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Structure-Based Design of Agents Targeting the Bacterial Ribosome

Justin Bower,[a] Martin Drysdale,[a] Richard Hebdon,[b] Allan Jordan,[a,*] Georg Lentzen,[c] Natalia Matassova,[d] Alastair Murchie,[d] Jenifer Powles[c] and Stephen Roughley[a]

[a]*Department of Medicinal Chemistry, RiboTargets Ltd., Granta Park, Abington, Cambridge CB1 6GB, UK*
[b]*Department of Bacteriology, RiboTagets Ltd., Granta Park, Abington, Cambridge CB1 6GB, UK*
[c]*Department of Screening, RiboTargets Ltd., Granta Park, Abington, Cambridge CB1 6GB, UK*
[d]*Department of Assay Development, RiboTargets Ltd., Granta Park, Abington, Cambridge CB1 6GB, UK*

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Abstract—Rational structure-based drug design has been applied to the antibiotic thiostrepton, in an attempt to overcome some of its' limitations. The identification of a proposed binding fragment allowed construction of a number of key fragments, which were derivatised to generate a library of potential antibiotics. These were then evaluated to determine their ability to bind to the L11 binding domain of the prokaryotic ribosome and inhibit bacterial protein translation.

The thiopeptide antibiotic thiostrepton **1** is a natural product employed in topical veterinary medicine,[1] but its low solubility and poor bioavailability have hampered its application in human infections. Active against Gram-positive bacteria, thiostrepton stabilizes the binding of the ribosomal protein L11 to a region of the 23S ribosomal RNA known as the L11 binding domain (L11BD).[2] The dynamics of this L11–L11BD complex are critical for stimulating the GTPase action of the elongation factors EF-G and EF-Tu, which in turn provide the energy required to drive protein translation. Though efforts to find novel therapeutics have recently been directed toward the L11–L11BD interaction,[3] rational exploitation of this vital region of RNA has been hampered by a lack of structural knowledge. Recent work in our laboratories has for the first time elucidated the structure of the thiopeptide antibiotics (in particular thiostrepton) bound to the L11BD and highlighted intimate details of those residues in close contact with this region of the ribosome (Fig. 1).[4] These studies indicated a low molecular weight fragment, based around the conserved dehydrodemethylvaline-containing macrocycle, which appears to be involved in the binding of the antibiotic to the L11BD and, further, that focused libraries based around this fragment may emulate the antibacterial activity of thiostrepton, or lead to simpler derivatives which may overcome some of its limitations.

Chemistry

The proposed binding fragment could be readily disconnected to three key fragments, namely a phenyl thiazole acid, a dehydrodemethylvaline unit or suitable mimic and a thiazole amino acid. From this premise, the synthetic approach detailed in Scheme 1 was derived. 2-Aminobutyric acid was *N*-Boc protected, then converted to the primary amide with ethyl chloroformate and ammonia. The thioamide, obtained by treatment with Lawesson's reagent, was cyclised to the required thiazole with ethyl bromopyruvate to give the hydroxy thiazoline and dehydrated to the thiazole with trifluoroacetic anhydride and 2,6-lutidine as per the literature method, avoiding racemisation of the stereocentre.[5] Deprotection of the amine, followed by facile coupling with the protected L-threonine **4**[6] and *iso*butyl chloroformate, gave the dipeptide mimetic. Finally, *iso*butyl chloroformate-mediated amide formation with 2-phenyl-thiazole-4-carboxylic acid,[7] followed by hydrolysis of the terminal ethyl ester gave the desired scaffold **2**. A parallel synthesis, starting from L-valine gave scaffold **3** in a similar manner.

*Corresponding author. Tel.: +(1)-223-895-315; fax: +(1)-223-895-556; e-mail: allan.jordan@ribotargets.com

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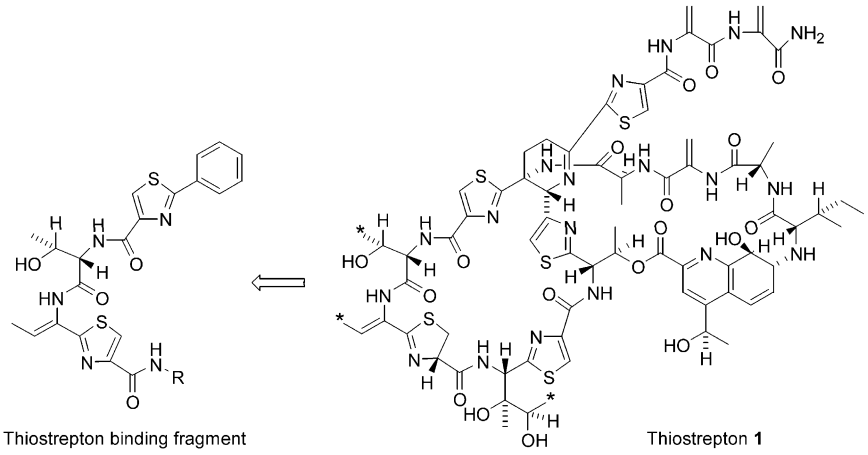
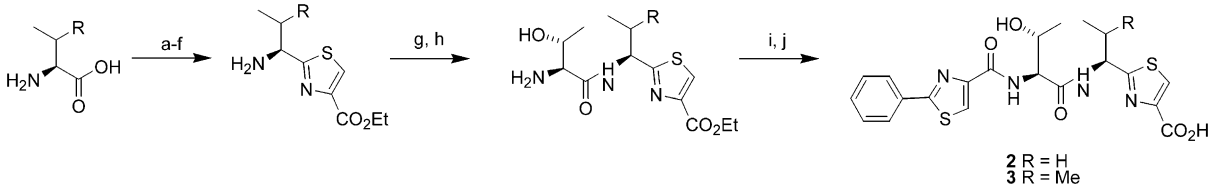
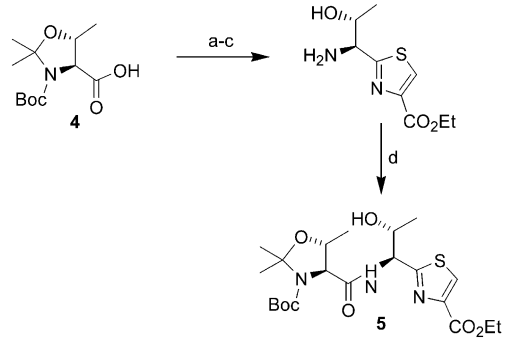


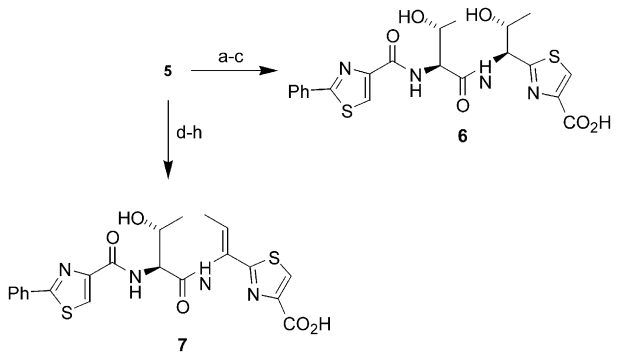
Figure 1. Thiostrepton, highlighting observed nOes to the ribosomal RNA (marked *), and the resultant proposed binding fragment.



Scheme 1. R = H or Me. Reagents: (a) Boc_2O, NaOH; (b) ethyl chloroformate, THF then NH_4OH; (c) Lawesson's reagent, toluene; (d) ethyl bromopyruvate, $KHCO_3$, 1,2-dimethoxyethane; (e) TFAA then 2,6-lutidine, 1,2-dimethoxyethane; (f) EtOH, AcCl; (g) **4**, *iso*-butyl chloroformate, *N*-methyl morpholine, THF; (h) TFA, DCM; (i) 2-phenyl-thiazole-4-carboxylic acid, *iso*-butyl chloroformate, *N*-methyl morpholine, THF; (j) LiOH, H_2O, THF.



Scheme 2. (a) Ethyl chloroformate, THF then NH_4OH; (b) Lawesson's reagent, toluene; (c) ethyl bromopyruvate, $KHCO_3$, 1,2-dimethoxyethane; (d) **4**, *iso*-butyl chloroformate, *N*-methyl morpholine, THF.



Scheme 3. (a) TFA, DCM; (b) 2-phenyl-thiazole-4-carboxylic acid, *iso*-butyl chloroformate, *N*-methyl morpholine, THF; (c) LiOH, H_2O, THF; (d) mesyl chloride, triethylamine, DCM; (e) TFA, DCM then aq HCl; (f) DBU, $CHCl_3$; (g) 2-phenyl-thiazole-4-carboxylic acid, *iso*-butyl chloroformate, *N*-methyl morpholine, THF; (h) LiOH, H_2O, THF.

The protected threonine derivative **4** was also employed in the preparation of key intermediate **5** (Scheme 2), which could be diversified to form scaffolds **6** and **7** (Scheme 3).

Thus, **4** was converted to the thioamide, via the primary amide and cyclised with ethyl bromopyruvate and TFAA/2,6-lutidine. The resultant thiazole acid was *N*-deprotected and coupled with a second equivalent of **4** to yield **5**. Deprotection of the *N*-Boc acetal unit, *iso*-butyl chloroformate mediated coupling with the phenyl thiazole acid and hydrolysis of the ethyl ester gave facile access to scaffold **6** (Scheme 3).

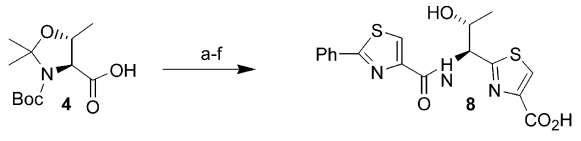
The preparation of the dehydrodemethylvaline-containing unit **7**, displayed in thiostrepton itself, proved to be more troublesome. Literature precedent suggested that the treatment of a protected threonine derivative with thionyl chloride or phosgene would generate a formation of a cyclic sulfamidite[8] or cyclic anhydride,[9] which could be treated with base to yield the desired dehydrodemethylvaline unit and sulfur dioxide or carbon dioxide respectively. Alternatively, it was reported that the cyclic anhydride derived from Cbz-2-amino-2-butenoic acid (itself formed from 2-ketobutyric acid and benzyl carbamate[10]) could be coupled directly with **4** and further elaborated to give a late-stage intermediate in the synthesis of **7**.[11] In our hands, however, these approaches failed to give satisfactory results. We also

hoped that the aforementioned protected 2-amino-2-butyric acid could be coupled directly to **4** and further derivatised to **7**, but again this proved unsuccessful.

The required unit was, however, successfully obtained by mesylation of the free hydroxyl of **5**, which we proposed could be eliminated with DBU to form the dehydrodemethylvaline moiety. We had assumed this would be a facile process and was immediately attempted on the acetal-protected material. These attempts initially proved fruitless but we reasoned that the cyclic acetal rigidifies the scaffold and restricts the conformation such that E2 elimination cannot occur. Indeed, we found that deprotection *prior* to treatment with DBU allowed the desired transformation to occur smoothly.

We had assumed to this point that the dehydrodemethylvaline moiety (or a suitable mimic thereof) would be crucially implicated in the interaction of our compounds with the L11BD. To test this hypothesis, we also prepared the truncated scaffold **8**, devoid of this central unit, as shown in Scheme 4. The common intermediate **4** was converted to the corresponding protected thiazole, deprotected and coupled to the phenyl thiazole acid and the ethyl ester hydrolysed to generate **8**.

Once prepared, the scaffolds **2**, **3**, **6**, **7** and **8** were derivatised with a diverse set of amino- and hydroxyamino-units (Fig. 2). We reasoned these units may interact in a similar manner to the ethanediol unit in thiostrepton,



Scheme 4. (a) Ethyl chloroformate, THF then NH_4OH; (b) Lawesson's reagent, toluene; (c) ethyl bromopyruvate, $KHCO_3$, 1,2-dimethoxyethane; (d) TFA, DCM; (e) 2-phenyl-thiazole-4-carboxylic acid, *iso*-butyl chloroformate, *N*-methyl morpholine, THF; (f) LiOH, H_2O, THF.

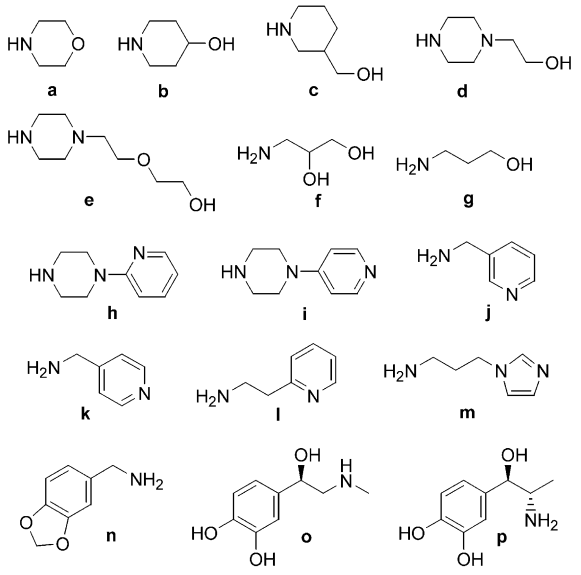


Figure 2. Amines incorporated into scaffolds **2**, **3**, **6**, **7** and **8**.

which we had previously shown to display nOes to the L11BD RNA (Fig. 1). These couplings were conducted in parallel in a Bohdan Mini-block™ and employed solid-supported DCC and catalytic HOBt, followed by treatment with solid-supported isatoic anhydride to capture the excess amine used to drive the reaction to completion. Careful optimisation of this chemistry allowed the required products to be isolated simply by filtration and concentration in vacuo, as single entities by LC-MS.

Results and Discussion

Site-specific methylation of the L11BD RNA has been shown to be a major mechanism of thiostrepton resistance,[12] occurring exclusively on *O*-2′ of A1067. This methylation prevents binding of the antibiotic, whilst still allowing formation of the L11–L11BD complex and resultant activation of the elongation factors EF-Tu and EF-G, in turn allowing normal ribosomal function. Methylation can be readily monitored using radiolabeled *S*-adenosyl methionine, both on the whole ribosome, and also on an artificial 58-nucleotide construct of the L11BD region. This allows the rapid detection of those compounds that interact with this region. Those which interact with the L11BDR 58-mer construct in a manner similar to thiostrepton will prevent incorporation of the radiolabel and this can be detected by scintillation counting.[13] The assay can be repeated on the entire ribosomal RNA, to ensure that this binding is selective for L11BD and confirm that the compounds do not bind in a non-selective manner. Those compounds found to interact selectively with the L11BD site can then be assessed for both their ability to inhibit protein translation and their effect on bacterial cell growth and proliferation.

From the synthesized library of 93 compounds, five were identified that prevented the incorporation of this radiolabel into the L11BD 58-mer construct (Table 1)

Compound **8**–**e** appeared initially to be the strongest inhibitor of L11BD methylation. However, the failure to inhibit radiolabel incorporation in the presence of the whole ribosomal RNA indicates that this example of the truncated series appears to interact with RNA in a non-selective manner. For those representatives of the more complex series, inhibition of methylation was reduced, but satisfyingly similar across both the 58-nucleotide

Table 1. Inhibition of methylation of A1067, on both the L11BD 58-mer construct and rRNA

Compd		% Inhibition of L11BD 58-mer methylation	% Inhibition of rRNA methylation
Scaffold	Amine		
8	**e**	59	—
3	**g**	37	37
6	(OH)	35	27
2	**n**	28	13
3	**n**	24	20

All inhibition studies conducted at a compound concentration of 50μM.

Table 2. Inhibition of *E. coli* in vitro protein translation (EcIVT)[14] and antibacterial activity (MIC_{95})

Compd		% EcIVT inhibition	MIC_{95}
Scaffold	Amine		
Thiostrepton **1**		98 (0.1)	0.015
8	**e**	—	>200
3	**g**	48	>200
6	(OH)	—	>200
2	**n**	31	>200
3	**n**	16	>200

EcIVT percentage inhibition measured at 200 μM. Figures in parentheses represent IC_{50} in μM.

construct and whole ribosomal RNA. This finding indicates that these simplified mimics of thiostrepton do indeed bind selectively to the L11BD region of the ribosome, albeit with reduced potency compared to the natural antibiotic.

Furthermore, it was found that inhibition of protein translation correlated closely with the results of the methylation assay, indicating that these specific binders were indeed interfering with the dynamics of L11–L11BD binding in a manner similar to Thiostrepton. Disappointingly, however, these compounds failed to display any in vitro antibacterial activity (Table 2).

Conclusions

These studies have yielded a number of novel ligands that have been shown to interact specifically with a vital functional region of the bacterial ribosome and inhibit protein translation. In conjunction with our earlier results elucidating the structure of this key region,[4] the work described here is the first reported example of rational, structure-based drug design targeted toward this complex but vital cellular machine. Employing computational techniques, NMR, molecular biology, biochemistry and medicinal chemistry in a unified manner has allowed the investigation of a functional site with under-exploited therapeutic potential, established critical details of the interaction of thiostrepton **1** with ribosomal RNA and generated rationally designed ligands which have been shown to interact selectively with the desired region. With the recent appearance of high-resolution crystal structures of the prokaryotic ribosome,[15–17] we are confident that this integrated approach will herald a new era in anti-infective research, generating a variety of novel antibacterial agents with strong clinical potential.

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