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Registry No. 1, 87781-93-9; 2, 83173-14-2; 3, 87781-96-2; 4, 87781-94-0; 5, 87781-95-1; 6, 87781-97-3; 7, 87781-98-4; 8, 87781-99-5; 9, 87782-00-1; 10, 87782-08-9; 11, 87782-09-0; 12, 87782-01-2; 15, 41623-91-0; 16, 87782-02-3; 17, 15373-27-0; 18, 87782-03-4; 18·2NH_3, 87782-04-5; 19, 87782-05-6; 19·4NH_3, 87782-06-7; 20·3NH_3, 87782-07-8; *p*-butylaniline, 104-13-2; 1-chloro-3,5-di-*p*-toluoyl-2-dioxyribofuranose, 3601-89-6; tetra-*O*-acetylribofuranose, 28708-32-9; DNA polymerase, 9012-90-2.

Quantitative Structure–Activity Relationships of 6-Anilinouracils as Inhibitors of *Bacillus subtilis* DNA Polymerase III

George E. Wright* and Joseph J. Gambino

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.
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Quantitative structure–activity relationships (QSAR) of a series of 6-anilinouracil derivatives were developed for their inhibitory activity against the wild-type DNA polymerase III (pol III) and a mutant enzyme, pol III/*azp-12*, derived from *Bacillus subtilis*. Interaction between inhibitors and both enzymes appears to result solely from hydrophobic binding. Comparison of the substituent contributions indicates increased hydrophobic character and a minor change of shape of the inhibitor binding site of the mutant enzyme. Because the two enzymes have identical K_m values for substrates, the inhibitor binding site is thought to be distinct from the enzyme active site.

6-(Phenylhydrazino)-, 6-(benzylamino)-, and 6-anilinouracils have been shown to be potent inhibitors of *B. subtilis* DNA polymerase III (pol III).[1-3] All three series of analogues inhibit pol III by the immobilization of the enzyme because of an interaction between the phenyl group of the inhibitors and a hydrophobic binding site on the enzyme thought to be physically near the active site. The qualitative data show several trends for substituents. Compounds with hydrophobic groups in both the meta and para positions of the phenyl ring are most active, and those with only meta substituents are generally more active than those with the same substituent in the para position. Compounds with large alkyl groups in the para position or with polar substituents tend to be the least active inhibitors.

A preliminary attempt to correlate substituent hydrophobic (π) constants with activity showed a rough linear relationship for meta-substituted anilinouracils but no direct correlation for para-substituted compounds.[3] A more detailed derivation of quantitative structure–activity relationships for these inhibitors was sought for two purposes. The first was to facilitate the synthesis of more potent inhibitors as potential antibacterial agents by indicating the type and position of substituent needed for strong interaction with the enzyme binding site. The second purpose was to determine the nature of and difference between the inhibitor binding sites of the wild-type enzyme (WT) and of a mutant, "inhibitor-resistant" enzyme, pol III/*azp-12*.[3,4] This mutant enzyme was obtained from *B. subtilis* colonies selected for resistance to 6-[(*p*-hydroxyphenyl)hydrazino]uracil and 6-[(*p*-hydroxyphenyl)hydrazino]isocytosine. Originally, this mutation was thought to be the result of a structural change in the inhibitor binding site, which repulsed polar groups in the phenyl ring, such as *p*-hydroxyl and *p*-amino, based on the observation that inhibitors with H and Me groups were equiactive against both wild-type and mutant polymerases.[1] Further study with other substituted inhibitors showed that the pattern of sensitivity of this enzyme was more complex. For example, in the 6-anilinouracil series, compounds with meta substituents had decreased inhibitory activity toward the mutant enzyme compared with the WT enzyme, while para-substituted compounds usually had increased inhibitory activity against the mutant enzyme.[3]

For the determination of QSAR's for these enzymes, 6-anilinouracil analogues presented several advantages over the 6-(phenylhydrazino)uracil[1] series: they show a wider range of inhibitory activity, they do not need reduction in the assay mixture as do the oxidizable 6-(phenylhydrazino)uracils, and they are expected to be conformationally less mobile than both the hydrazino and benzylamino analogues.

K_i, the concentration of 6-anilinouracil required to achieve 50% inhibition of enzyme activity, was used in the calculation of the QSARs because it has been shown to be identical with the concentration needed for half-maximal complex formation between DNA, inhibitor, and enzyme.[4] It is thus thought to represent a true binding constant for the inhibitor–enzyme interaction.

QSAR of Inhibitors of DNA Polymerase III. Equation 1 was derived for the compounds listed in Table I by using the substituent constants tabulated by Hansch[5] and multiple regression analysis. The equation was developed by the sequential addition and deletion of parameters thought to be important in binding based on the qualitative assessment of the experimental data. The *F* test was used to determine the significance of each change, and Table II shows the final sequence used.

$$\log (1/K_i) = 1.00\ (\pm 0.13)\ \pi_m + 0.71\ (\pm 0.12)\ \pi_p + 3.87\ (\pm 0.48)\ MR_m - 2.30\ (\pm 0.27)\ (MR_m)^2 + 2.86\ (\pm 0.54)\ MR_p - 2.18\ (\pm 0.26)\ (MR_p)^2 + 2.50\ (\pm 0.30) \quad (1)$$

$$n = 37;\ r = 0.942;\ F_{(6,30)} = 40.01\ (0.001)$$

The hydrophobic parameter π_m contributed the most to binding among all single parameters, but it accounted for only 18% of the variance in the data (eq 1a). The

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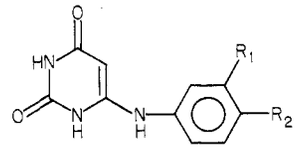
Table I. Experimental and Calculated Inhibition Constants of 6-Anilinouracils against *B. subtilis* DNA Polymerase III and Parameters Used in the Derivation of Equation 1



compd no.	R_1	R_2	log $(1/K_i)$ exptl	log $(1/K_i)$ calcd	Δ log $(1/K_i)$	substituent parameters[a] π_m	π_p	MR_m	$(MR_m)^2$	MR_p	$(MR_p)^2$
1	Et	Me	6.721	6.386	0.335	1.02	0.56	1.03	1.06	0.565	0.319
2	CH_2Cl	Me	6.585	5.992	0.593	0.64[b]	0.56	1.05	1.10	0.565	0.319
3	I	Me	6.420	5.878	0.542	1.12	0.56	1.39	1.93	0.565	0.319
4	$-(CH_2)_3-$		6.377	6.040	0.337	0.6[c]	0.6[c]	0.697[c]	0.486	0.697[c]	0.486
5	Cl	Me	6.201	6.024	0.177	0.71	0.56	0.603	0.364	0.565	0.319
6	Me	Me	6.036	5.831	0.205	0.56	0.56	0.565	0.319	0.565	0.319
7	Cl	Cl	6.032	6.142	−0.110	0.71	0.71	0.603	0.364	0.603	0.364
8	Me	Br	6.000	5.944	0.055	0.56	0.86	0.565	0.319	0.888	0.788
9	Et	H	5.538	5.339	0.199	1.02	0	1.03	1.06	0.103	0.011
10	I	H	5.328	4.816	0.512	1.12	0	1.394	1.943	0.103	0.011
11	CH_2Cl	H	5.119	4.944	0.175	0.64[b]	0	1.05	1.10	0.103	0.011
12	Br	H	4.987	5.255	−0.268	0.86	0	0.888	0.788	0.103	0.011
13	*n*-Pr	H	4.770	4.963	−0.193	1.55	0	1.496	2.238	0.103	0.011
14	H	Me	4.757	4.191	0.566	0	0.56	0.103	0.011	0.565	0.319
15	Me	NH_2	4.721	4.549	0.172	0.56	−1.23	0.565	0.319	0.542	0.294
16	Me	Et	4.721	5.872	−1.151	0.56	1.02	0.565	0.319	1.03	1.06
17	H	Cl	4.602	4.308	0.294	0	0.71	0.103	0.011	0.603	0.364
18	Cl	H	4.602	4.977	−0.375	0.71	0	0.603	0.364	0.103	0.011
19	H	Br	4.500	4.306	0.194	0	0.86	0.103	0.011	0.888	0.788
20	Me	H	4.369	4.783	−0.414	0.56	0	0.565	0.319	0.103	0.011
21	H	Et	4.182	4.233	−0.051	0	1.02	0.103	0.011	1.03	1.06
22	CH_2OH	Me	4.105	4.382	−0.277	−1.03	0.56	0.719	0.517	0.565	0.319
23	Me	*n*-Pr	4.009	5.013	−1.004	0.56	1.55	0.565	0.319	1.496	2.238
24	H	CF_3	3.996	4.384	−0.388	0	0.88	0.103	0.011	0.502	0.252
25	H	OMe	3.921	3.761	0.160	0	−0.02	0.103	0.011	0.787	0.619
26	NH_2	Me	3.914	4.009	−0.095	−1.23	0.56	0.542	0.294	0.565	0.319
27	H	*i*-Pr	3.466	3.359	0.107	0	1.53	0.103	0.011	1.496	2.238
28	CH_2OH	H	3.384	3.334	0.050	−1.03	0	0.719	0.517	0.103	0.011
29	H	*n*-Pr	3.345	3.374	−0.029	0	1.55	0.103	0.011	1.496	2.238
30	Me	NHCOMe	3.333	3.235	0.098	0.56	−0.97	0.565	0.319	1.493	2.229
31	NHCOMe	Me	3.333	3.499	−0.166	−0.97	0.56	1.493	2.229	0.565	0.319
32	H	NH_2	3.193	2.909	0.285	0	−1.23	0.103	0.011	0.542	0.294
33	Me	*n*-Bu	3.160	3.261	−0.101	0.56	2.13	0.565	0.319	1.959	3.838
34	*n*-Bu	H	3.158	3.655	−0.497	2.13	0	1.959	3.838	0.103	0.011
35	H	OH	2.752	3.036	−0.284	0	−0.67	0.103	0.011	0.285	0.081
36	H	CH_2OH	2.495	3.071	−0.576	0	−1.03	0.103	0.011	0.719	0.517
37	H	*n*-Bu	2.305	1.622	0.683	0	2.13	0.103	0.011	1.959	3.838
			standard deviation		0.410						

[a] Substituent parameters were obtained from ref 5. Published MR values were scaled by 0.1. [b] C. Hansch, personal communication. [c] π and MR for the $(CH_2)_3$ group were divided by 2 to give values for m and p positions.

addition of π_p was of minor significance, although inclusion of the term $(MR_p)^2$ showed a negative parabolic correlation between the MR (a measure of group size[6]) of substituents in the para position and binding (eq 1b). This is in accord with the experimental data in which increasing group size leads to a rapid loss of activity, e.g., in the 4-alkylphenyl derivatives **14**, **21**, **27**, **29**, and **37**. Inclusion of the term MR_p gave no significant improvement relative to eq 1b (data not shown).

Addition of the term π_p gave an equation (1c) with significant improvement over eq 1b. The magnitude of this term along with that of π_m indicates that the binding site will accommodate hydrophobic groups in both meta and para positions. Incorporation of the $(MR_m)^2$ term into eq 1c gave a marginally significant increase in the correlation coefficient (eq 1d). Further inclusion of MR_m gave a highly significant increase in eq 1e, accounting for 78% of the variance and indicating a larger capacity of the inhibitor binding site for meta than for para substituents.

When MR_p was added to eq 1e to give eq 1, a significant increase in the correlation coefficient was observed. Equation 1 accounted for 89% of the variance in the data. The positive MR_p term shows that the parabolic relationship indicated by $(MR_p)^2$ in eq 1b–e corresponds to a methyl group having optimum size in this position. The experimental data in Table I confirm this, the 4-methylphenyl derivative (**14**) being the most potent inhibitor in the series of para-substituted compounds.

Several other parameters were tried during the determination of eq 1. For example, σ_m, σ_p, R_m, and R_p, terms for electronic contributions to binding, had no significant effect on the equation (results not shown).

K_i values calculated from eq 1 (Table I) agree reasonably well with the experimental data, the standard deviation of calculated log $(1/K_i)$ being 0.410. Calculated values for several compounds differed widely from experimental values, e.g., **16** and **23**, but the ordering of inhibitor potencies calculated from eq 1 closely paralleled the experimental K_i's.

QSAR of Inhibitors of the Mutant Enzyme Pol III/*azp-12*. The pol III/*azp-12* enzyme showed a pattern

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Table II. Development of Equation 1

eq no.	π_m	π_p	MR_m	$(MR_m)^2$	MR_p	$(MR_p)^2$	constant	r	F	F of difference between eq
1a	0.76 (±0.27)[a]						4.23 (±0.21)	0.431	$F_{(1,35)}$ 8.03 (0.01)	
1b	0.69 (±0.25)					−0.48 (±0.17)	4.60 (±0.23)	0.581	$F_{(2,34)}$ 8.77 (0.001)	$F_{(1,34)}$ 7.93 (0.01)
1c	0.73 (±0.22)	0.80 (±0.24)				−0.88 (±0.19)	4.54 (±0.20)	0.714	$F_{(3,33)}$ 11.44 (0.001)	$F_{(1,33)}$ 11.41 (0.01)
1d	1.02 (±0.24)	0.87 (±0.22)		−0.49 (±0.21)		−1.03 (±0.19)	4.81 (±0.22)	0.762	$F_{(4,32)}$ 10.98 (0.001)	$F_{(1,32)}$ 5.21 (0.05)
1e	0.92 (±0.18)	0.80 (±0.16)	3.44 (±0.64)	−2.28 (±0.37)		−0.89 (±0.14)	3.75 (±0.26)	0.884	$F_{(5,31)}$ 21.87 (0.001)	$F_{(1,31)}$ 28.16 (0.001)
1	1.00 (±0.13)	0.71 (±0.12)	3.87 (±0.48)	−2.30 (±0.27)	2.86 (±0.54)	−2.18 (±0.26)	2.50 (±0.30)	0.942	$F_{(6,30)}$ 40.01 (0.001)	$F_{(1,30)}$ 29.75 (0.001)

[a] The number in parentheses following each coefficient is the standard deviation.

Table III. Experimental and Calculated Inhibition Constants of 6-Anilinouracils against *azp-12* DNA Polymerase III from Equation 2

compd no.	log $(1/K_i)$ exptl	log $(1/K_i)$ calcd	Δ log $(1/K_i)$
1	5.854	6.317	−0.463
2	6.824	5.822	1.002
3	6.553	5.894	0.659
4	5.710	6.011	−0.301
5	6.076	6.017	0.059
6	5.456	5.808	−0.352
7	6.481	6.210	0.271
8	6.071	6.136	−0.065
9	4.730	4.822	−0.092
10	4.690	4.387	0.303
11	5.260	4.327	0.933
12	4.660	4.718	−0.058
13	4.848	4.692	0.156
14	4.161	4.457	−0.296
15	3.801	3.766	0.035
16	5.377	6.153	−0.776
17	4.851	4.650	0.201
18	4.380	4.522	−0.142
19	5.398	4.785	0.613
20	3.750	4.313	−0.563
21	4.699	4.802	−0.103
22	3.699	3.861	−0.162
23	4.860	5.523	−0.663
24	4.947	4.762	0.185
25	3.921	3.848	0.073
26	3.526	3.537	−0.011
27	4.658	4.149	0.509
28	2.290	2.365	−0.075
29	4.278	4.172	0.096
30	2.810	2.686	0.124
31	2.726	3.020	−0.294
32	2.409	2.415	−0.006
33	3.879	3.917	−0.038
34	3.333	3.913	−0.580
35	2.420	2.669	−0.249
36	2.710	2.717	−0.007
37	2.660	2.566	0.094
		standard deviation	0.398

of response to 6-anilinouracils that was very different from that of the wild-type enzyme (cf. Table III). For example, compounds with large para substituents were more potent inhibitors of the mutant enzyme than of the wild-type enzyme. The opposite was generally true for meta-substituted compounds. In order to obtain some insight into the structural basis of the mutation, we developed the QSAR of the series of 6-anilinouracils against *azp-12* DNA polymerase III.

Equation 2 was developed for inhibition of the pol

$$\log (1/K_i) = 1.26\ (\pm 0.13)\ \pi_m + 1.13\ (\pm 0.12)\ \pi_p + 2.51\ (\pm 0.46)\ MR_m - 1.67\ (\pm 0.26)\ (MR_m)^2 + 3.48\ (\pm 0.52)\ MR_p - 2.42\ (\pm 0.25)\ (MR_p)^2 + 2.39\ (\pm 0.29) \quad (2)$$

$$n = 37;\ r = 0.948;\ F_{(6,30)} = 45.35\ (0.001)$$

III/*azp-12* enzyme by the compounds summarized in Table III. This equation accounts for 90% of the variance. Its sequential development is shown in Table IV.

As with the wild-type enzyme, the dominant single parameter was π_m, although the addition of π_p to produce eq 2b showed the greatest significance. Sequential addition of MR terms generated eq 2 (Table IV), containing the same parameters as eq 1, although the magnitudes of the coefficients are very different.

The most obvious difference between eq 1 and 2 is the increased contribution to binding with the mutant enzyme by both hydrophobic parameters π_m and π_p, with the term

Table IV. Development of Equation 2

eq no.	π_m	π_p	MR_m	$(MR_m)^2$	MR_p	$(MR_p)^2$	constant	r	F	F of difference between eq
2a	0.81 (±0.27)[a]						4.17 (±0.21)	0.448	$F_{(1,35)}$ 8.86 (0.01)	
2b	0.89 (±0.25)	0.62 (±0.21)					3.88 (±0.21)	0.600	$F_{(2,34)}$ 9.64 (0.001)	$F_{(1,34)}$ 8.52 (0.01)
2c	0.85 (±0.20)	1.19 (±0.22)				−0.75 (±0.18)	4.19 (±0.19)	0.761	$F_{(3,33)}$ 15.12 (0.001)	$F_{(1,33)}$ 17.00 (0.001)
2d	1.12 (±0.17)	1.13 (±0.17)			3.47 (±0.73)	−2.45 (±0.37)	2.93 (±0.30)	0.872	$F_{(4,32)}$ 25.38 (0.001)	$F_{(1,32)}$ 24.23 (0.001)
2e	1.31 (±0.17)	1.20 (±0.16)		−0.40 (±0.16)	3.01 (±0.71)	−2.31 (±0.35)	3.36 (±0.32)	0.895	$F_{(5,31)}$ 24.64 (0.001)	$F_{(1,31)}$ 5.96 (0.05)
2	1.26 (±0.13)	1.13 (±0.12)	2.51 (±0.46)	−1.67 (±0.26)	3.48 (±0.52)	−2.42 (±0.25)	2.39 (±0.29)	0.948	$F_{(6,30)}$ 45.35 (0.001)	$F_{(1,30)}$ 30.73 (0.001)

[a] The number in parentheses following each coefficient is the standard deviation.

Table V. Correlation between Parameters Used To Derive Equations 1 and 2

	π_m	π_p	MR_m	$(MR_m)^2$	MR_p	$(MR_p)^2$
π_m	1	0.191	0.268	0.275	0.040	0.011
π_p		1	0.021	0.020	0.377	0.389
MR_m			1	0.888	0.218	0.128
$(MR_m)^2$				1	0.187	0.108
MR_p					1	0.906
$(MR_p)^2$						1

π_p increasing by about 60% in eq 2. This indicates an increased capacity for hydrophobic binding by the inhibitor binding site of the pol III/*azp-12* enzyme relative to wild-type polymerase. The smaller increase in hydrophobic contribution in the meta position for the mutant enzyme is offset, however, by a decrease in contribution of MR_m of 54%. The change in the MR_p and $(MR_p)^2$ terms between eq 1 and 2 implies a slight increase in capacity for large groups to bind at the para position. Thus, the 4-bromophenyl derivative (**19**) is the most potent of the series of para-substituted compounds against the mutant enzyme.

The change in the inhibitor binding site of pol III/*azp-12* reflected in eq 2 is apparently twofold. First, the volume into which meta substituents fit has been reduced, even though it has become more hydrophobic, to the extent that a compound with a meta group has less activity against pol III/*azp-12* than the same compound has against the WT enzyme, e.g., compounds **9**, **10**, and **12**; however, compounds with large meta substituents do not differ in potency significantly (**13** and **32**). Second, the hydrophobic contribution of the para substituents to binding has increased, indicating that the corresponding region of the enzyme is more hydrophobic. The volume of the binding site for para substituents also appears to have increased in the mutant enzyme: large para groups provide greater activity against the mutant polymerase, e.g., in compounds **23**, **27**, **29**, and **34**.

Among the parameters used in eq 1 and 2, strong correlations were observed between MR and $(MR)^2$ terms, as is shown in Table V. The slight correlation between π and MR may be explained by the predominance of bulky substituents that are also hydrophobic.

Discussion

The QSAR's developed give some insight into the nature of the inhibitor binding site of both the wild-type polymerase and the pol III/*azp-12* enzyme. Equation 1 shows the importance of substituents in the meta position to binding. Substituents in this position have the most significant effect on activity, while the contribution of para substituents to binding seems to be less. An important fact shown by eq 1 is that the nature of the inhibitor–enzyme interaction apparently results only from hydrophobic binding. The inclusion into the equation of different electronic parameters had no effect on the equation, demonstrating that any binding due to electrostatic interactions is minimal.

Equation 2 derived for the pol III/*azp-12* enzyme indicates a change in the binding site structure of this mutant enzyme relative to the wild-type enzyme. The pocket into which para substituents intrude has increased its ability to bind hydrophobic groups, as indicated by the large increase in π_p relative to eq 1, at the same time suggesting an increased binding site volume as well. The pocket for meta groups, although appearing to be more hydrophobic, is less able to accommodate large substituents, as evidenced by the large reduction in the value of MR_m.

Table VI. 6-Anilinouracils

compd no.	yield,[a] %	crystn solvent	mp, °C	formula
1	50	EtOH	304-305	$C_{13}H_{15}N_3O_2 \cdot 0.5H_2O$
13	44	EtOH	287-289	$C_{13}H_{15}N_3O_2 \cdot 0.5H_2O$ [b]
16	48	EtOH	297-299	$C_{13}H_{15}N_3O_2 \cdot 0.5H_2O$
23	44	75% HOAc	294-295	$C_{14}H_{17}N_3O_2$
33	37	75% HOAc	291-293	$C_{15}H_{19}N_3O_2$
34	37	EtOH	261-263	$C_{14}H_{17}N_3O_2$

[a] Compounds were synthesized by the method described in ref 2. [b] N: calcd, 6.34; found, 5.90.

The subtle change in shape of the binding site detected by the differences between eq 1 and 2 shows that the mutant enzyme is not strictly an "inhibitor-resistant" enzyme but rather one that has a different pattern of response to the same series of inhibitors. The evidence from both the QSAR's and the qualitative inhibition data indicates a change in the structure of this binding site in the mutant enzyme that may result from a single hydrophobic amino acid replacement. Indeed, pol III/*azp-12* and other mutants of pol III[7] may represent a series of isoenzymes from *B. subtilis* that differ structurally from one another only as revealed by their resistance to a particular inhibitor, 6-[(*p*-hydroxyphenyl)hydrazino]uracil, and their different pattern of inhibition by the series of 6-anilinouracils reported here. The pol III/*azp-12* enzyme and other mutants of pol III are not deficient in their ability to synthesize DNA because their K_m values for deoxyribonucleoside 5'-triphosphates are the same as those of wild-type DNA polymerase III.[4,7] Consequently, the pol III inhibitor binding site and its substrate binding (active) site are structurally distinct.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were done by Schwarzkopf Microanalytical Laboratory, Woodside, NY; all agreed to ±0.4% of calculated values except where noted in Table VI. Nuclear magnetic resonance spectra were obtained with a Perkin-Elmer R-12B/Nicolet TT7 Fourier-transform instrument at 60 MHz; proton spectra obtained in dimethyl-d_6 sulfoxide or chloroform-*d* were consistent with the proposed structures.

m-Propylaniline and *m*-*n*-butylaniline were obtained by amination of the alkylbenzenes with trichloramine.[8] 4-Acetyl-3-methylaniline, 4-propionyl-3-methylaniline, and 4-*n*-butyryl-3-methylaniline were prepared by acylation and subsequent hydrolysis of *m*-acetamidotoluene.[9] The 3,4-dialkylanilines were made by reduction of these ketones with the Wolff–Huang–Minlon method.[10] 3-Acetyl-4-methylaniline was synthesized by acetylation of *p*-acetamidotoluene.[11]

3-Ethyl-4-methylaniline Hydrochloride. A solution of 3-acetyl-4-methylaniline (1.7 g, 0.011 mol), sodium hydroxide (1.3 g), and 85% hydrazine hydrate (1.4 mL) in diethylene glycol (25 mL) was heated at reflux for 1 h. Water was then distilled from the reaction mixture until the solution temperature reached 210 °C. The solution was heated at reflux for 3 h and, after cooling to room temperature, the viscous solution was extracted with benzene (3 × 50 mL). The benzene extracts were washed with water, dried ($MgSO_4$), and concentrated in vacuo. The dark oil was dissolved in ethanol (20 mL) and treated with concentrated hydrochloric acid (1.5 mL). The solvent was removed in vacuo, and the resulting semisolid was crystallized from ethanol/diethyl ether (3:1), yielding 1.0 g (51%) of colorless crystals: mp 185–187 °C; NMR (Me_2SO-d_6) δ 7.15 (s, 3 H, 2,5,6-H), 2.61 (q, 2 H, CH_2, J = 7.2 Hz), 2.27 (s, 3 H, Ar CH_3), 1.15 (t, 3 H, CH_3, J = 7.2 Hz). Anal. ($C_9H_{14}NCl$) C, H, N.

6-Anilinouracils. The synthesis of 6-anilinouracils employed 6-chlorouracil and the appropriate aniline in refluxing 2-methoxyethanol as previously described.[2] Most of the compounds used in this study were reported previously;[3] physical data for new inhibitors are summarized in Table VI.

Inhibition Data. Inhibition constants, K_i, define the inhibitor concentration causing half-maximal enzyme inhibition and were determined directly by assaying wild-type and *azp-12* DNA polymerase III from *Bacillus subtilis* using activated DNA as template but in the absence of dGTP as previously described.[1] Some of the K_i values were taken from ref 3.

Inhibitory potency for developing the QSAR equations is expressed as log $(1/K_i)$; the standard deviation of log $(1/K_i)$ in replicate assays was 0.2. Derivation of QSAR equations and statistical analyses were done on a Digital Equipment PDP8e computer with BASIC programs written by the authors.

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Registry No. **1**, 87986-17-2; **2**, 72255-68-6; **3**, 72255-66-4; **4**, 64054-69-9; **5**, 72255-65-3; **6**, 41648-10-6; **7**, 6972-74-3; **8**, 72255-62-0; **9**, 72255-48-2; **10**, 72255-50-6; **11**, 72255-53-9; **12**, 72255-49-3; **13**, 87986-18-3; **14**, 6948-11-4; **15**, 72255-63-1; **16**, 87986-19-4; **17**, 21333-02-8; **18**, 7269-03-6; **19**, 21333-03-9; **20**, 21332-93-4; **21**, 21332-95-6; **22**, 72255-67-5; **23**, 87986-20-7; **24**, 72255-58-4; **25**, 72255-57-3; **26**, 72255-69-7; **27**, 72255-55-1; **28**, 72255-52-8; **29**, 72255-54-0; **30**, 72255-64-2; **31**, 72255-70-0; **32**, 72255-59-5; **33**, 87986-21-8; **34**, 87986-22-9; **35**, 72255-56-2; **36**, 72255-61-9; **37**, 21332-96-7; 6-chlorouracil, 4270-27-3; 3-ethyl-4-methylaniline hydrochloride, 87986-23-0; *m*-propylaniline, 2524-81-4; 3-methyl-4-propylaniline, 87986-24-1; 4-butyl-3-methylaniline, 87986-25-2; *m*-*n*-butylaniline, 5369-17-5; DNA polymerase III, 37217-33-7; 4-ethyl-3-methylaniline, 69450-94-8.

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