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The Antimicrobial Natural Product Chuangxinmycin and Some Synthetic Analogues are Potent and Selective Inhibitors of Bacterial Tryptophanyl tRNA Synthetase

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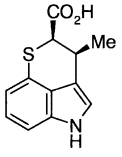
Abstract—The antimicrobial natural product chuangxinmycin has been found to be a potent and selective inhibitor of bacterial tryptophanyl tRNA synthetase (WRS). A number of analogues have been synthesised. The interaction with WRS appears to be highly constrained, as only sterically smaller analogues afforded significant inhibition. The only analogue to show inhibition comparable to chuangxinmycin also had antibacterial activity. WRS inhibition may contribute to the antibacterial action of chuangxinmycin.

Chuangxinmycin (**1**) is a natural product first isolated from *Actinoplanes tsinanensis*.[1,2] The compound was reported to have in vitro antibacterial activity against a number of Gram-positive and Gram-negative bacteria and to show in vivo efficacy in mouse infection models against *Escherichia coli* and *Shigella dysenteriae*.[1] Preliminary clinical results indicated that chuangxinmycin was effective in the treatment of septicaemia, urinary and biliary infections caused by *E. coli*.[1] Chuangxinmycin is reported to interact with the tryptophan biosynthetic pathway, but the molecular target for its antibacterial activity has not been described.[3]

The aminoacyl tRNA synthetases are a family of enzymes that catalyse attachment of amino acids to their cognate tRNA in protein biosynthesis. The activity and fidelity of these enzymes are essential to living cells. Inhibition of bacterial isoleucyl tRNA synthetase is the mode of action of the antibacterial agent mupirocin (marketed as Bactroban®) and selective inhibitors of other tRNA synthetases are of interest as they could afford new antibacterials with a novel mode of action.

From high throughput screening, we have found that novel natural product inhibitors of tRNA synthetase frequently contain a structural fragment that is readily recognisable for its relatedness to the amino acid substrate.[4–6]

Chuangxinmycin has some structural similarity to tryptophan (**2**), the substrate of tryptophanyl tRNA synthetase (WRS). It also bears some structural resemblance to the known WRS inhibitor indolmycin (**3**). We hypothesised that chuangxinmycin might be exerting its antibacterial activity via inhibition of WRS. In this report, we describe studies of the inhibition of WRS by chuangxinmycin, as well as the synthesis and testing of some analogues designed to elucidate the role of the dihydrothiopyran carboxylic acid in binding.



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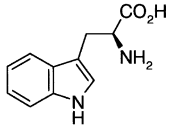

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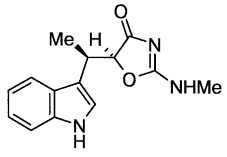

3

Chuangxinmycin was found to potently inhibit the aminoacylation of tRNA by purified *Staphylococcus*

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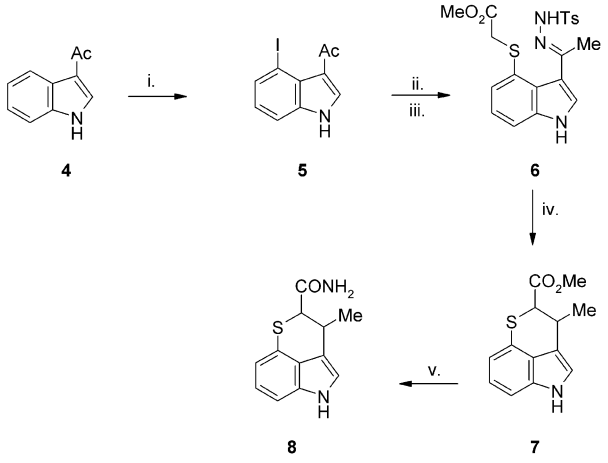
aureus WRS,[7,8] and in a pyrophosphate exchange assay[9] at varying concentrations of the amino acid substrate, chuangxinmycin was found to be competitive with respect to tryptophan with a K_i of 20 nM. Chuangxinmycin was highly selective for the bacterial enzyme, with no inhibition of ovine WRS observed at concentrations up to 30 μM.

To further investigate the interactions of chuangxinmycin with bacterial WRS, we decided to synthesise some key analogues of the inhibitor. We concentrated on modification of the substituents on the characteristic dihydrothiopyran ring of chuangxinmycin. A number of derivatives of chuangxinmycin, some of which are products of modification of the carboxylic acid group, have been reported in the Chinese literature.[10]

We decided to use synthetically derived (±)-chuangxinmycin methyl ester (**7**) as the starting point for the preparation of analogues. This material was prepared using a modification of the route reported by Matsumoto and Watanabe,[11] starting from 3-acetylindole (Scheme 1). The primary amide (**8**) was formed by treatment of the methyl ester with methanolic ammonia, although the reaction was very slow. In general, mixtures containing similar amounts of the respective *cis* and *trans* derivatives were obtained.[12]

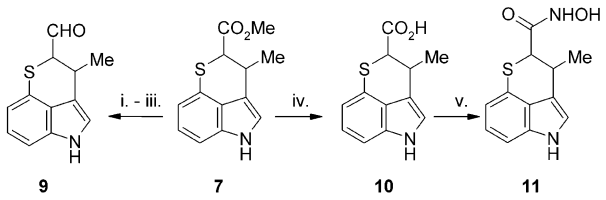


Scheme 1. Reagents and conditions: (i) $Tl(CF_3CO_2)_3/CuI/I_2$, quant; (ii) $Me_3SnSCH_2CO_2Me$, quant; (iii) $TsNHNH_2$, 97%; (iv) NaH then Δ, 44%; (v) NH_3/MeOH, 21 d, 50%.

The ester **7** and the primary amide **8** were tested for inhibition against a crude preparation of wild type WRS from *S. aureus* Oxford. Neither compound showed significant inhibition up to 20 μM.

The ester was hydrolysed to the free acid **10** with sodium hydroxide (Scheme 2). The hydroxamic acid **11** was formed from the acid by carbodiimide-HOAt mediated coupling with hydroxylamine. The aldehyde **9** was prepared from the ester **7** by first protecting the indole nitrogen with a triisopropylsilyl group, followed by DIBAL-H reduction and TBAF deprotection.

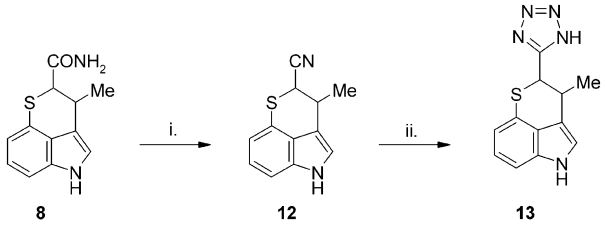
Compounds **9** and **11** were tested in a standard aminoacylation assay against purified recombinant *S. aureus*



Scheme 2. Reagents and conditions: (i) LiHMDS/THF/−78 °C then TIPS-Cl, 82%; (ii) DIBAL-H/toluene/DCM(4:1), 84%; (iii) TBAF/THF, 73%; (iv) $NaOH/H_2O$/EtOH, quant; (v) $NH_2OH.HCl$/EDCI/HOAt/N-methylmorpholine/DMF, 88%.

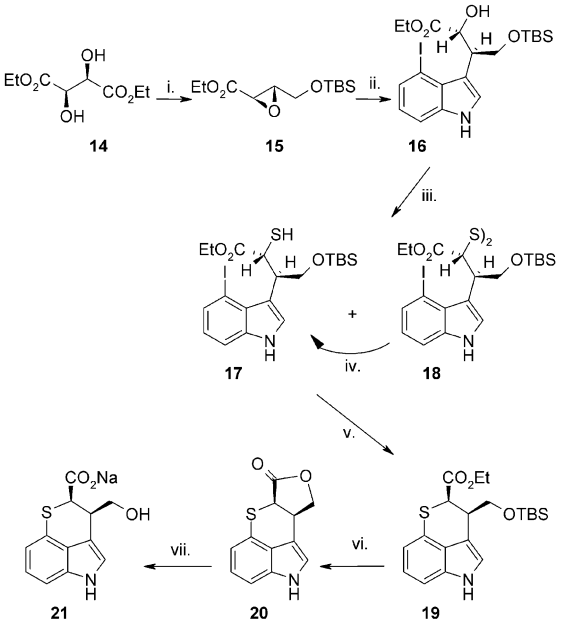
WRS in which chuangxinmycin gave an IC_{50} value of about 30 nM. The hydroxamic acid gave almost no inhibition up to 10 μM but the aldehyde was a potent inhibitor with an IC_{50} of 90 nM. It was shown that the aldehyde was stable in the DMSO stock solution used for the assay and was not oxidised to chuangxinmycin itself. The possibility exists that the aldehyde could form a covalent adduct, for example by Schiff base formation with an enzymatic lysine residue. However, following incubation with sodium borohydride[13] no evidence for a covalent adduct was obtained by LC–MS.

Dehydration of the primary amide to the nitrile resulted in a 1:7 ratio of *cis*-***12***: *trans*-***12*** (Scheme 3). The ratio could be changed to a 2.1:1 ratio in favour of the desired *cis* isomer by epimerisation with catalytic DBU. Cycloaddition of azide to the nitrile afforded the tetrazole analogue **13**.



Scheme 3. Reagents and conditions: (i) $POCl_3$/pyridine/0–10 °C, 61%, then DBU (0.2 equiv)/DMSO, quant; (ii) $NaN_3/Et_3N.HCl$/DMF/110 °C, 78%.

In order to investigate functionalisation of the methyl group, we adapted a diastereo- and enantioselective synthesis of chuangxinmycin described by Akita and coworkers[14] in order to include a silyl protected hydroxymethyl in place of the methyl group (Scheme 4). Chiral epoxide **15**, available from **14** in five steps,[15,16] alkylates 4-iodoindole in the presence of Lewis acid to give **16** in virtually identical yield to that reported for the parent compound.[14] Conversion of the α-hydroxy group in **16** to thiol **17** was assumed to proceed with retention of configuration by analogy to the literature precedent.[14] A significant amount of disulfide **18** was formed as a by-product of the reaction, however this was smoothly converted to **17** by heating with dithiothreitol in triethylamine. The synthesis was completed by palladium-mediated cyclisation followed by desilylation with concomitant lactonisation, to afford lactone **20**.[17] This was converted to the hydroxymethyl analogue **21** by treatment with lithium hydroxide. Compound



Scheme 4. Reagents and conditions: (i) refs 15 and 16, followed by TBS-Cl; (ii) 4-I-indole/$SnCl_4$, 35%; (iii) MsCl/pyridine/0–25 °C then KSAc/DMF/40 °C then K_2CO_3/MeOH, 35% **17** and 25% **18**; (iv) dithiothreitol/Et_3N/60 °C, 78%; (v) $Pd(PPh_3)_4$/Et_3N/THF 86%; (vi) TBAF/THF, 74%; (vii) LiOH/MeOH then HCl then NaOH 27%.

21 was isolated as virtually exclusively the *cis* isomer, the unusually high stereoselectivity probably being due to intramolecular hydrogen bonding effects.

Compounds **12**, **13**, **20** and **21** were tested for inhibition in a scintillation proximity assay for WRS activity.[18] In this assay format chuangxinmycin gave a higher IC_{50} value of 90–200 nM. The tetrazole analogue **13** and the hydroxymethyl derivative **21** afforded no inhibition up to the highest concentration tested, 10 μM. However, significant inhibition was shown by the nitrile **12** (IC_{50} 1100 nM) and by the lactone **20**, which had an IC_{50} value of 230 nM, comparable to that of chuangxinmycin itself.

It is noteworthy that the only active analogues of chuangxinmycin, the aldehyde **9**, the nitrile **12**, and the lactone **20**, all have smaller steric footprints than chuangxinmycin, indicative of a tight constraint to binding in this region of WRS. The lack of activity of the tetrazole, hydroxamic acid, ester and amide, as well as of the hydroxymethyl analogue **21**, confirm this steric constraint. Rather than an ionisable feature, the presence of a small dipolar group with Lewis base character seems to be critical for binding in this region of WRS.

In a standard antibacterial assay to determine minimum inhibitory concentration (MIC), the only analogue to show activity was the compound that showed comparable inhibition to chuangxinmycin, the lactone **20**. This compound was active against the bacterial pathogens *S. aureus* Oxford, MIC 4 μg/mL, *Haemophilus influenzae* Q1, MIC 16 μg/mL, and *Moraxella catarrhalis* 1502, MIC 16 μg/mL. The activity is likely to be due to the intact lactone as the potential hydrolysis product **21** was completely inactive at concentrations up to 32 μg/mL.

In summary we have shown that chuangxinmycin is a potent and selective inhibitor of bacterial WRS. The interaction is highly constrained with only sterically smaller analogues showing significant inhibition. The only analogue to show inhibition comparable to chuangxinmycin, the lactone **20**, also had antibacterial activity. We suggest that WRS inhibition is likely to contribute to the mechanism of antibacterial activity of chuangxinmycin.

Acknowledgements

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12. The ratio of *cis*:*trans* isomer in samples that were tested for biological activity was as follows: **7** 1:1, **8** 6:1, **9** 2:1, **11** 1:1, **12** 1.3:1, **13** 1.1:1, **20** >20:1, **21** >20:1.
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17. The lactone **20**, has a coupling constant S-CH-CH of 6.2 Hz, significantly larger than normally observed values (around 3 Hz for *cis* isomers, around 4–4.5 Hz for *trans* isomers). Since a *trans* fusion of the lactone would geometrically be highly disfavoured this suggests a close-to-eclipsed conformation of the two hydrogen atoms (Karplus curve). Indeed, modelling **20** and chuangxinmycin shows a reduced dihedral angle (from 52.6 to 34.1°) in the tetracyclic compound. The hydrolysed hydroxycarboxylate **21** has a normal *cis* coupling constant of 3.0 Hz.
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