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Structure–activity relationships of novel antibacterial translation inhibitors: 3,5-Diamino-piperidinyl triazines

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Abstract—Structure–activity relationships of the 3,5-diamino-piperidinyl triazine series, a novel class of bacterial translation inhibitors, are described. Optimization was focused on the triazine C-4 position in which aromatic substituents that contained electron-withdrawing groups led to potent inhibitors. The initial lack of antibacterial activity was correlated with poor cellular penetration. Whole cell antibacterial activity was achieved by linking additional aromatic moieties at the triazine C-4 position.
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Bacterial infections are a leading cause of morbidity and are major contributors toward top causes of mortality in the United States, including lower respiratory diseases, pneumonia, and sepsis.[1] Coupled with mounting bacterial resistance, an aging population, and a dearth of novel therapeutics, there is an important unmet clinical need to develop new antibiotics.[2] One challenge in antibiotic discovery is developing compounds with broad-spectrum activity. A proven drug target against diverse bacterial groups is the ribosome, where a number of clinically important antibiotic classes bind including macrolides, tetracyclines, aminoglycosides, and oxazolidinones. Importantly, all of these antibiotics, which were identified in whole cell screens, specifically target rRNA, which represents a macromolecule class that has largely been ignored in target driven drug discovery programs.[3] The recently solved co-structures of antibiotics bound to the ribosome have opened structure-guided strategies to discover small molecules that bind rRNA.[4] Emerging from such an approach we have reported on a novel series of synthetic aminoglycoside mimetics that inhibit bacterial translation, presumably by interfering with the ribosomal decoding site (A-site).[5] Here we expand on the series structure–activity relationships (SAR).

The aminoglycosides are currently the best characterized class of small molecules that interact specifically with RNA. In prior studies the two-amine groups of the 2-deoxy-streptamine (2-DOS) scaffold, which is conserved among aminoglycoside antibiotics, have been identified as a key pharmacophore for binding rRNA.[6] Medicinal chemistry efforts to improve the toxicological, microbiological, and pharmacological properties of aminoglycosides have been hindered by their complex chemistry. To circumvent this problem, we have previously developed a synthetic mimetic of 2-DOS, the *cis*-3,5-diamino-piperidine (DAP).[5] The DAP ring retains the characteristic *cis*-1,3-diamine configuration of 2-DOS which is an important feature for RNA recognition via hydrogen bonding to base edges.[6] In contrast to aminoglycosides, the reduced chemical complexity of the DAP heterocycle renders this series amenable to rapid parallel synthesis. In one optimal configuration two symmetrically positioned DAP moieties are directly linked to a triazine core to form the di-DAP substituted triazine (DAPT) (**1**) (Scheme 1). Similar to aminoglycosides, DAPT molecules have been shown to bind the A-site within 16S rRNA, inhibit translation in vitro, promote translational miscoding in vivo, act as cidal agents and protect mice from bacterial infections.[5] Molecular modeling studies based on crystal structures of aminoglycosides bound to the A-site suggest that the DAP groups serve as a key pharmacophore for rRNA binding. Here we describe the optimization of the DAPT series around R^1 (**1**), which plays a critical role for obtaining antibacterial activity.

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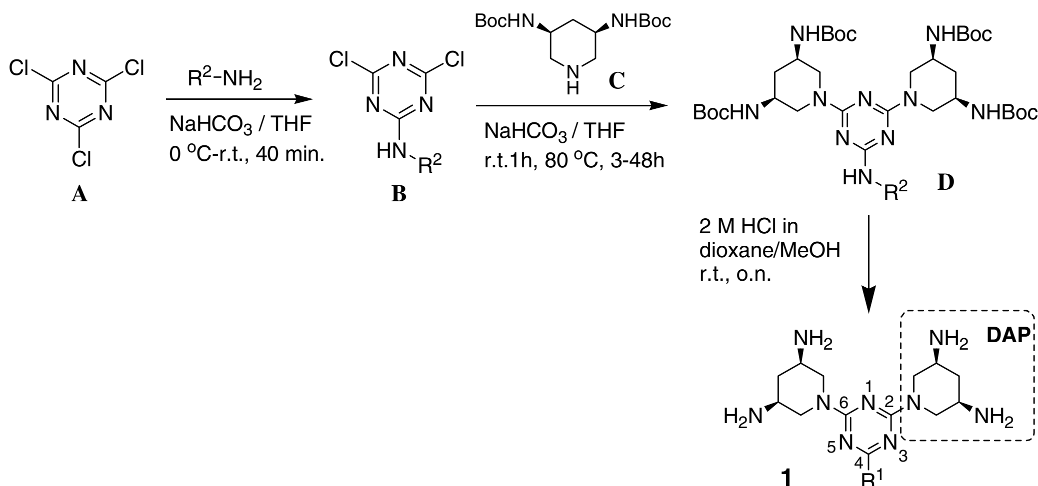

Scheme 1. Parallel synthesis of DAPT (**1**) compounds.

In these studies, lead optimization was focused on substitutions at the triazine C-4 position (**1**), which for convenience of discussion, this region of the molecule was termed tailpiece. Scheme 1 shows a general route for parallel synthesis of DAPT library compounds. In this method, cyanuric chloride (**A**) was treated with an amine (e.g., aromatic amine) at low temperature to form intermediate **B**, followed by treatment with Boc-protected DAP (**C**) under heating conditions to give the tri-substituted 1,3,5-triazine derivative **D**. Removal of the Boc-protecting group with HCl in dioxane yielded compound **1**.[7] Early library compounds showed that halide, hydroxyl, amine or alkyl tailpieces (compounds **2**–**11**) resulted in poor to modest activity in a bacterial in vitro translation assay (Table 1). In contrast, aromatic tailpieces, as exemplified by compounds **14** and **15**, resulted in potent translation inhibitors. Compounds with aromatic heterocyclic tailpieces had mixed activities; an isoxazole tailpiece (**13**) exhibited potent inhibitory activity, while a pyridine tailpiece (**12**) had moderate activity (Table 1). These combined data suggest that planar aromatic amine tailpieces (R^1) improved rRNA binding. We hypothesize that stacking interactions with rRNA base residues could account for improved activity.

Although compound **14** was a reasonable inhibitor of bacterial translation, it did not inhibit bacterial growth (Table 2). To improve potency and establish antibacterial activity, optimization was focused around the phenyl tailpiece of compound **14**. The results in Table 2 show that electron-withdrawing substitution on the phenyl ring of R^2 improved inhibitory potency against bacterial translation as exemplified in compounds **16**–**21**. In contrast, electron-donating substitutions, including compounds **22**–**26**, resulted in reduced potency compared to the parent molecule (**14**). We hypothesize that substituent-modulated changes in electronic properties of the phenyl ring likely influence the stacking interactions with rRNA bases.

Importantly, certain compounds (**16**, **18**, and **20**) that were potent protein synthesis inhibitors in vitro showed modest antibacterial activity on standard test strains of

Table 1. Bacterial protein synthesis inhibitory activity for compounds **1**

Compound[a]	R^1	IC_{50} (μM)[b]
2	Cl	42
3	NH_2	92
4	NHOH	45
5	$NHCH_3$	62
6	$NHNH_2$	170
7	$N(CH_3)_2$	290
8	N	180
9	HN	63
10	N NH	23
11	H N NH	36
12	HN N	32
13	HN N–O	9
14	HN	7
15	HN NH_2	8
Kan		0.4
Gent		0.03
Tet		2.8
Cm		1.5

[a] Kan, kanamycin; Gent, gentamicin; Tet, tetracycline; and Cm, chloramphenicol.
[b] Coupled in vitro transcription–translation assay using *Escherichia coli* extracts.[5]

Escherichia coli and *Staphylococcus aureus* (Table 2). To determine if the weak antibacterial activity was caused by poor cellular penetration, this series of compounds was tested against an *imp*$^-$ permeability mutant.[8] Indeed, the MIC values of a number of the potent translation inhibitors (**16**, **17**, **18**, and **20**) were significantly

Table 2. Antibacterial activity[a] and protein synthesis inhibitory activity

DAPT–NH–R^2

Compound	R^2	IC_{50} (μM)	*Sa*	*Ec*	*Ec imp*$^+$	*Ec imp*$^-$
14		7	>64	>64	>64	>64
16		4	32	32	32	4
17	Br	4	>64	>64	>64	4
18	NH, F	3	16	64	32	4
19	HN, F	5	>64	>64	>64	>64
20	F, F, F	5	16	32	32	4
21	NH_2	4	>64	>64	>64	>64
22		13	32	64	>64	>64
23		15	32	>64	64	8
24	OH	11	>64	>64	>64	>64
25	O	14	>64	>64	>64	>64
26	N	11	>64	>64	>64	>64

Sa, *Staphylococcus aureus* ATCC 25923; *Ec*, *Escherichia coli* ATCC 25922; *Ec imp*$^+$, *E. coli* LMG194 (wild type); *Ec imp*$^-$, *E. coli* LMG194 (isogenic permeable mutant).
[a] MIC (minimum inhibitory concentration) μg/mL.[5]

lowered when tested on the *imp*$^-$ mutant as compared to the isogenic *imp*$^+$ strain (Table 2). These results suggest that the poor antibacterial activity of this series was caused by poor cell penetration or efflux.

To improve the antibacterial activity of this series, subsequent libraries were made focusing on the modification of the R^2 phenyl ring. Initially, sample libraries of about 30 compounds were synthesized with substitutions at the *ortho* (R^3), *meta* (R^4) or *para* (R^5) positions of the phenyl ring that is directly linked to the triazine core (Table 3). The synthesis of these compounds was accomplished by first coupling the aniline linker with R^5 followed by the same triazine chemistry shown in Scheme 1.[7] SAR of these library compounds revealed that *ortho* substitutions universally correlated with weak antibacterial activity, while substitutions at the *meta* and *para* positions often resulted in compounds with substantially improved MIC values. Table 3 illustrates that a *para* substitution (**27**) is more potent than an identical *ortho* substitution (**28**). Similar antibacterial differences can be seen between compounds **18** and **19** (Table 2). In contrast, substituents in the *meta* or *para* position often offer little discernable differences in potencies as seen between compounds **29** and **30** (Table 3).

The SAR in Table 3 suggested that longer tailpieces, which contained two aromatic rings, could improve antibacterial activity on a standard test strain (e.g., **27**, **29**, and **30**). To further test this SAR, the role of the phenyl 'linker' was examined. Using a similar synthetic route,[7] Table 4 shows a comparison of the antibacterial activity between compounds with and without an aromatic linker (X). In all cases the inclusion of an aromatic linker (compounds **31**, **33**, **35**, and **37**) results in improved antibacterial activity. These results are consistent with the prior SAR suggesting the phenyl linker in the tailpiece plays an important role in bacterial cell penetration.

Based on the established SAR small focused libraries were synthesized around the DAPT series containing a phenyl linker. In most cases substituents were attached in the *para* position. Table 5 shows representative compounds from focused libraries where the phenyl linker substituents (R^5) consisted of aromatic heterocycles (**38**), sulfonamides (**39**), α-ketoamides (**40**), β-ketoamides (**41**–**45**), and amides (**46**, **47**). Again, these compounds were prepared using a similar synthetic approach described above.[7] As shown in Table 5 the expansion of the tailpiece resulted in a set of compounds that exhibited favorable antimicrobial activity. The MICs against *E. coli* were typically in the range of 1–4 μg/mL, while activity against *Pseudomonas aeruginosa* was generally more potent (MICs frequently <1 μg/mL). In general, activity against *S. aureus* and other Gram-positive bacteria was less potent, with exceptions of compounds **38** and **46**, suggesting that there is room for further optimization. The IC_{50} values for in vitro translation inhibition remained in a similar single digit μM range, and these compounds generally exhibited low toxicity towards human cells.

In summary, the DAPT series described here originated from a structure-guided approach to discover novel aminoglycoside mimetics that bind rRNA and act as bacterial protein synthesis inhibitors. This series evolved through an iterative process of high throughput library synthesis and testing, in part described here, which discovered promising leads. To date over 1000 compounds have been synthesized and utility has been shown in a mouse septicemia model.[5] An important feature of this series is the potent antimicrobial activity against a number of Gram-negative

Table 3. Antibacterial activity and protein synthesis inhibitory activity

DAPT–NH–(Linker: benzene ring with R^3, R^4, R^5)

Compound	$R^3_{(ortho)}$	$R^4_{(meta)}$	$R^5_{(para)}$	IC_{50} (μM)	*Escherichia coli*[a]
27	H	OCH_3	HN, O, OH, Cl	8	4
28	HN, O, OH, Cl	OCH_3	H	30	>64
29	H	H	O, NH, HO, OH	5	8
30	H	O, NH, HO, OH	H	4	8

[a] MIC (μg/mL) of strain ATCC 25922.

Table 4. Antibacterial activity and protein synthesis inhibitory activity

DAPT–X–Y

Compound	X[a]	Y	IC_{50} (μM)	*Escherichia coli*[b]
14	NH		7	>64
31	NH		9	16
32	NH	Cl, Cl	7	32
33	NH, O, NH, OH	Cl, Cl	10	2
34	NH	H, N, N	14	64
35	NH, O, NH, OH	H, N, N	4	8
36	—	N, Br	5	16
37	HN, OH, O	N, Br	9	2

[a] Left sides of the molecules are bonded to DAPT via the designated N atom and the right sides are bonded to Y.
[b] MIC (μg/mL) of strain ATCC 25922.

pathogens with especially striking activity against *P. aeruginosa*, a pathogen for which there is a clear unmet clinical need to develop new antibiotics.[2] Further attractions of this novel class include activity against aminoglycoside-resistant bacteria and the relative ease in which the series can be synthesized.

Table 5. Antibacterial activity, protein synthesis inhibitory activity, and eukaryotic cytotoxicity[a]

Compound	R^4	R^5	IC_{50} (μM)	MIC (μg/mL)			CC_{50} (μM)[a]
				Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853	
38	H		10	2	2	ND	33
39	OH		4	4	16	16	68
40	OH		4	1	16	⩽0.5	92
41	H		6	4	16	⩽1	24
42	H		4	4	16	⩽1	71
43	H		9	4	16	1	179
44	H		6	4	8	1	146
45	H		5	2	16	⩽1	124
46	H		10	1	2	1	140
47	OH		7	2	16	⩽0.5	180
Gent			0.03	<0.5	<0.5	0.5	>300
Cm			1.5	4	8	>64	ND

ND, not determined.

[a] Eukaryotic cytotoxiccity was determined in a proliferation assay with CEM T-cells after 72 h.[5]

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