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Active site directed inhibitors of replication-specific bacterial DNA polymerases

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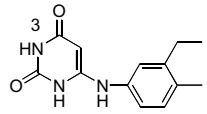
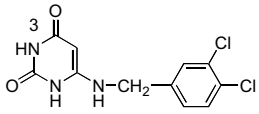
Abstract—7-Substituted-N^2-(3,4-dichlorobenzyl)guanines potently and competitively inhibit DNA polymerases IIIC and IIIE from Gram+ bacteria. Certain derivatives are also competitive inhibitors of DNA polymerase IIIE from Gram− bacteria.

The replicative DNA polymerases of eubacteria—the pol IIIs—have evolved from a common ancestor into two distinct types with respect to primary structure—a 'C' type encoded by the gene *pol*C, and an 'E' type, encoded by *dna*E.[1] Significantly, *dna*E-like genes are ubiquitous, present in all classes of eubacteria, while *pol*C is confined to a single group—the low G:C Gram-positives (Gram+).[2] In those organisms in which *dna*E is the *sole* pol III gene, that is in Gram-negatives (Gram−) and high G:C Gram-positives, pol IIIE is the *sole* replication-specific polymerase.[3] In its narrow, low G:C Gram+ domain, pol IIIC is well established as a replication-specific enzyme.[3] However, it is now clear from recent work in *B. subtilis*,[4,5] *E. faecalis*[6] and *S. aureus*[1] that the low G:C Gram+ pol IIIE is also an essential replication protein.

The replication of chromosomal DNA is required for eubacterial cell division, and inhibition of this process is rapidly bactericidal.[7,8] We have expanded our study of potential antibiotics based on pol IIIC inhibition to include assays of recombinant pol IIIE from the Gram+ *B. subtilis* and, in addition, a commercial preparation of pol IIIE from the Gram− *E. coli*. As reported below, we have discovered a class of compounds that potently and non-selectively inhibits *both* Gram+ pol IIIC and pol IIIE, and a subset of that class that also inhibits Gram− pol IIIE.

6-Anilinouracils and 6-benzylaminouracils are well established as active-site directed inhibitors of pol IIIC.[9] The active compounds have two molecular 'domains' required for inhibition: a 'base-pairing domain' responsible for hydrogen bonding of the molecule with cytosine bases in the DNA template, and an 'aryl domain' responsible for selectivity and affinity to the enzyme. The phenyl ring substitution patterns in the 6-anilinouracils, for example, 'EMAU' (**1**), and the 6-benzylaminouracils, for example, 'DCBAU' (**2**) have been identified as optimal for binding of the respective families to pol IIIC. Base pairing with cytosines explains the competitive inhibition by these compounds with respect to dGTP, and inhibition is caused by the formation of an inactive inhibitor/DNA/enzyme complex.[9] Indeed,


EMAU, **1**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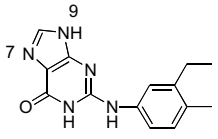
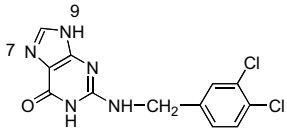

EMPG, **3**


DCBG, **4**

Keywords: DNA polymerase; Replication; Competitive inhibitors; Antibacterial; Broad spectrum.
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Table 1. Inhibition of pol III species by substituted 6-aminouracils and guanines

No.	Compd/substituent	K_i (μM)[a]		
		B.s. pol IIIC	B.s. pol IIIE	E.c. pol IIIE
1	EMAU	0.31	>315[b]	>323
2	DCBAU	0.42	1.5	>280
EMPGs:				
3	EMPG	2.7	>248	In[c]
3a	9-$(CH_2)_4OH$	In	In	In
3b	7-$(CH_2)_4OH$	0.47	In	In
3c	7-$(CH_2)_5OH$	0.28	318	In
DCBGs:				
4	DCBG	0.72	0.32	95
4a	7-$(CH_2)_4OH$	0.19	0.063	86
4b	9-$(CH_2)_4OH$	712	1.6	In
4c	7-$(CH_2)_2OEt$	0.33	0.072	56
4d	9-$(CH_2)_2OEt$	116	19.4	>209
4e	7-$(CH_2)_3CO_2Et$	0.15	0.047	16.9
4f	9-$(CH_2)_3CO_2Et$	825	64	>189
4g	7-$(CH_2)_3CO_2H$	0.17	0.13	15.3
4h	9-$(CH_2)_3CO_2H$	28	0.24	101
4i	7-$(CH_2)_4OAc$	0.066	0.12	33
4j	7-$(CH_2)_4$-N[$(CH_2)_4$]O	0.051	0.047	26
4k	7-$(CH_2)_5OAc$	0.052	0.058	13.8
4l	7-$(CH_2)_4CH(CH_3)OH$ (R)	0.052	0.035	41
4m	7-$(CH_2)_4CH(CH_3)OAc$ (R)	0.08	0.031	20.3
4n	7-$(CH_2)_5O(CO)iPr$	0.25	0.071	27.2
4o	7-$(CH_2)_5OH$	0.052	0.091	27
4p	7-$(CH_2)_2O(CH_2)_2OH$	0.37	0.11	45
4q	7-$(CH_2)_5OMe$	0.07	0.088	47
4r	7-$(CH_2)_5SMe$	0.23	0.18	128
4s	7-$(CH_2)_5SOMe$	0.093	0.068	36
4t	7-$(CH_2)_5SO_2Me$	0.28	0.09	51

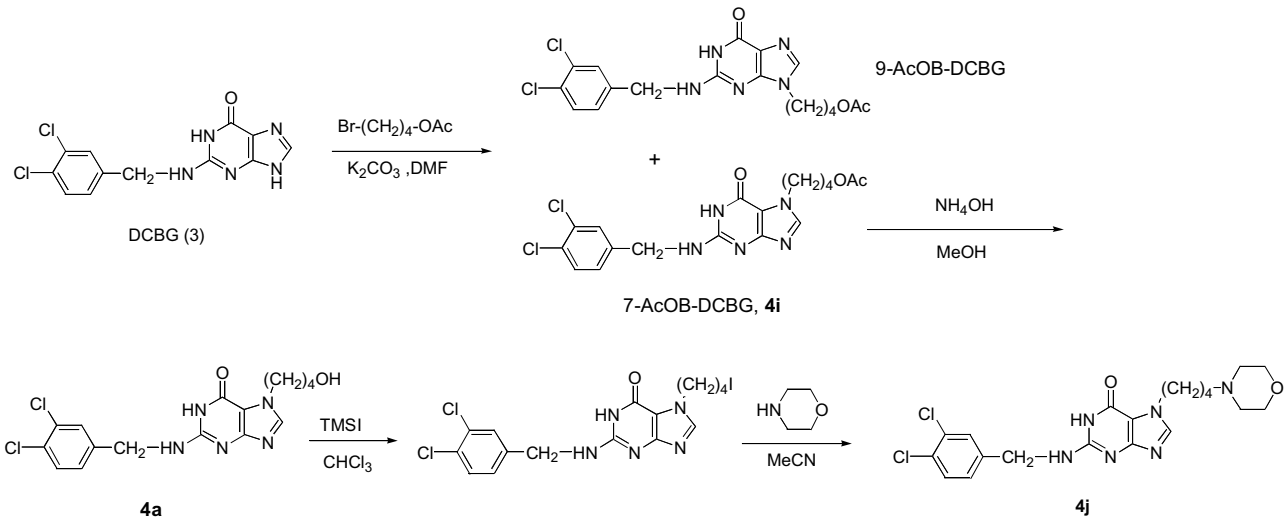
[a] Assayed under truncated (-dGTP) conditions.[12] Results are averages of duplicate assays (SD ≈30%).
[b] Lower limit of estimated K_i.
[c] <10% inhibition at highest concn. tested.

analogous N^2-phenylguanines such as 'EMPG' (**3**) and the N^2-benzylguanines such as 'DCBG' (**4**) appear to inhibit pol IIIC via the same mechanism.[10,11]

Screening of an extensive library of related compounds has shown that 6-anilinouracils such as **1** are potent and selective inhibitors of pol IIIC, while certain 6-benzylaminouracils inhibit *both B. subtilis* pol IIIC and pol IIIE (Table 1).[12] Among an extensive series of 6-(substituted)amino-uracils, halo-substituted 6-benzylaminoracils were the only derivatives that inhibited pol IIIC and pol IIIE, and, among these, compounds bearing the 3,4-dichloro substitution pattern of **2** were the most potent (Table 1).

In the guanine inhibitor series, **3** was a moderately potent but selective inhibitor of *B. subtilis* pol IIIC (Table 1), while N^2-*benzyl* guanines showed significant activity against *both B. subtilis* pol IIIs. Among these compounds, the 3,4-dichlorobenzyl derivative **4** emerged as the most potent inhibitor of the enzymes, with K_i values of <1 μM (Table 1). The results of further screening of other N^2-phenyl, benzyl and variously substituted guanines showed that only halo-substituted N^2-benzylguanines had pol III inhibitory activity. These included 3-CF_3–4-Cl, 3-Cl–4-CF_3, 4-Cl, 4-CF_3, and 3,4-OCH_2O benzyl compounds, but their K_i values were >10 μM for pol IIIC and 25–100 μM for pol IIIE (data not shown).

The results of studies with 3-substituted-6-anilinouracils[7] prompted us to prepare N-substituted inhibitor analogs in the guanine series. The general synthetic strategy for these derivatives is illustrated in Scheme 1. DCBG (or EMPG, by analogous chemistry) is alkylated to give 9 and 7 isomers, in ratios of ≈2–3:1.[13] Although favoring the 9 isomers, this direct alkylation increases the overall yield of 7 isomers compared with classical alkylation of the 6-chloropurines via the sodium salt method.[14,15] Modification of the N-substituents provided additional derivatives, as illustrated in Scheme 1. Among numerous N^2-phenylguanines and N^2-benzylguanines, the 7-isomers, not the expected 9-isomers, were the most active pol III inhibitors. 7-(4-Hydroxybutyl)-EMPG (**3b**) potently and selectively inhibited *B. subtilis* pol IIIC, but, surprisingly, the 9-isomer **3a** was inactive (Table 1). Among N-alkylated derivatives of **4**, the 7-substituted compounds, for example, 7-(4-hydroxybutyl)-DCBG (**4a**), were considerably more active than the 9-substituted compounds, for example, **4b**, against both *B. subtilis* pol IIIC and pol IIIE. The observation that **4a** inhibited *both* Gram+ pol III species prompted synthesis and assay of additional DCBG derivatives (Table 1). With few exceptions,



Scheme 1.

7 isomers are more active than the corresponding 9 isomers. A notable exception is 9-carboxypropyl-DCBG (**4h**), which is 100-fold more active against Gram+ pol IIIE than pol IIIC. Active EMPG derivatives are highly selective for inhibition of pol IIIC, whereas DCBG derivatives are potent but non-selective inhibitors of Gram+ pol IIIC and pol IIIE. Certain 7-substituted DCBGs, for example, **4e**, **4i**, **4j**, **4m**, **4o**, inhibited *E. coli* pol IIIE, a hitherto resistant bacterial pol III, although the best compounds in this limited collection display K_i values some 100-fold greater than the analogous Gram+ pol III inhibitors.

Inhibition of pol IIIC by uracil and simple guanine inhibitors is strictly competitive with dGTP.[8–10] That this is true for the 7-substituted EMPGs was demonstrated by variable substrate assays with 7-(5-hydroxypentyl)-EMPG (**3c**). This compound showed competitive kinetics with respect to dGTP (Fig. 1, panel A), and the calculated K_i 0.28 μM, is essentially the same as that derived directly from the 'truncated' assay, 0.32 μM (Table 1). We next asked if inhibition of pol IIIC by a 7-substituted DCBG compound was also competitive with dGTP, and if inhibition of the pol IIIEs was also competitive. Assays using 7-(5-hydroxypentyl)-DCBG (**4o**) and varying dGTP concentrations gave the results displayed in panels B–D of Figure 1. In each case, the double reciprocal plot suggested competitive inhibition. K_i values for *B. subtilis* pol IIIC (0.091 μM) and pol IIIE (0.21 μM) and for *E. coli* pol IIIE (21 μM) were essentially identical with those from the direct assay of **4o** (Table 1). We thus confirmed the active site-directed mechanism of the DCBG compounds against the Gram+ pol IIIs, and demonstrate in this paper the first reported active site-directed inhibitors of a Gram− pol IIIE.

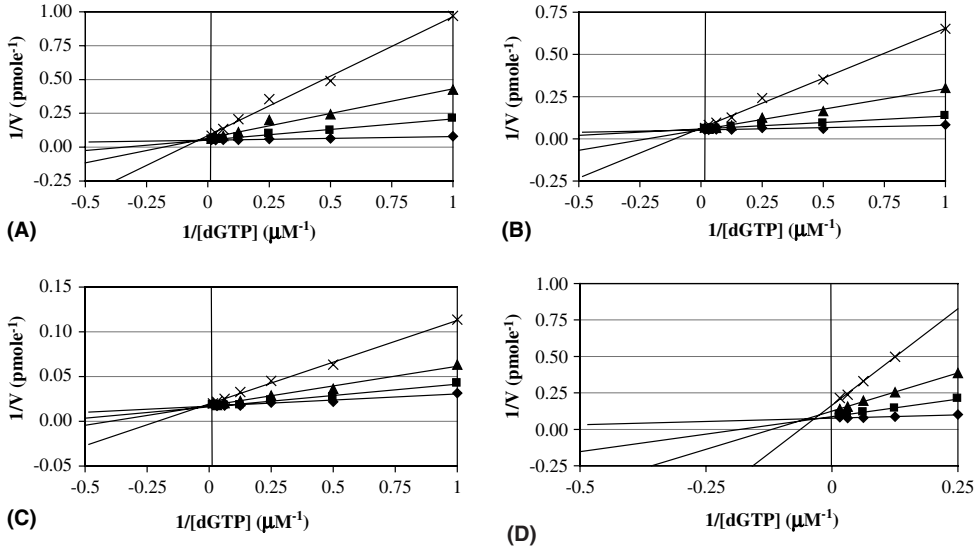


Figure 1. Double reciprocal (Lineweaver–Burk) plots demonstrating effect of varying dGTP concentration on inhibition of pol III species.[12] Panel A, B.s. pol IIIC + **3c**. Panel B, B.s. pol IIIC + **4o**. Panel C, B.s. pol IIIE + **4o**. Panel D, E.c. pol IIIE + **4o**. Inhibitor concentrations: (■) $3 \times K_i$; (▲) $10 \times K_i$; (×) $30 \times K_i$; (♦) no inhibitor.

At first glance it is unusual that the 9-substituted guanines are weak or inactive pol III inhibitors, given that the polymerase substrate dGTP has the deoxyribonucleotidyl group at N9. However, substituents in the 7 position of the guanines are nearly isosteric with those in the 3 position of the uracil-based inhibitors. (Compare the structures of **1** and **2** with those of **3** and **4**.) The 3 substituents in the uracil compounds enhance pol III inhibitory activity, and, in certain cases, impart potent antibacterial activity.[16] The results of screening the guanines for antibacterial activity in vitro will be presented elsewhere.

Acknowledgements

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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.2004.11.016.

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13. All new compounds were fully characterized by ^{1}H NMR spectra, and elemental (CHN) or HRMS analyses. A typical synthesis follows: A mixture of DCBG (**4**) and 1.25 equiv K_2CO_3 was stirred in DMF for 15 min. 4-Bromobutyl acetate (1.1 equiv) was added, and the mixture was stirred at 45 °C for 18 h. The mixture was poured into water, extracted with $CHCl_3$, and the residue from the organic layer purified on a silica gel column. Elution with 2% MeOH in $CHCl_3$ gave the 7 isomer **4i** (25%), and elution with 4% MeOH in $CHCl_3$ gave the 9 isomer (62%). [Characteristic ^{1}H NMR patterns distinguished between 7 and 9 isomers (Kjellberg, J.; Johansson, N. *Tetrahedron* **1986**, *42*, 6541).] A solution of **4i** in MeOH was stirred with concd. NH_4OH at rt for 6 h, the solution concentrated, and the residue purified on a silica gel column. Elution with 15% MeOH in $CHCl_3$ gave **4a** (90%). A solution of **4a** and a 5-fold excess of TMSI in $CHCl_3$ was heated at reflux for 6 h. After coming to rt, the solution was treated with MeOH and Na_2SO_3, stirred for 0.5 h, and concentrated. Water was added and the suspension was filtered, giving the 4-iodobutyl intermediate (93%). A solution of the 4-iodobutyl intermediate and excess morpholine in MeCN was stirred at rt for 18 h. Concentration and crystallization of the residue from MeOH gave **4j** (71%). Yields and properties of all new compounds are in Supporting Information.
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