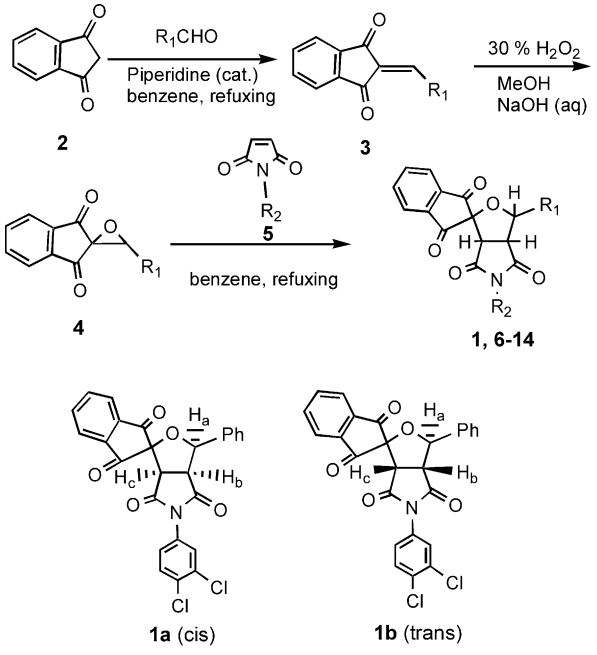
Abstract—We have identified a series of spirocyclic furan and pyrrolidine inhibitors of *Enterococeus faecalis* and *Staphylococcus aureus* phenylalanyl-*t*RNA synthetases. The most potent analogue **1b** showed $IC_{50} = 5$ nM (*E. faecalis* PheRS) and $IC_{50} = 2$ nM (*S. aureus* PheRS) with high selectivity over the human enzyme. The crystal X-ray structure of analogue **1b** was determined.

Emergence of resistant bacteria has given new urgency to the search for new antibacterial agents that act via novel mechanisms of action.[1–3] Aminoacyl-*t*RNA synthetases are essential enzymes for biological cell growth.[4–8] We have previously described several series of aminoacyl-*t*RNA synthetase inhibitors.[9–11] As part of our high throughput screening efforts identifying *t*-RNA synthetase inhibitors, we discovered that a series of spirocyclic compounds had good inhibition of *Enterococcus faecalis* and *Staphylococcus aureus* Phenylalanyl-*t*RNA synthetases (EfPheRS and SaPheRS). The lead compound **1a** inhibited EfPheRS ($IC_{50} = 0.82$ μM) and SaPheRS ($IC_{50} = 0.38$ μM) with high selectivity over the human enzyme ($IC_{50} > 100$ μM). In this communication, we report on the structure–activity relationships of the series of spirocyclic analogues based on compound **1a**.

The spirocyclic furan analogues were prepared by synthetic route outlined in Scheme 1. Reaction of 1,3-indandione **2** with aldehydes in the presence of piperidine produced 2-benzylidene-1,3-indandiones **3**. Treatment with 30% hydrogen peroxide in methanol formed 3-phenylspirocyclic [oxirane-2,2′-indan]-1′,3′-dione **4**.[12] Heating the epoxide **4** gave an intermediate carbonyl ylide which underwent 1,3-dipolar cycloaddition with maleimides **5**[13] to give spirocyclic isomers **1** and **6–14**.[14]

In the case of the 3,4-dichlorophenyl analogue, the two isomers, *cis* (**1a**) [15] and *trans* (**1b**), were formed in a ratio of 2:1 and separated by silica gel chromatography (20% hexane in dichloromethane).



Scheme 1.

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The structure of these compounds was determined by ^{1}H NMR experiments and mass spectra. X-ray crystallography of analogue **1b** (Fig. 1) further confirmed the stereochemistry.

Scheme 2 shows the preparation of spirocyclic pyrrolidine analogues. Reaction of ninhydrin **15a**, phenyl glycine **16** and substituted maleimide **5a** provided two isomers (**17a** and **b**),[16] which were separated by Florisil® chromatography. Analogue **22** was synthesized by coupling with amine **20**, aldehyde **21** and substituted maleimide **5a**. Because formation of carbomethoxy analogues in one step was unsuccessful, analogue **25** was prepared stepwise by generating ylide first followed by treatment with **5a**.[17,18]

Spirocyclic analogues were evaluated for inhibition of the aminoacylation activity[19] of EfPheRS and SaPheRS and the results of spirocyclic furan analogues are shown in Table 1. The stereochemistry dramatically affects the activity. The *trans* spirocyclic analogues are significantly more potent than the *cis* spirocyclic analogues. The *trans* 3,4-dichlorophenyl analogue **1b** is the most potent compound in this series. It inhibits EfPheRS ($IC_{50}=5$ nM) and SaPheRS ($IC_{50}=2$ nM) with high selectivity over the human enzyme ($IC_{50}>100$ μM). This trend is also evident in comparing the *cis* 3-chloro-4-methylphenyl analogue **6a** and the *trans* chloro-4-methylphenyl analogue **6b**. The substitution pattern on the R_2 position also affects the potency. 3,4-Methylenedioxy benzene analogue **7** has moderate activity whereas 3-(trifluoromethyl)-4-chlorophenyl analogue **9** is less active. Analogue **13** with an electron-donating methoxy group and analogue **14** with an electron-withdrawing nitro group show a significant loss in potency. Interestingly, only limited variations appear to be tolerated at the R_1 position. The 4-methyl and 4-chlorophenyl analogues (**8** and **10**) showed a significant loss in potency. The 2-methyl-phenyl analogue **12** is ten times less active than the phenyl analogue **11**.

The results of spirocyclic pyrrolidine analogues are shown in Table 2. It is worthy to note that the *trans* spirocyclic pyrrolidine analogues were also more potent than the *cis* spirocyclic analogues. 3,4-Dichlorophenyl *cis* analogue **17a** is 100 times less active than *trans* analogue **17b**. Analogues without carbonyl groups on the spirocyclic ring (**19a** and **b**) were inactive. Benzoyl analogues **22a** and **22b** were only moderately active whereas the carbomethoxy analogue **25** was inactive.

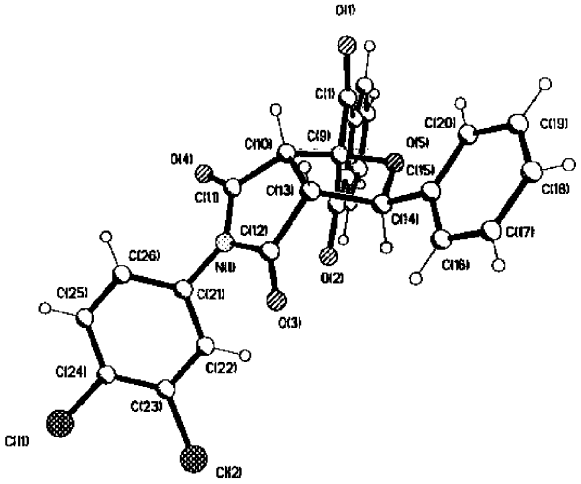


Figure 1. The crystal X-ray structure of analogue **1b**.

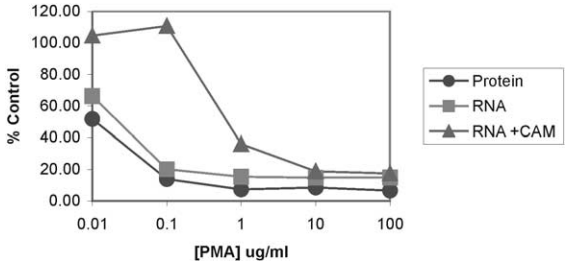
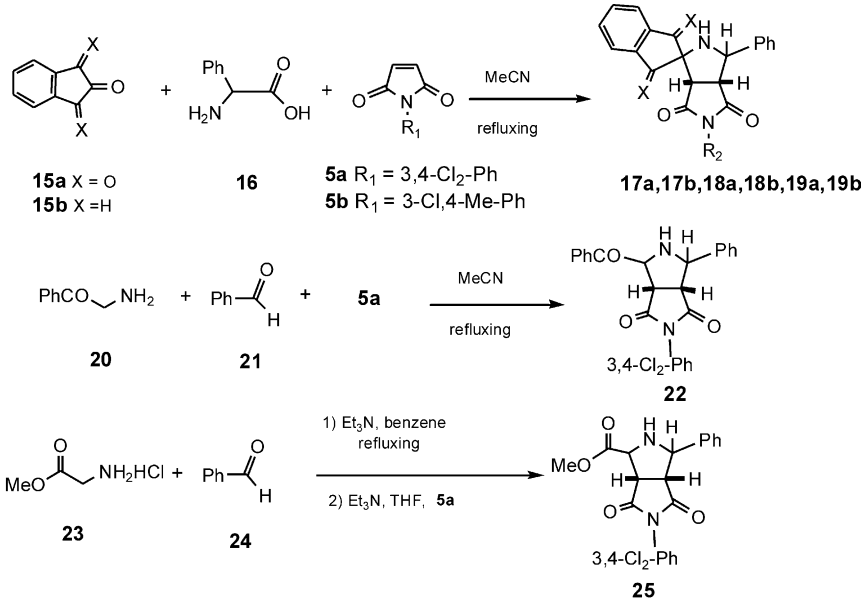
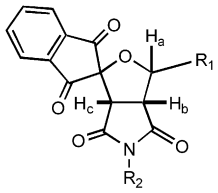


Figure 2. WT *S. aureus* macromolecular labeling with PMA.



Scheme 2.

Table 1. Inhibition of EfPheRS and SaPheRS by furan analogues

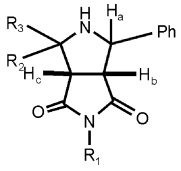


Compd	R^1	R^2	IC_{50} (μM) (EfPheRS)	IC_{50} (μM) (SaPheRS)	IC_{50} (μM) (Human PheRS)
1a (*cis*)	Ph	3,4-Cl_2-Ph	0.85	0.56	>100
1b (*trans*)	Ph	3,4-Cl_2-Ph	0.005	0.002	>100
6a (*cis*)	Ph	3-Cl-4-Me-Ph	8.3	13.7	>100
6b (*trans*)	Ph	3-Cl-4-Me-Ph	0.22	0.07	>100
7 (*cis*:*trans* = 25:1)	Ph	3,4-(OCH_2O)-Ph	2.7	0.97	>100
8 (*cis*)	4-Me-Ph	3,4-(OCH_2O)-Ph	>100	>100	>100
9 (*cis*)	Ph	3-CF_3-4-Cl-Ph	32	45	>100
10 (*cis*)	4-Cl-Ph	3-CF_3-4-Cl-Ph	>100	>100	>100
11 (*cis*:*trans* = 18:1)	Ph	2-OMe-Ph	6.1	1.8	>100
12 (*cis*)	2-Me-Ph	2-OMe-Ph	57	13	>100
13 (*cis*)	Ph	4-OMe-Ph	>100	>100	>100
14 (*cis*)	Ph	4-NO_2-Ph	>100	>100	>100

These spirocyclic furan and pyrrolidine compounds also exhibit *Escherichia coli* PheRS inhibitory activity. For example, the most potent analogue **1b** possesses good activity against *E. coli* PheRS (IC_{50} = 30 nM).

The analogues were tested for antimicrobial activity against a panel of organisms. Analogue **1b** has weak whole cell activity (MIC = 50 μg/mL, *S. aureus* ATCC6538P). Inhibitors of tRNA synthetase activity produce a unique profile in assays monitoring macromolecular synthesis in *S. aureus*. As shown in Figure 2, pseudomonic acid (PMA),[20–24] a known inhibitor of isoleucine-*t*RNA synthetase, inhibits synthesis of both protein and RNA. RNA synthesis is inhibited due to

Table 2. Inhibition of EfPheRS and SaPheRS by pyrrolidine analogues



Compd	R^1	R^2/R^3	IC_{50} (μM) (EfPheRS)	IC_{50} (μM) (SaPheRS)	IC_{50} (μM) (Human PheRS)
17a (*cis*)	3,4-Cl_2Ph		4.0	3.0	>100
17b (*trans*)	3,4-Cl_2Ph		0.04	0.04	>100
18a (*cis*)	3-Cl,4-Me-Ph		8.7	6.1	>100
18b (*trans*)	3-Cl,4-Me-Ph		0.99	0.73	>100
19a (*cis*)	3,4-Cl_2Ph		>100	>100	>100
19b (*trans*)	3,4-Cl_2Ph		>100	>100	>100
22a (*cis*)[a]	3,4-Cl_2Ph	PhCO/H	15.7	59	>100
22b (*trans*)[a]	3,4-Cl_2Ph	PhCO/H	6.7	30	>100
25 (*cis*)[a]	3,4-Cl_2Ph	CO_2Me/H	>100	>100	>100

[a] The stereochemical center adjacent to the benzoyl or methoxy carbonyl centers was not assigned.

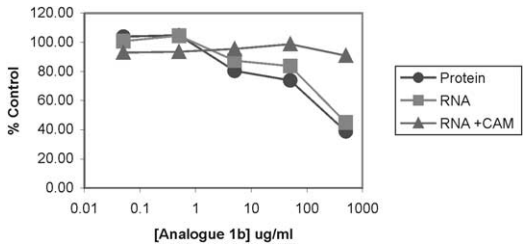


Figure 3. WT *S. aureus* macromolecular labeling with analogue **1b**.

induction of the stringent response.[25] Induction of the stringent response is inhibited by addition of the protein synthesis inhibitor chloramphenicol (CAM), allowing RNA synthesis to continue uninhibited until the dose of PMA is increased by 10–100-fold. As shown in Figure 3, analogue **1b** also triggers the stringent response, as indicated by the altered profile of RNA synthesis inhibition in the presence of CAM. This result suggests a synthetase component to the mechanism of action.

In conclusion, a novel structural class of spirocyclic furan and pyrrolidine analogues with potent activity against *E. faecalis* and *S. aureus* PheRSs with high selectivity over the human enzyme has been discovered. These compounds also exhibit *E. coli* PheRS inhibitory activity. Further study shows that an analogue **1b** was unstable in Mueller Hinten Broth (MHB) bacterial media. The results of our efforts to improve the stability and the antibacterial activity of this series are described in the next paper.

Acknowledgements

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