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# Antibacterial activity in serum of the 3,5-diamino-piperidine translation inhibitors

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**Abstract**—Translation inhibitors of the 3,5-diamino-piperidine series act as aminoglycoside mimetics that inhibit bacterial growth. Here we show antibacterial SAR in the presence and absence of serum with a particular focus toward *Pseudomonas aeruginosa*.
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The increasing prevalence of antibiotic resistance represents a global medical threat. In the United States about two million patients are infected from hospital visits.[1] The majority of these nosocomial pathogens are resistant to at least one antibiotic and cause over 90,000 deaths per year. *Pseudomonas aeruginosa* has been identified as a particularly problematic pathogen.[2] It is an invasive, Gram-negative bacteria that causes a wide range of severe infections including; a leading cause of hospital acquired pneumonias, bloodstream and surgical wound infections and infections of immunocompromised patients. In cystic fibrosis patients *P. aeruginosa* incites inflammation that destroys lung tissue and leads to respiratory failure. Compounding the problem of causing life-threatening infections, *P. aeruginosa* has a strong propensity to develop resistance to virtually any antibiotic. For example, clinical isolates of *P. aeruginosa* are frequently resistant to commonly used antibiotics including fluoroquinolones (33%), imipenem (22%), and ceftazidime (30%).[2] Some isolates are resistant to all FDA clinical approved antibiotics. For these reasons, there is an urgent clinical need to develop new antibiotics that work by novel mechanisms to treat *P. aeruginosa*.

To address this important clinical need, we used a rational approach to develop new antibiotics for the treatment of severe and drug resistant bacterial infections. This approach was based on three-dimensional structural information of aminoglycosides interactions with the ribosomal decoding site, or A-site, to develop mimetics that are amenable to rapid optimization by parallel synthesis.[3] Structural studies showed that 2-deoxystreptamine (2-DOS), the common core scaffold among aminoglycoside antibiotics, binds in a conserved configuration regardless of the 4,5- or 4,6-disubstitutions found in the neomycin or gentamicin families, respectively.[4] The *cis*-1,3- amino groups of 2-DOS are involved in base recognition via conserved hydrogen bonds with A1493, G1494, and U1495 of the 16S rRNA. These interactions anchor the aminoglycoside scaffold within the A-site internal loop and displace residues A1492 and A1493 from the RNA interior. These two adenine residues act as a molecular switch that is involved in securing the fidelity of translation by interacting with the first two base pairs of the mRNA–tRNA codon–anticodon hybrid. Thus, aminoglycoside binding impairs the ribosome's ability to discriminate against near-cognate tRNA–mRNA pairings, which causes an accumulation of defective peptides and, ultimately, cell death.

While 2-DOS is recognized as a key pharmacophore for binding the rRNA target, its synthesis and modification is challenging due to the five contiguous stereogenic

*Keywords*: Aminoglycosides; Antibiotics; Translation inhibitors; 2-Deoxy-streptamine; Ribosome; Decoding site; 3,5-Diamino-piperidine.
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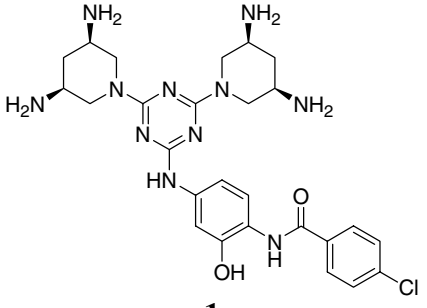
centers.[5] Therefore, we developed simplified synthetic mimetics of 2-DOS, one of which is *cis*-3,5-diaminopiperidine (DAP).[4b,6] The DAP ring retains the characteristic *cis*-1,3-diamine configuration of 2-DOS which is required for RNA recognition. Typically, two symmetric DAP moieties are attached to a triazine core (termed DAPT).[6] To optimize antibacterial activity, the so-called 'tailpiece' substituents were attached at the triazine 4-position via an *N*-linkage.[7] Prior studies described the synthesis and structure–activity relationships of the DAP series as inhibitors of *in vitro* translation and antibacterial potency.[3,6,7] Here, we describe the potent antibacterial activity against *P. aeruginosa* and the effect of serum on MIC (minimal inhibitory concentration).

Table 1 shows the antibacterial activity of *N*-{4-[4,6-bis-(3,5-diamino-piperidin-1-yl)-[1,3,5]triazin-2-ylamino]-2-hydroxy-phenyl}-4-chloro-benzamide (compound **1**)[3a] against 53 clinical isolates of *P. aeruginosa*. For comparison, the activity of seven diverse clinical antibiotics, most of which are used to treat *P. aeruginosa* infections, are shown as well. Compound **1** shows a reasonable distribution in activity with the highest MIC value as good as or better than any other antibiotic. Fluoroquinolones, such as ciprofloxacin, can show potent activity toward *P. aeruginosa*, but as discussed above, resistance is common and consequently the $MIC_{90}$ value of ciprofloxacin is poor. In contrast, compound **1** shows robust and broadly consistent activity against a panel of clinical isolates and has the most potent $MIC_{80}$ and $MIC_{90}$ values among the eight antibiotics tested. These results demonstrate that the DAP series exhibits exceptional anti-*P. aeruginosa* activity that justifies further exploration.

**Table 1.** Antibacterial activity against 53 clinical *P. aeruginosa* isolates



**1**

| Antibiotic | MIC[a] (μg/ml) | | | | |
|---|---|---|---|---|---|
| | Distribution | Mean | $MIC_{50}$ | $MIC_{80}$ | $MIC_{90}$ |
| Compound **1** | 1 32 | 4 | 4 | 4 | 8 |
| Amikacin | 1 128 | 6 | 4 | 8 | 32 |
| Tetracycline | 1 >128 | 27 | 32 | 64 | 64 |
| Chloramphenicol | 64 >128 | >128 | >128 | >128 | >128 |
| Ciprofloxacin | 0.06 128 | 1 | 0.25 | 32 | 64 |
| Imipenem[b] | 0.5 32 | 3 | 2 | 16 | 16 |
| Piperacillin[c] | 1 128 | 12 | 8 | 32 | 64 |
| Ceftazidime | 0.5 32 | 4 | 2 | 8 | 16 |

Susceptibility test was done by agar dilution using Mueller–Hinton (MH) agar by Fujisawa Pharmaceuticals Co., Ltd.

[a] Minimum inhibitory concentration. Subscript designation of 50, 80 or 90 refers to percent of isolates inhibited at a given concentration.

[b] Imipenem/cilastatin sodium.

[c] Piperacillin/tazobactam.

Although many DAP compounds show potent antibacterial activity, their potencies are significantly reduced by serum. Figure 1 plots the fold shift in MIC values for a set of 45 compounds incubated in the presence or absence of 50% serum against *Escherichia coli* and *P. aeruginosa*. These results show that serum dramatically increased MIC values. Moreover, serum had a differential effect between organisms; *E. coli* has approximately a 10-fold mean MIC shift, while *P. aeruginosa* has a 50-fold mean shift with serum. The reason for the species difference is not clear, though it may reflect different properties in outer membrane composition. Similarly, the aminoglycosides tobramycin and gentamicin exhibited a more pronounce serum shift against *P. aeruginosa* (4- to 8-fold, respectively) compared to *E. coli* (1- to 2-fold). Although antibiotic interactions with serum proteins are usually attributed to albumin binding,[8] this protein appears to have a minimal effect on the DAP series as incubations with 8% albumin typically resulted in modest MIC changes. Interestingly, some compounds actually exhibited a synergistic effect that lowered *E. coli* MIC values with serum. Such results were reproducible and titratable. While serum protein binding can have positive effects on antibiotic pharmacokinetics,[8] we found that the compounds with high serum MIC shifts correlated with no or very poor efficacy in mice. In contrast, a DAP compound that was not inhibited or shifted by serum fully protected mice from an *E. coli* septicemia infection.[3] For these reasons, we sought to understand serum SAR to improve activity.

Table 2 shows that simple addition of a nitrogen atom in aromatic rings of the tailpiece substituent could significantly reduce serum effect on MIC for two bacterial species (*E. coli* and *P. aeruginosa*). This modest structural change resulted in a 32-fold increase in serum MIC potencies (compare compounds **2** and **4**). The position of the nitrogen atom in the aromatic ring had little impact on antibacterial activity (compare **3** and **4**). Similar SAR were found in other cases (compare **7** and **8** or **9**,

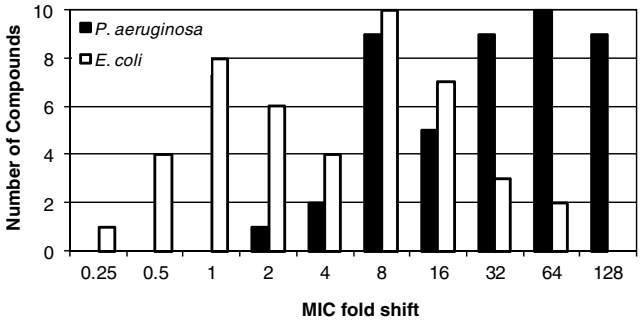
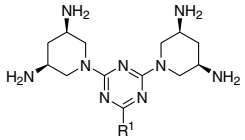
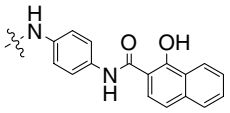
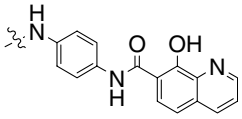
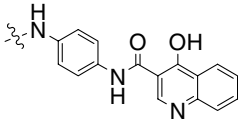
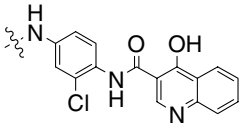
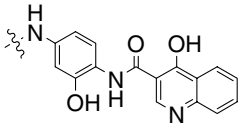
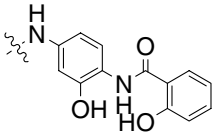
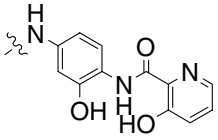
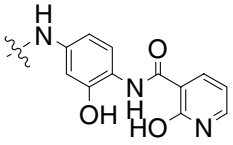
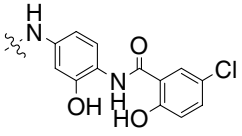
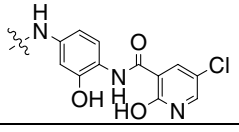



**Figure 1.** The effect of 50% mouse serum on a set of 45 DAP compounds. MIC (μg/ml) values were determined by microdilution method in MH media according to National Committee for Clinical Laboratory Standards. Strains: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

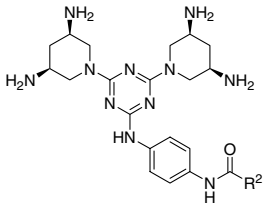
**Table 2.** DAPT serum SAR with nitrogen heterocycle tailpieces

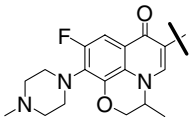
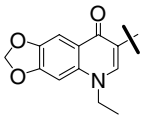
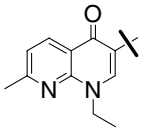
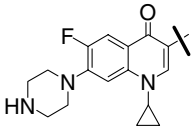
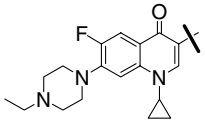
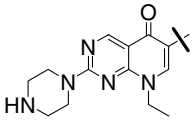
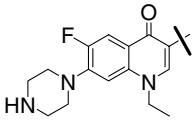


| Compound | $R^1$ | IVT $IC_{50}$[a] (μM) | *E. coli* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | | | MIC[b] | Serum fold shift | MIC | Serum fold shift |
| **2** |  | 24 | 1 | 32 | ⩽0.5 | ⩾128 |
| **3** |  | NA[c] | 4 | 2 | 2 | 16 |
| **4** |  | 5 | 4 | 1 | 1 | 8 |
| **5** |  | 24 | 4 | 1 | 1 | 8 |
| **6** |  | 5 | 1 | 4 | 1 | 8 |
| **7** |  | 11 | 4 | 4 | 2 | 32 |
| **8** |  | 10 | 8 | 2 | 4 | 8 |
| **9** |  | 4 | 8 | 1 | ⩽0.5 | ⩾32 |
| **10** |  | 9 | 4 | 8 | 1 | ⩾64 |
| **11** |  | 3 | 4 | 2 | ⩽0.5 | ⩾32 |

[a] Coupled *in vitro* transcription-translation assay with *E. coli* extracts.[3]
[b] MICs (μg/ml) as described in Figure 1.
[c] NA, not available.

**Table 3.** Quinolone tailpieces and serum shift



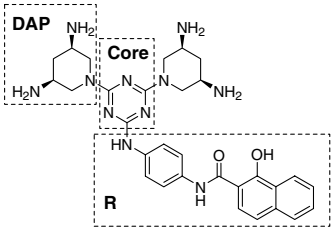
| Compound | $R^2$ | IVT $IC_{50}$ (μM) | *E. coli* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | | | MIC | Serum fold shift | MIC | Serum fold shift |
| **12** |  | 1 | 16 | 2 | 8 | 2 |
| **13** |  | 3 | 1 | 4 | 1 | 16 |
| **14**[a] |  | 2 | 4 | 4 | 2 | 8 |
| **15** |  | 0.4 | 64 | 0.06 | 2 | 2 |
| **16** |  | 1.3 | ⩽0.5 | ⩾4 | 2 | 8 |
| **17** |  | 0.9 | 32 | 0.13 | 1 | 4 |
| **18** |  | 2 | ⩽0.5 | 1 | 1 | 4 |

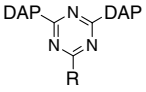
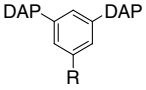
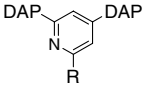
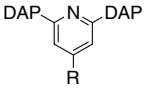
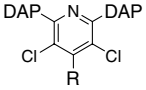
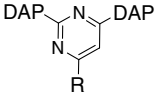
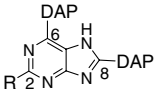
[a] The corresponding nalidixic acid molecule has an IVT $IC_{50} > 100$.

**10** and **11**), prompting us to synthesize a small focused library that contained nitrogen heterocycles as tailpieces (Table 3). Since at least two aromatic rings in the tailpiece imparted an optimal configuration for activity,[7] we chose to make a focused library using quinolones as tailpieces. Table 3 indeed shows that nitrogen containing heterocycles from quinolones result in potent DAPT compounds that had modest serum MIC shifts. In some cases the *E. coli* MIC values were dramatically reduced by serum (compounds **15** and **17**), suggesting synergistic antibacterial effects with serum, which is consistent with known antibacterial activity in serum.[8] These results show that nitrogen containing heterocycles as tailpieces lead to potent *in vitro* translation inhibitors that have no or modest shifts in antibacterial activity in the presence of serum.

Aside from tailpiece SAR, we also explored substitution of the triazine core. As shown in Table 4, when the DAP and tailpiece moieties were kept constant, a variety of core substitutions including benzene, pyridine, pyrimidine and purine rings had a minimal effect on translation

**Table 4.** Core SAR




| Compound | Product | IVT $IC_{50}$ (μM) | *E. coli* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | | | MIC | Serum fold shift | MIC | Serum fold shift |
| **19** |  | 24 | 1 | 32 | ⩽0.5 | ⩾128 |
| **20** |  | 12 | 2 | ⩾32 | 2 | ⩾32 |
| **21** |  | 16 | 2 | 32 | ⩽0.5 | ⩾128 |
| **22** |  | 26 | 2 | 16 | 1 | 64 |
| **23** |  | 14 | 2 | 32 | 1 | ⩾64 |
| **24** |  | 18 | ⩽0.5 | ⩾16 | 8 | ⩾8 |
| **25**[a] |  | 18 | 1 | 8 | 2 | 32 |

[a] A mixture of 2 regio-isomers (other: 2,6-di-DAP-8-*R*-purine) as intermediates of 6,8-di-DAP-2-Cl-purine and 2,6-di-DAP-8-Cl-purine were not isolated. Synthesis follows either method A or B as described in supplementary material.

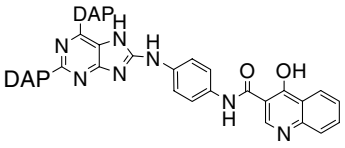
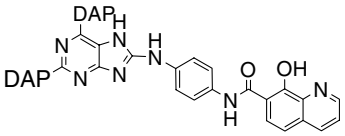
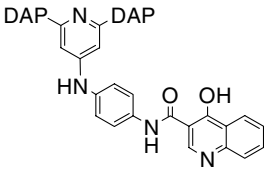
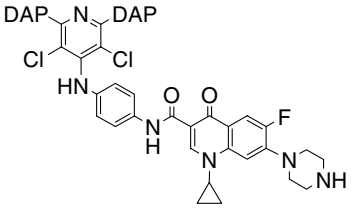
inhibition, suggesting that the aromatic core acts as a scaffold for the DAP functional groups to bind at the A-site RNA.[3,6] With respect to MIC in the presence of serum, a purine core (compound **25**) resulted in a moderate improvement as compared with **19**. The replacement of the triazine core with a pyridine core (compounds **22** and **23**) may also have a modest effect on the MIC shift with serum.

To explore possible synergistic or additive effects between core and tailpiece substitutions, a selected number of compounds were synthesized. As shown in Table 5, the combination of a triazine core replacement with a purine or pyridine core with nitrogen-containing heterocycle tailpieces resulted in potent translation inhibitors with improved serum MICs. Of particular note, addition of a nitrogen atom in the tailpiece aromatic ring of a pyridine compound **28** (Table 5) resulted in a significant improvement in serum MIC as compared to compound **22** (Table 4).

*Summary and perspective:* This work expands on our efforts toward rationally designed 2-DOS mimetics.[3,9] The active DAP pharmacophore was derived from structural information of the molecular recognition between the conserved 2-DOS scaffold of aminoglycosides and the A-site target RNA.[3,6] After the initial design of DAPT, the series then evolved through an iterative process of parallel synthesis and high-throughput testing that led to promising lead compounds. Rapid medicinal chemistry elaboration can now be used to potentially generate a clinical candidate that may improve on the pharmaceutical properties of aminoglycosides, including persisting resistance, poor bioavailability and undesirable toxicity.

This letter focused on antibacterial optimization in the presence of serum of our novel DAP translation inhibitors. Improvements in potency correlated to compound structure were achieved with nitrogen containing heterocycle tailpieces. Substitutions of the

**Table 5.** Purine and pyridine core optimization

| Compound | Product | IVT $IC_{50}$ (μM) | *E. coli* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | | | MIC | Serum fold shift | MIC | Serum fold shift |
| **26**[a] |  | NA | 1 | 8 | 1 | 16 |
| **27**[a] |  | 0.4 | 8 | 2 | 16 | 4 |
| **28** |  | 2.2 | ⩾64 | ⩽0.06 | ⩽0.5 | ⩾4 |
| **29** |  | 0.8 | 8 | 2 | 8 | 4 |

[a] Compounds **26** and **27** were synthesized with a different method than **25** in Table 4. Synthesis follows either method A or B as described in supplementary material.

triazine core with a purine or pyridine ring also showed clear SAR. A promising feature of the DAP derivatives[10] is their potent antimicrobial activity against clinical *P. aeruginosa* isolates (Table 1). This pathogen is notorious for developing antibiotic resistance.[2] Our description of the novel DAP series that exhibits exceptional $MIC_{90}$ value against a panel of clinically resistant *P. aeruginosa* is significant. However, enthusiasm for the series was initially diminished by the loss of antibacterial activity in the presence of serum. Our current results show that this obstacle can be addressed and that certain compounds have acceptable antibacterial activity with serum. We believe that these and other findings warrant further development of the DAP series as antibiotics.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.023.

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