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A series of heterocyclic inhibitors of phenylalanyl-*t*RNA synthetases with antibacterial activity

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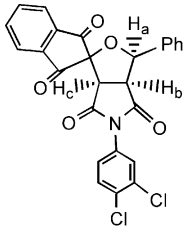
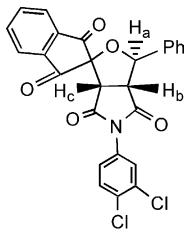
Abstract—A series of novel heterocyclic analogues have been synthesized and evaluated for their ability to inhibit phenylalanyl-*t*-RNA synthetases and act as antibacterial agents. Several analogues have good antibacterial activity against *Staphylococcus aureus*.

In the preceding paper,[1] we described a series of spirocyclic furan and pyrrolidine inhibitors of *Enterococci faecalis* and *Staphylococcus aureus* phenylalanyl-*t*RNA synthetases (EfPheRS and SaPheRS). These compounds also exhibit *Escherichia coli* PheRS inhibitory activity. Based on lead compound **1a**, generated by high throughput screening, a number of spirocyclic analogues were prepared. We discovered the isomeric compound **1b** with potent activity against EfPheRS ($IC_{50}=2$ nM) and SaPheRS ($IC_{50}=5$ nM). Analogue **1b** exhibited a relatively weak antibacterial activity (MIC = 50 μg/mL, *S. aureus*). Further study showed that both analogues **1a** and **1b** were unstable in Mueller Hinton Broth (MHB) bacterial media. The half-life time for **1a** and **1b** in MHB at 37 °C is 117 min and 206 min respectively. To improve the stability and whole cell activity of this series, we sought to modify the structure of the molecule. Herein, we report the structure–activity relationship of a novel series of heterocyclic PheRS inhibitors with antibacterial activity against *S. aureus*.



1a (cis)



1b (trans)

It was found that derivatives generated by sodium borohydride reduction of spirocyclic analogues were stable in MHB. Scheme 1 shows the chemistry of these novel heterocyclic analogues. The preparation of spirocyclic compound **1b** was described in the preceding paper.[1] Reduction[2] of analogue **1b** with sodium borohydride in methanol gave two isomers, which were purified by silica gel chromatography (15% ethyl acetate in dichloromethane). Elution of the column gave the slower moving isomer **3a** and the faster moving isomer **3b**. The ratio for isomer **3a** to isomer **3b** was 1:1.8. Both carbonyl groups on the spiro cyclic ring of compound **1b** were reduced to the hydroxyl groups. Steric effects govern the stereochemistry of reduction. The ketone *cis* to the malimide ring is reduced selectively from less hindered face of the molecule to provide compounds **3a**

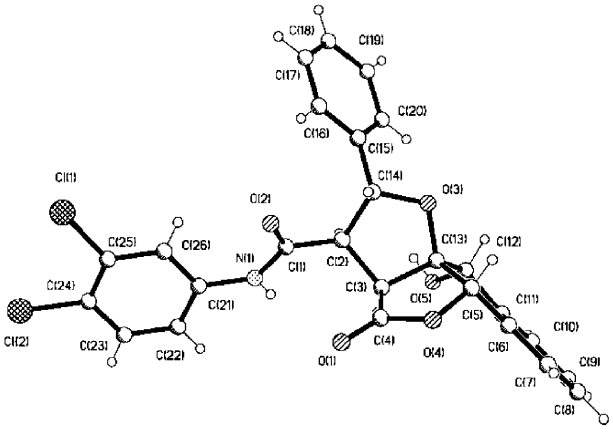


Figure 1. The crystal X-ray structure of analogue **3a**.

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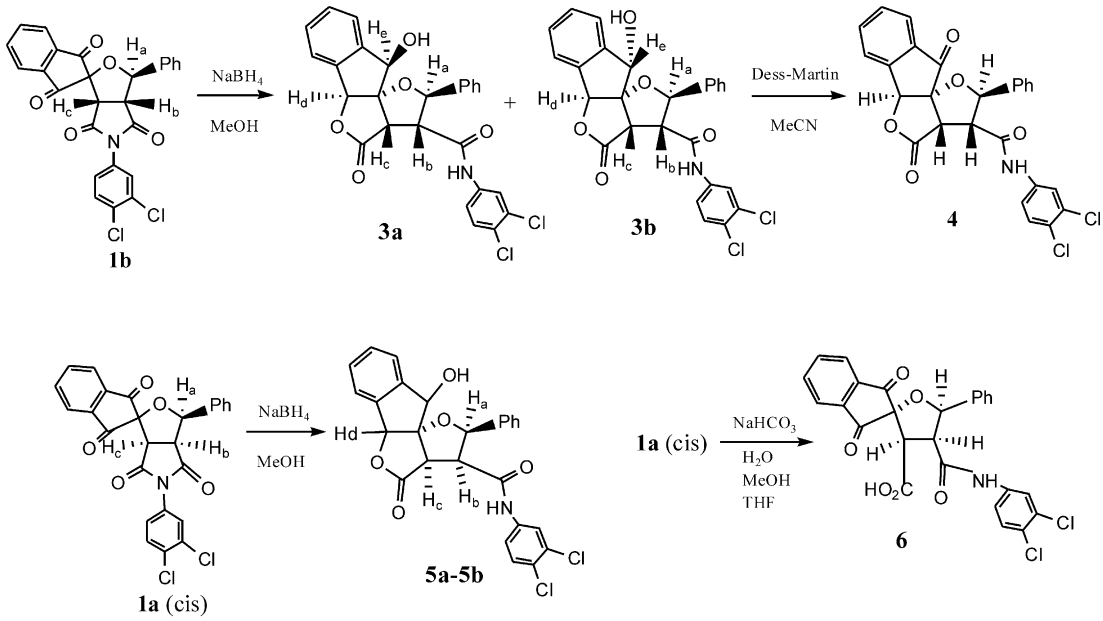


Scheme 1.

and **3b** where H_d is *cis* to the furan oxygen. The structure of these compounds was characterized by ^{1}H NMR experiment and mass spectra. X-ray crystallography (Fig. 1) confirmed the stereochemistry. The protons H_d and H_e in analogue **3a** are *cis* to each other. Oxidation of the mixture of analogues **3a** and **b** with Dess–Martin reagent[3] in acetonitrile generated carbonyl butyrolactone derivative **4**. Reduction of the *cis* spirocyclic analogue **1a** provided **5a–b**. Basic hydrolysis (NaOH/MeOH) of *cis* compound **1a** led to the maleimide ring open analogue **6**.

Heterocyclic analogues were evaluated for inhibition of the aminoacylation activity of EfPheRS and SaPheRS. Analogues were further tested for antibacterial activity against a panel of organisms. Table 1 shows the enzyme and whole cell activity. Both reduced hydroxyl butyrolactone derivatives **3a** and **b** were less potent enzyme inhibitors than the parent compound **1b**. However, they each demonstrated a significant increase in antibacterial activity against *S. aureus*. Minimum inhibitory concentration (MIC) for analogues **3a** and **b** was 3.1 μg/mL and 6.2 μg/mL respectively as compared with the corresponding compound **1b** (MIC = 50 μg/mL). Carbonyl butyrolactone derivative **4** retained moderate enzyme activity, but had no activity against *S. aureus*. This result suggests the importance of the hydroxyl group in analogues **3a** and **b** to the antibacterial activity. For hydroxyl butyrolactone derivatives **5a** and **5b** from reduction of compound **1a**, only one isomer had the whole cell activity (MIC = 6.2 μg/mL). The stereochemistry of **5a** and **b** was not assigned. Hydrolysis analogue **6** has moderate enzyme activity, but it was found to be inactive (MIC > 100 μg/mL).

Inhibitors of *t*RNA synthetase activity produce a unique profile in assays monitoring macromolecular synthesis in *S. aureus*. As demonstrated in Figure 3, pseudomonic acid (PMA),[4,5] a known inhibitor of isoleucine-*t*RNA synthetase, inhibits synthesis of protein, RNA, and DNA. RNA and DNA inhibition are due to induction of the stringent response.[6] The addition of the protein synthesis inhibitor chloramphenicol (CAM) prevents induction of the stringent response, allowing RNA and DNA synthesis continues uninhibited until the dose of PMA is increased by 10–100-fold. As shown in Figure 2, analogue **3a** has a similar dose–response profile to PMA. Inhibition of RNA synthesis is clearly

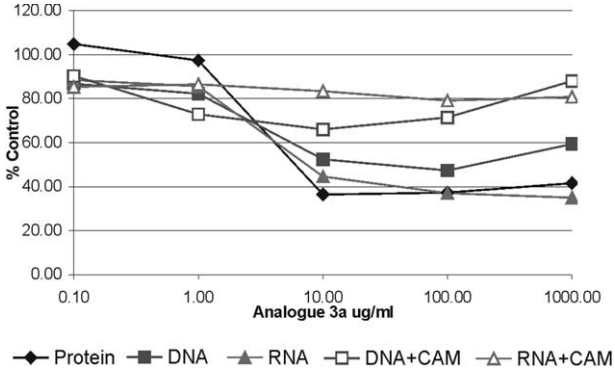


Figure 2. WT *S. aureus* macromolecular labeling with analogue **3a**.

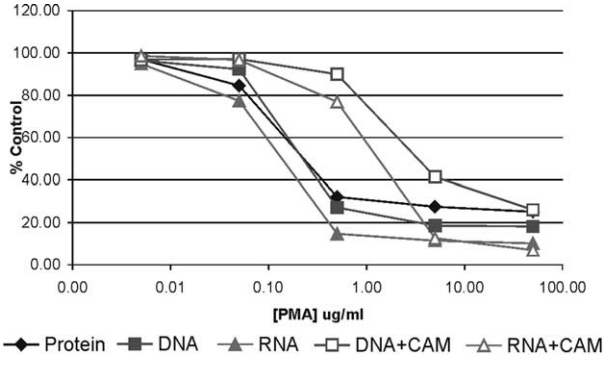
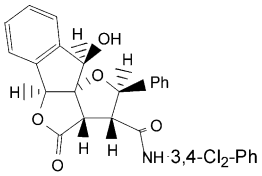
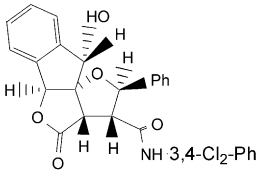
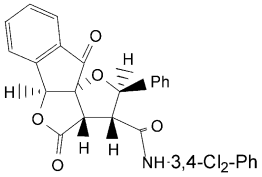
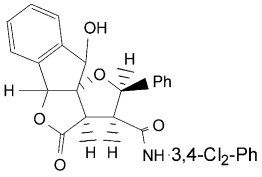
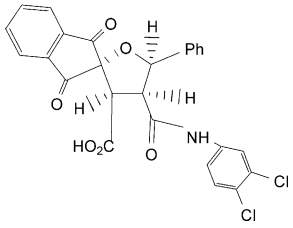


Figure 3. WT *S. aureus* macromolecular labeling with PMA.

Table 1. Inhibition of EfPheRS, SaPheR and minumn inhibitory concentration (MIC)

Compd	Structure	Ic_{50} (μM) (EfPheRS)	Ic_{50} (μM) (SaPheRS)	MIC (μg/mL) (*S. aureus*)
3a		0.47	0.51	3.1
3b		0.17	0.26	6.2
4		3.1	2.6	> 100
5a[a]		15	14	> 100
5b		4.9	> 100	63.2
6		11	33	> 100

[a] The stereochemistry of the alcohol in **5a** and **5b** was not assigned.

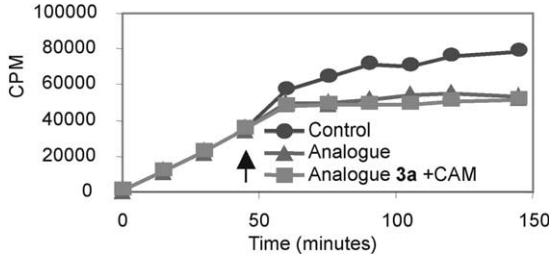


Figure 4. DNA Synthesis-analogue.

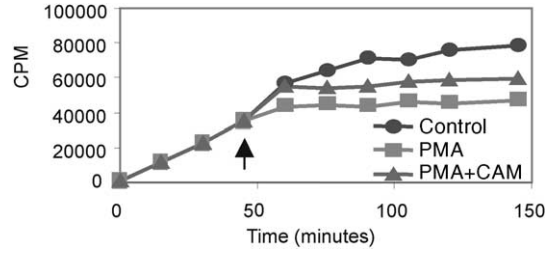


Figure 5. DNA Synthesis-PMA.

relieved by the addition of chloramphenicol, consistent with a phenylalanyl-*t*RNA synthetase as the primary target. Relief of DNA synthesis, however, was less striking, raising the possibility that the compound has an additional mode of action. This was confirmed by using a more sensitive kinetic assay to monitor DNA synthesis. As shown in Figure 5, addition of PMA results in an immediate cessation of DNA synthesis, whereas in the presence of chloramphenicol DNA synthesis continues for an additional 10 min, indicative of induction of the stringent response. In the presence of analogue **3a**, however, DNA synthesis is inhibited immediately both with and without CAM (Fig. 4) indicating that DNA synthesis inhibition is due to activities other than inhibition of PheRS.

In conclusion, a novel structural class EfPheRS and SaPheRS heterocyclic inhibitors with in vitro antibacterial activity has been discovered. The best analogue **3a** with activity against EfPheRS ($IC_{50} = 0.17$ μM)

and SaPheRS ($IC_{50} = 0.26$ μM) has potent antibacterial activity against *S. aureus* (MIC = 3.1 μg/mL).

Acknowledgements

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References and notes

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