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Design and Synthesis of Novel Antibacterial Agents with Inhibitory Activity against DNA Polymerase III

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Abstract—4-Substituted 2-amino-6-(anilino)pyrimidines have been found to be selective inhibitors of DNA polymerase III, a replicative enzyme known to be essential in the DNA synthesis of Gram-positive bacteria. Among the analogues, **18** displayed an IC_{50} of 10 μM against DNA polymerase III from *Staphylococcus aureus*.

The last decade of the 20th century has seen an alarming rise in the incidence of multi-antibiotic resistant strains of pathogenic Gram-positive (Gr+) bacteria, including vancomycin resistant *Staphylococcus aureus*.[1,2] Curtailing this rise will depend largely on the development of chemotherapeutic agents capable of selectively attacking novel bacterial targets. One such target that has received considerable attention over recent years is DNA polymerase III (Pol III), an enzyme known to be essential in the replicative DNA synthesis of Gr+ bacteria.[3,4] We have recently synthesized and characterized a series of 4-substituted 2-amino-6-(anilino)pyrimidine based inhibitors of Pol III with the goal of identifying novel, potent and selective antibacterial agents. In this Letter, we detail our initial efforts in this area.

The potential utility of Pol III inhibitors as antibacterial agents was first described by Langley in 1962.[5] The prototype inhibitors [e.g., 6-(phenylhydrazino)uracils **1**,[6] 6-(benzylamino)uracils **2**,[7] and 6-anilinouracils **3**[8]] were shown to inhibit Pol III of *Bacillus subtilis* by promoting the formation of a catalytically inactive ternary complex with DNA and the enzyme. The essential features of the mechanism of complex formation have been worked out by Cozzarelli[9] and by Wright[10] and involve: (1) hydrogen bonding of the uracil and its 6-NH moiety with cytosine in the DNA template; and (2) binding of the phenyl group with a hydrophobic site on the enzyme. These early prototype inhibitors were unattractive candidates for further development since they were insoluble, simple uracil derivatives, many of which contained objectionable functionality such as the hydrazino group. In an attempt to overcome some of these limitations, we decided to replace the 6-anilinouracil moiety with a 4-substituted 2-amino-6-(anilino)pyrimidine subunit, a hitherto unexplored area. We postulated that introduction of a lipophilic 4-substituent such as a substituted phenoxy group would aid solubility whilst maintaining a degree of activity towards the Pol III target. Our design strategy for selecting the 2-amino-6-(anilino) pyrimidine platform was based on the pioneering work of Wright and co-workers. In his studies, Wright[8] has shown that 6-anilinouracil derivatives such as **3** hydrogen bond with cytosine residues in the DNA template and that their action is competitive with 2′-deoxyguanosine-5′-triphosphate (dGTP) (Fig. 1, panel A). We reasoned that 4-substituted 2-amino-6-(anilino) pyrimidines (e.g., **4**) should also retain the capacity to form hydrogen bonds, albeit with thymine bases, and would therefore be competitive with 2′-deoxyadenosine-5′-triphosphate (dATP) (Fig. 1, panel B).

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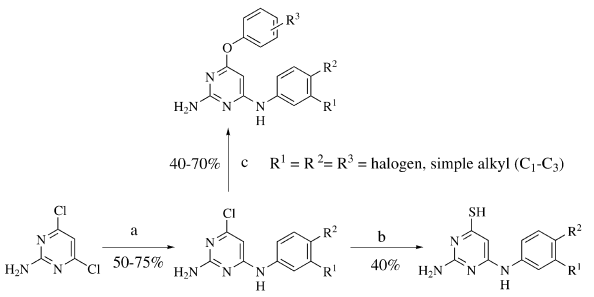
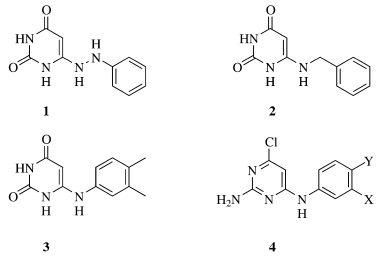


Scheme 1. (a) 3,4-Disubstituted anilines, EtOH, 60 °C; (b) NaSH, $(CH_2OH)_2$, 100 °C; (c) substituted phenols, Cs_2CO_3, DMF, 120 °C.

Our initial studies focused on determining whether the 2-amino-6-(anilino)pyrimidine platform would be viable for inhibitor design. Although we were confident that the 2-amino-6-(anilino) pyrimidine platform would impart inhibitory activity against Pol III according to our working model depicted in B, the extent of this activity was unclear. Thus, a series of 2-amino-4-chloro-6-(anilino)pyrimidines was initially prepared by reaction of 2-amino-4,6-dichloropyrimidine with the appropriately substituted aniline, according to Scheme 1 (path a).[11] The resulting 2-amino-4-chloro-6-(anilino)pyrimidine analogues were shown to be weakly active Pol III inhibitors with IC_{50} values in the 50–120 μM range.[12] A number of these analogues also displayed encouraging levels of antibacterial activity against several species of relevant Gr+ bacteria (Table 1) and were uniformly inactive against an outer-membrane permeable strain of *Escherichia coli*, a Gram-negative Pol III deficient organism.[13]

The ability of these compounds to selectively inhibit the DNA synthesis of Gr+ bacteria was also investigated by whole-cell, dose–response labeling.[14] Vancomycin, rifampicin and ciprofloxacin, which selectively inhibit bacterial cell wall, RNA synthesis and DNA synthesis, respectively, were run as control compounds. The results are summarized in Figure 2.



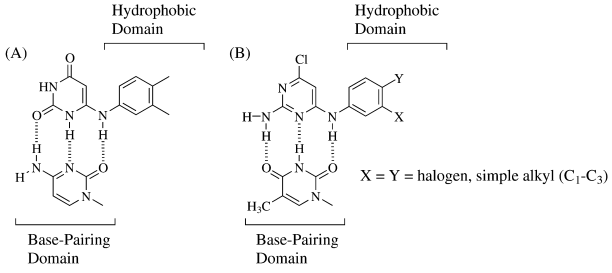
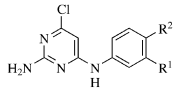


Figure 1. (A) Hydrogen bonding of a 6-aminouracil with cytosine; (B) hydrogen bonding of a 2-amino-6-(anilino)pyrimidine with thymine.

Each compound was tested versus five different labeled precursors to investigate the effect on DNA synthesis (6-^{3}H thymidine), RNA synthesis (5,6-^{3}H uracil), protein synthesis (4,5-^{3}H leucine), phospholipid synthesis (2-^{3}H glycerol) and cell wall synthesis (2,3-^{3}H alanine). As shown in Figure 2, panel D, the 2-amino-4-chloro-6-(3′-ethyl-4′-methyl)anilinopyrimidine derivative **11** selectively inhibits DNA synthesis of *S. aureus* without affecting any of the other macromolecules investigated. Panels E and F confirm these findings for 2-amino-4-chloro-6-(3′-bromo-4′-methyl)anilinopyrimidine **6** and 2-amino-4-chloro-6-(3′,4′-dimethyl)anilinopyrimidine **9**, respectively.

Variation of substituents in the 4-position of the 2-amino-6-(anilino)pyrimidines (paths b and c, Scheme 1) gave mixed results.[15] Data for inhibition of Pol III by a series of 2-amino-4-substituted-6-(anilino)pyrimidines are presented in Table 2.

Table 1. In vitro anti-Pol III and antibacterial activity of 2-amino-4-chloro-6-(anilino)pyrimidines



Compd	R^1	R^2	IC_{50} (μM)	MIC (μg/mL)[a]			
				B. subtilis ATCC 6633	*E. faecium* RLAI[d]	*S. aureus* MB2985[e]	*S. pneumonia* CL5350[e]
5	I	CH_3	51	5	36	>36[b]	18
6	Br	CH_3	94	8	31	31	31
7	Cl	CH_3	126	13	27	27	27
8	Cl	Cl	98	7	29	29	>29[c]
9	CH_3	CH_3	115	6	>25[b]	>25[b]	>25[c]
10	3′,4′-$(CH_2)_3$–		119	3	>26[b]	>26[b]	>26[c]
11	CH_2CH_3	CH_3	53	3	13	>26[b]	26
12	H	^{t}Bu	89	7	14	7	14

[a]Minimum inhibitory concentration (mg/mL).
[b]Growth unaffected at the highest level tested.
[c]Growth affected but incomplete inhibition at the highest level tested.
[d]RLAI (ampicillin and imipenem-resistant) was obtained from Dr. Barbara Murray.
[e]MB2985 (the Staph Smith strain) and CL5350 are from Merck collections.

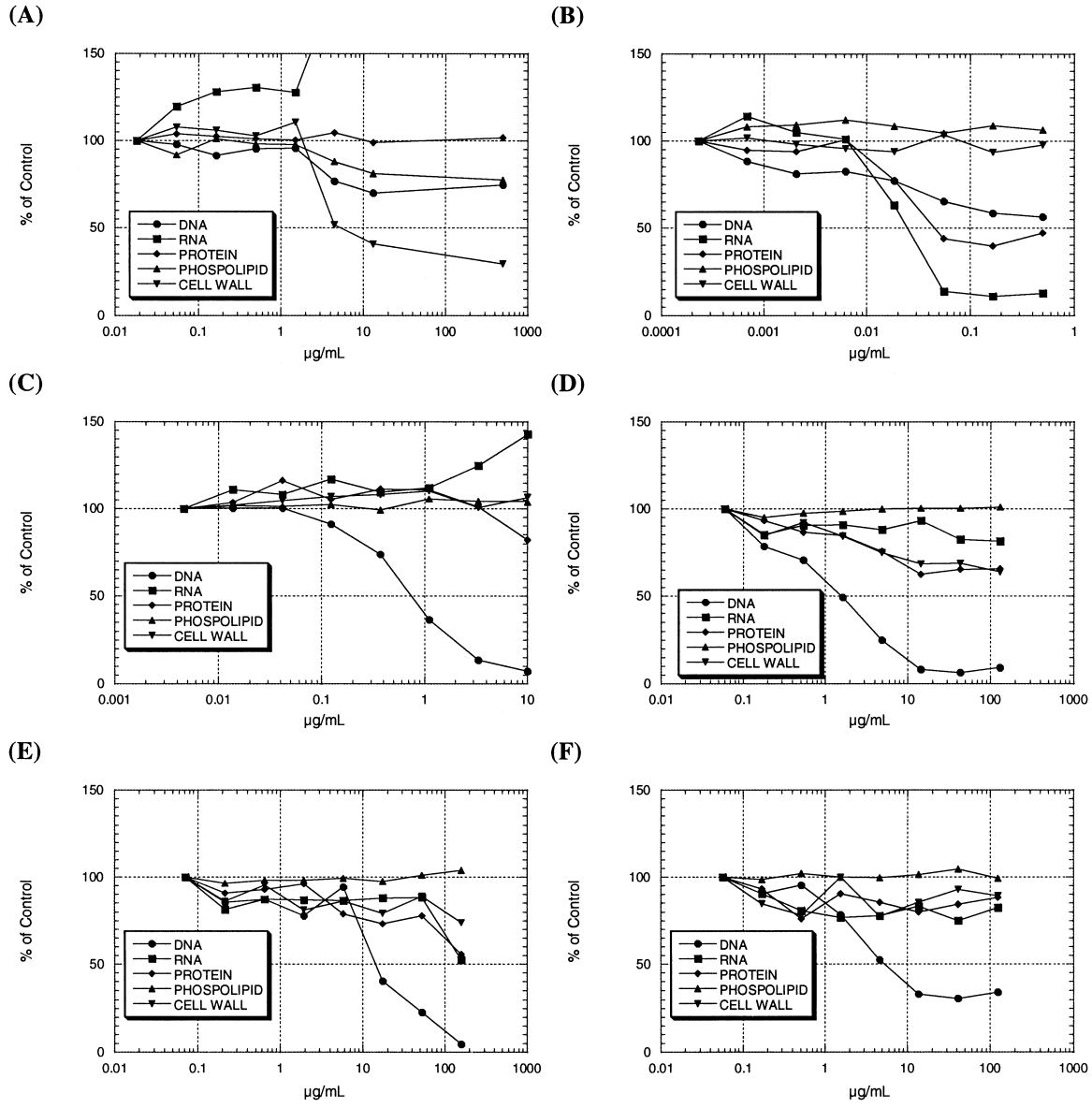
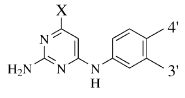


Figure 2. Mode of action study by whole-cell dose–response labeling. (A) vancomycin; (B) rifampicin; (C) ciprofloxacin; (D) 2-amino-4-chloro-6-(3′-ethyl-4′methyl)anilinopyrimidine **11**; (E) 2-amino-4-chloro-6-(3′-bromo-4′-methyl)anilinopyrimidine **6**; (F) 2-amino-4-chloro-6-(3′,4′-dimethyl)anilinopyrimidine **9**.

The tautomeric 4-thio derivative **13**[16] was found to be 2-fold less active than the prototype 4-chloro analogue **5**. Etherification of the 4-thio compounds decreased activity in general (e.g., **14** and **15**), as did replacement of the 4-chloro moiety by electron-releasing 4-methyl and 4-*N*,*N*-dimethylamino groups; all these derivatives were essentially inactive. The substituted 4-phenoxy analogues (path c, Scheme 1) gave interesting results. In general, the phenoxy derivatives were weaker inhibitors than the parent 4-chloro compounds. However, 3- or 4-substituted phenoxy analogues (e.g., **18** and **21**) often exhibited improved activity. We found that small hydrophobic groups in the 3- and 4-positions of the phenoxy ring gave compounds equivalent to or better than the parent 4-chloro inhibitors. Indeed, the *p*-bromo analogue **18** was 5-fold more active than the prototype 4-chloro compound. An IC_{50} determination of **18** produced a value of 10 µM, which represented our

Table 2. In vitro anti-Pol II activity of 2-amino-4-substituted-6-(anilino)pyrimidines



Compd	X	3′	4′	% Inhibition[a]
13	SH	I	CH_3	54
14	SCH_3	I	CH_3	20
15	SC_6H_5	I	CH_3	2
16	CH_3	I	CH_3	21
17	$N(CH_3)_2$	CH_3	CH_3	27
18	4-Br-C_6H_4O	C_2H_5	CH_3	97 (10 µM)[b]
19	4-Cl-C_6H_4O	C_2H_5	CH_3	13
20	4-F-C_6H_4O	C_2H_5	CH_3	33
21	3-CH_3-C_6H_4O	C_2H_5	CH_3	88 (54 µM)[b]

[a]% Inhibition against Pol III of *S. aureus* at 500 µM concentration of inhibitor.
[b]IC_{50} value.

best Pol III inhibitor. Interestingly, close analogues of **18** (e.g., **19** and **20**) were surprisingly poor inhibitors of Pol III. With respect to antibacterial activity, the 4-phenoxy derivatives generally demonstrated disappointing levels of potency against our panel of gram-positive organisms (MICs were in the $\sim$20 to $>$40 μg/mL range). The reasons behind this apparent discrepancy between affinity for the Pol III target in vitro and growth of its host bacterial cell in vivo are not known, but could be due to permeability barriers or to differences between the strain used for whole-cell work versus that used for cloning the enzyme. Such discrepancies between in vitro versus whole-cell activity have been noted by other researchers for different Pol III inhibitors.[3b]

In summary, we have discovered a novel series of Pol III inhibitors based on the 2-amino-6-(anilino)pyrimidine platform. We have shown that small hydrophobic substituents on the 3- and 4-positions of the anilino group combined with 4-halosubstituted phenoxy substituents (e.g., **18**) lead to increases in the anti-Pol III activities of these Gram-positive selective inhibitors.

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11. All new compounds showed satisfactory NMR spectroscopic and mass spectrometry data.
12. The enzyme inhibition assay was performed at 30 °C for 20 min in 0.1 mL reaction mix (5% final DMSO concentration of inhibitor solutions) containing 30 mM Tris HCl, pH 7.5, 20% glycerol, 4 mM dithiothreitol, 10 mM magnesium acetate, 0.003 mM dATP, 0.003 mM dGTP, 0.001 mM dCTP, 0.001 mM ^{3}H-dTTP, and 0.35 mg/mL activated calf thymus DNA purchased from Worthington Enzymes. *S. aureus* DNA polymerase was cloned from a conveniently available source of chromosomal DNA, strain R27.
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14. Compounds which were active in the MIC assay were tested for the mode of action (MOA) by whole-cell dose–response labeling. All labeled precursors were purchased either from Amersham or New England Nuclear.
15. For SARs in a related series see: Trantolo, D. J.; Wright, G. E.; Brown, N. C. *J. Med. Chem*. **1986**, *29*, 676.
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