Simple Aminols as Aminoglycoside Surrogates

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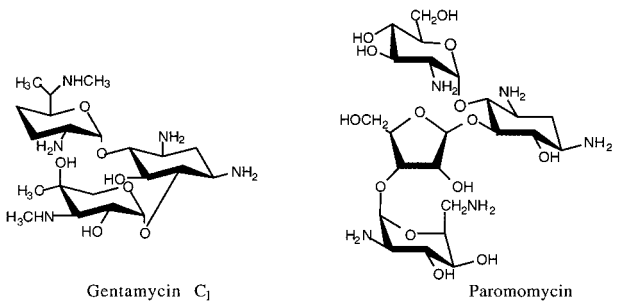
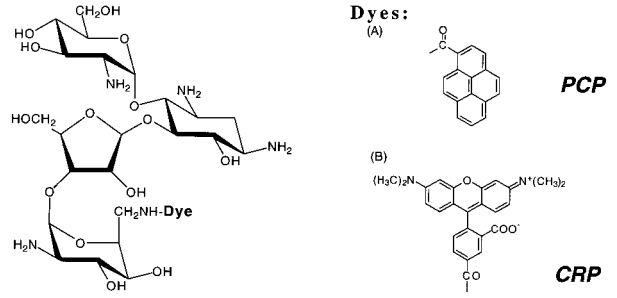
Aminoglycoside antibiotics are antibacterial drugs which function by specifically binding to prokaryotic 16S rRNA causing mistranslation and premature termination of mRNA translation.[1] The target binding site for aminoglycosides is the A-site decoding region of prokaryotic 16S rRNA.[2] Aminoglycosides are low affinity antagonists with dissociation constant (K_D) values in the low micromolar range, and the toxicities encountered in the use of these drugs are substantial.[3] In addition, bacterial resistance readily develops to aminoglycosides.[3] Aminoglycosides are also exceedingly difficult to synthesize, and consequently, analogues which avoid many of the shortcomings of the natural products have not been forthcoming. It is of substantial interest to prepare aminoglycoside surrogates which avoid the deficiencies of the natural products, both for use as antibacterial agents and as platforms in the de novo design of general RNA antagonists. In the current studies we have used a high throughput fluorescence assay method to discover simple 1,3-(2)-aminol-containing molecules which act as potent, competitive inhibitors of aminoglycoside binding to the bacterial A-site decoding region. These studies pave the way for the design and synthesis of new classes of synthetically approachable antibiotics.

Miniature constructs of the A-site decoding region molecule have been prepared which behave in biochemically predictable ways, suggesting that local structure is preserved.[4−6] For example, aminoglycosides, such as paromomycin and gentamycin C_1 (Scheme 1), bind to these constructs in the 1−2 μM range.[6] This is the range that is expected from in vivo studies.[1,2] Previously, we had shown that aminoglycoside binding to RNA constructs could be rapidly and accurately measured using either fluorescence quenching[7] or fluorescence anisotropy measurements.[6,8] Fluorescence quenching methods are readily adapted to 96-well plate assays as preliminary screens for RNA ligand interactions. In the current studies, approximately 60 compounds were selected from the Aldrich catalog and incubated in a 96-plate well format with an A-site decoding region construct[5] and (PCP) (Scheme 2), a pyrene-labeled paromomycin analogue whose fluorescence is quenched through specific interactions with the RNA[9] (Figure 1A). The quenching by A-site RNA is fit to a 1:1 binding isotherm[7] and provides a $K_D = 0.21 \pm 0.02$ μM for PCP. The quenching is specifically reversed by competition with paromomycin, yielding a $K_D = 1.87 \pm 0.14$ μM for this aminoglycoside (Figure 1B). PCP is analogous to the pyrene-labeled tobramycin analogue previously used to study aminoglycoside interactions

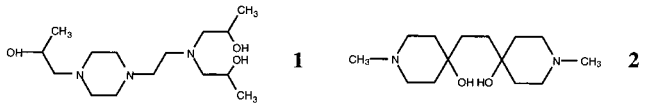
Scheme 1. Structures of Aminoglycosides Gentamycin C_1 and Paromomycin



Scheme 2. Fluorescent Paromomycin Analogues Used in Binding Assays



Scheme 3. Structures of Bioactive Aminols



with tobramycin-binding RNA constructs.[7] Of the approximately 60 compounds tested at 100 μM, only the two molecules **1** and **2** shown in Scheme 3 produced a fluorescent signal, thus showing that they were able to bind to the complex as paromomycin did. To obtain quantitative information on the binding of these two analogues to the A-site construct, full binding isotherms (Figure 2, parts A and B) were generated by the methods previously described using carboxy-tetramethylrhodamine-labeled paromomycin (CRP) to monitor anisotropy changes.[6] The measured dissociation constant for the binding of CRP to the constructs is 0.21 ± 0.01 μM. The competitive binding studies with molecules **1** and **2** yielded K_D values of 1.01 ± 0.03 μM for **1** and 2.95 ± 0.04 μM for **2**. The binding is also saturable and fits a calculated curve based on a binding stoichiometry of 1:1.[6] The measured K_D values are quite in the range of dissociation constants measured for aminoglycoside binding to the decoding region A-site. For example, paromomycin binds with a $K_D = 1.87$ μM, so that aminol **1** is almost 2-fold more potent than this antibiotic. It also should be noted that compound **1** contains three asymmetric centers. If the interactions between **1** and the A-site are stereospecific, then the potency of the active diastereomer would be substantially higher than quoted.

The studies reported above show that very simple 1,3- and 1,2-aminols can bind to the A-site decoding region as potently as the more complicated aminoglycosides. The binding of these aminols to the A-site construct is specific because of the high affinity measured and the fact that the binding is saturable and stoichiometric. Further evidence for specificity comes from structure−activity studies. The approximately 60 compounds studied as A-site antagonists included the three aminols shown in Scheme 4. When assayed at concentrations of 100 μM these analogues were inert as antagonists of PCP binding to the A-site construct.

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(1) Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. J. *The molecular basis of antibiotic action*, 2nd ed.; John Wiley & Sons: London, Great Britain, 1981; Chapter 6, pp 419−439.

(2) Woodcook, J.; Moazed, D.; Cannon, M.; Davies, J.; Noller, H. F. *EMBO J.* **1991**, *10*, 3099−3103.

(3) Chambers, H. F.; Sande, M. A. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th ed.; Hardman, J. G.; Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Goodman Gilman, A., Eds.; McGraw-Hill: New York, 1996; Chapter 46, pp 1103−1121.

(4) Purohit, P.; Stern, S. *Nature* **1994**, *370*, 659−662.

(5) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367−1371.

(6) Wang, Y.; Hamasaki, K.; & Rando, R. R. *Biochemistry* **1997**, *36*, 768−779.

(7) Wang, Y.; Rando, R. R. *Chem. Biol.* **1995**, *2*, 281−290.

(8) Hamasaki, K.; Killian, J.; Cho, J.; Rando, R. R. *Biochemistry* **1997**, *37*, 6656−663.

(9) Hamasaki, K.; Rando, R. R. *Anal. Biochem.*, in press.

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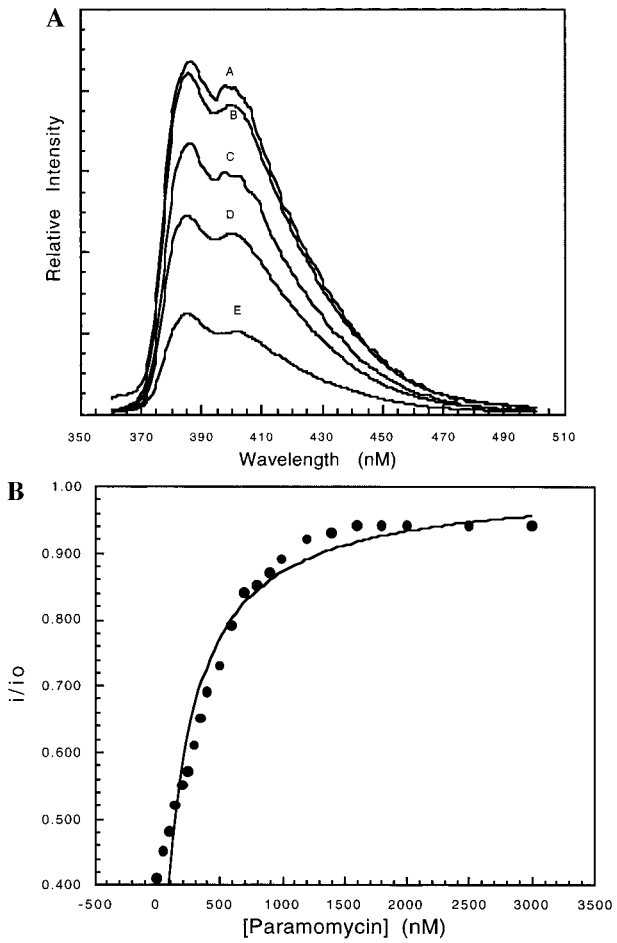


Figure 1. (A) Fluorescence emmision spectra of a PCP solution in the presence of varying concentrations of the A-site 16S rRNA construct. [A-site 16S rRNA]: A, 0 nM; B, 50 nM; C, 200 nM; D, 400 nM; E, 600 nM. (B). Relative fluorescence intensity of PCP (10 nM) solution containing 1 μM of the A-site 16S rRNA construct as a function of paramomycin concentrations. Curve fitting[6] (solid line) gives the dissociation constant of paramomycin-16S rRNA interaction as 1.87 ± 0.14 μM. i/io refers to the ratio of the fluorescence intensity with the addition of PCP to without the addition of PCP.

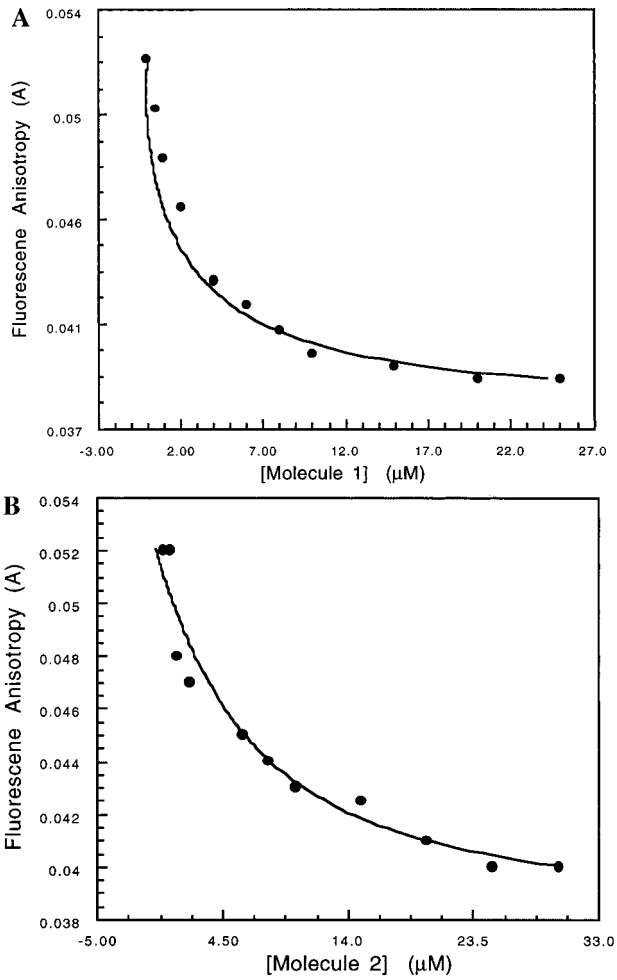
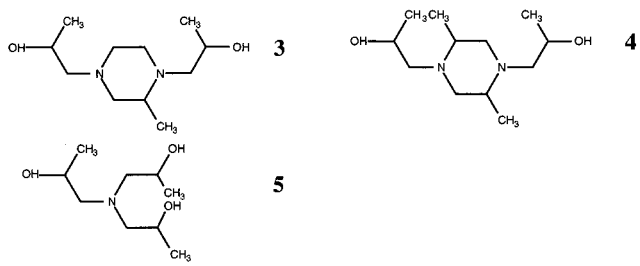


Figure 2. (A) Fluorescence anisotropy of a CRP (10 nM) solution containing the A-site 16S rRNA construct (500 nM) as a function of **1** concentrations. Curve fitting[6] (solid line) gives the dissociation constant as 1.01 ± 0.03 μM. (B) Fluorescence anisotropy of a CRP (10 nM) solution containing the A-site 16S rRNA construct (500 nM) as a function of **2** concentrations. Curve fitting (solid line) gives the dissociation constant as 2.95 ± 0.04 μM.

Thus, not only are non-aminol-containing molecules, which included polyamines, unable to interfere with PCP binding, but there is considerable specificity of binding with respect to the aminols themselves.

The finding that simplified aminols, discovered through the use of a novel high throughput screen, are potent antagonists of the A-site is significant for several reasons. First, this finding suggests that the 1,3-(2)-aminol moiety represents the essential pharmacophore of aminoglycosides with respect to interactions with RNA molecules. This idea is supported by a number of observations relevant to aminoglycoside function, including studies on the development of resistance to aminoglycosides,[10] NMR spectroscopic investigations of aminoglycoside RNA binding,[5] and studies on the general binding of aminols to RNA molecules.[11] Second, the simple structures of the aminol inhibitors described here should facilitate meaningful structure−activity studies for RNA-antagonist design and the discovery of new classes of synthetically accessible drug molecules able to specifically interfere with RNA function. Finally, the simple structures of the aminols suggests that these molecules may have been, or are, regulators of RNA function. The RNA-binding tetracycline antibiotics contain 1,2-aminol moieties. It is also interesting to note that hypusination, a posttranslational modification which allows interaction between the modified protein and RNA, involves a 1,3-(2)-aminolation reaction.[12]

Scheme 4. Structures of Inactive Aminols



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Supporting Information Available: Experimental details (1 page, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(10) Shaw, K. J.; Rather, P. N.; Hare, R. S.; Miller, G. H. *Microbiol. Rev.* **1993**, *57*, 138−163.

(11) Hendrix, M.; Alper, P. B.; Priestley, E. S.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 95−98.

(12) Liu Y. P.; Nemeroff, M.; Yan, Y. P.; Chen, K. Y. *Biol. Signals* **1997**, *6*, 166−174.