Inhibitors of *Bacillus subtilis* DNA Polymerase III. 6-Anilinouracils and 6-(Alkylamino)uracils

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Substituted 6-anilinouracils were found to be potent inhibitors of the replication-specific enzyme, DNA polymerase III, from *Bacillus subtilis*. Inhibition potency was maximized by inclusion of small alkyl groups or halogens in the meta and para positions of the phenyl ring; polar substituents decreased activity considerably. Qualitative structure–activity relationships indicated that the meta position can tolerate larger groups, suggesting that this position may be suitable for the introduction of a group capable of irreversibly binding to the enzyme. Several 6-(alkylamino)uracils were weak inhibitors of DNA polymerase III; the optimum alkyl groups for enzyme binding were *n*-pentyl and *n*-hexyl, which apparently can occupy the planar enzyme binding site. The varied activities of 6-anilinouracils on a mutant DNA polymerase, resistant to 6-(phenylhydrazino)- and 6-(benzylamino)uracils bearing a *p*-OH or NH_2 group, have altered previous postulates for the structural basis of inhibitor resistance and have permitted construction of a refined model for inhibitor conformation in the latter series.

The systematic study[1,2] of the mechanism of inhibition of *B. subtilis* DNA polymerase III (pol III) by 6-(phenylhydrazino)- and 6-(benzylamino)pyrimidines has demonstrated two major structural features required for inhibitor action: a substituted pyrimidine ring which permits base pairing to a pyrimidine in the DNA template (cf. Figure 1, panel A, for binding to cytosine and panel B for binding to thymine) and a planar (aryl) ring situated at or near the 6-NH group, i.e., R in Figure 1. Inhibitory action of the 6-substituted pyrimidines is reversed by specific purine deoxyribonucleoside 5′-triphosphates, dGTP in the case of uracil derivatives (panel A, Figure 1) and dATP in the case of isocytosines (panel B, Figure 1). Enzyme inhibition results directly from the immoblization of pol III in a ternary complex composed of the DNA template, the inhibitor, and the enzyme.[3-6]

A detailed study employing phenylhydrazino and benzylamino derivatives has revealed that immobilization of pol III occurs via the specific interaction between the phenyl group of the inhibitor and a planar, hydrophobic site proximal to the active catalytic site of the enzyme. The phenylhydrazino and benzylamino derivatives have been useful in determining the existence of the inhibitor binding site. However, they have had only limited value as probes with which to conduct a detailed analysis of its structure. The limitation of these compounds resides in the conformation of the phenyl ring relative to that of the pyrimidine ring; it cannot be rigorously defined and, therefore, direct substituent effects on inhibitor–enzyme binding cannot be distinguished from those affecting the conformation of the phenyl ring.

To circumvent the problem of conformational mobility inherent in the use of phenylhydrazino and benzylamino compounds, we have developed a series of active, site-specific inhibitors composed of 6-anilinopyrimidines. In these compounds, the phenyl and pyrimidine rings are likely to be mutually perpendicular, as depicted in panel C of Figure 1. Baker has suggested[7] that some charge-transfer character involving the uracil ring as donor favors such a conformation for 6-anilinouracil, 6-benzyluracils, and 6-(benzylamino)uracils. The results of this paper (see Discussion) support this contention.

This report summarizes the inhibitory activity of an extensive series of 6-anilinouracils on *B. subtilis* pol III and describes their use in the further characterization of the inhibitor binding site of wild-type and mutant *B. subtilis* pol III.

Results

Inhibitor constants (K_i) of 6-anilinouracil inhibitors of *B. subtilis* pol III are presented in Table I. While 6-anilinouracil itself (**1**) was essentially inactive, substituents in the phenyl ring generated pol III inhibitors with K_i values covering four orders of magnitude. In general, ortho-substituted derivatives were inactive as inhibitors, whereas compounds with a single meta or para substituent were active. Disubstituted compounds in which one of the substituents is in an ortho position were weak inhibitors. The simultaneous presence of substituents in the meta and para positions, especially when these are both hydrophobic groups, yielded potent inhibitors. We have proposed[2] that such substituents project into a hydrophobic enzyme site, increasing the binding energy between inhibitor and enzyme. Polar substituents in the meta or para positions generally conferred weak activity on inhibitors. For example, *m*-OH (**9**) and *p*-OH (**19**) derivatives were 60- and 100-fold weaker than the corresponding methyl analogues (**4** and **12**).

Monosubstituted Compounds. As indicated above, an *o*-methyl (**2**) or *o*-ethyl (**3**) group did not confer inhibitory activity on the 6-anilinouracil molecule. The K_i data for meta-substituted compounds (Table I, compounds **4**–**11**) demonstrated that hydrophobic substituents (alkyl, halogens) produce strong inhibitors, whose potency varies directly with the lipophilicity of the substituent. This trend is observed in Figure 2A, which depicts a plot of log K_i vs. substituent π values.[8] Substituents with one row II atom correlated well with π, whereas those with two row II atoms [**5** (Et), **10** (CH_2OH), and **11** (CH_2Cl)] were more potent than would be predicted on the basis of π alone; this observation indicated that there is an extended region of hydrophobic enzyme space near the meta position of the anilino ring. Compounds containing a single para hydrophobic substituent also correlated well with π (Figure 2B), but the correlation was opposite to that obtained with meta substituents; i.e., increasing π values led to decreasing

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(4) N. C. Brown and G. E Wright in "Drug Action at the Molecular Level", G. C. K. Roberts, Ed., Macmillan, London, 1977, pp 151–166.
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(6) N. R. Cozzarelli, *Annu. Rev. Biochem.*, **46**, 641 (1977).
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(8) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

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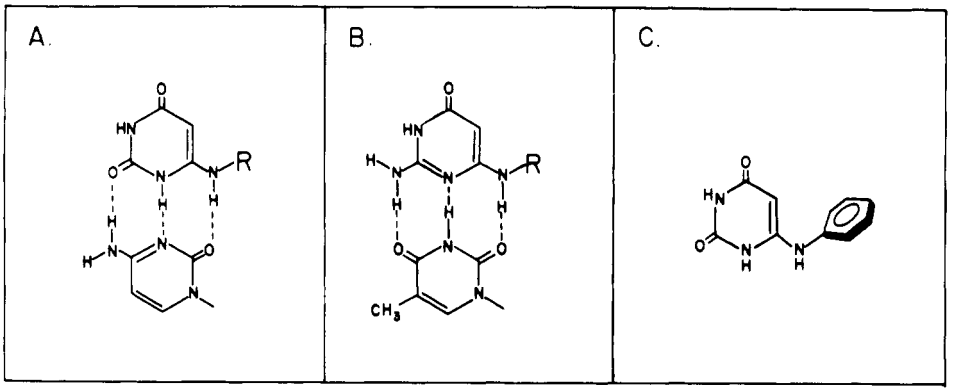
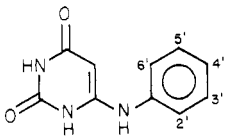


Figure 1. (A) Hydrogen bonding of a 6-aminouracil with cytosine; (B) hydrogen-bonding of a 6-aminoisocytosine with thymine; (C) 6-anilinouracil.

Table I. Activity of 6-Anilinouracils aganist *B. subtilis* DNA Polymerase III



no.	Ph substituent(s)	K_i, μM[a]
1		inact[b]
2	2′-Me	inact
3	2′-Et	inact
4	3′-Me	42.8
5	3′-Et	2.9
6	3′-Cl	25.0
7	3′-Br	10.3
8	3′-I	4.7
9	3′-OH	2630
10	3′-CH_2OH	413
11	3′-CH_2Cl	7.6
12	4′-Me	17.5
13	4′-Et	65.7
14	4′-*n*-Pr	452
15	4′-*i*-Pr	342
16	4′-*n*-Bu	4960
17	4′-Cl	25.0
18	4′-Br	31.6
19	4′-OH	1770
20	4′-OMe	120
21	4′-CF_3	101
22	4′-NH_2	640
23	4′-NHCOMe	inact
24	4′-CH_2OH	3200
25	2′,3′-Me_2	223
26	2′,4′-Me_2	1990
27	2′,5′-Me_2	1360
28	3′,4′-Me_2	0.92
29	3′,4′-$(CH_2)_3$-	0.42
30	3′-Me-4′-Br	1.0
31	3′-Me-4′-NH_2	19.0
32	3′-Me-4′-NHCOMe	464
33	3′-Cl-4′-Me	0.63
34	3′-I-4′-Me	0.38
35	3′,4′-Cl_2	0.93
36	3′-CH_2OH-4′-Me	78.5
37	3′-CH_2Cl-4′-Me	0.26
38	3′-NH_2-4′-Me	122
39	3′-NHCOMe-4′-Me	460
40	3′,5′-Me_2	124
41	2′,4′,6′-Me_3	inact
42	2′,3′-(C_4H_4)-	82.6
43	3′,4′-(C_4H_4)-	6.8

[a] K_i is the inhibitor concentration producing 50% inhibition of enzyme activity. [b] Compounds producing <10% inhibition at 1000 μM were considered inactive.

activity. However, polar para substituents (Figure 2B) also decreased activity. This suggests that group size may be a limiting factor in binding of para substituents with the enzyme, because group size, as measured by molar refraction (MR)[8] or E_s[9] values, strongly correlates with π for hydrophobic substituents. Steric repulsion of the large hydrophobic groups in the para position, therefore, is the most likely explanation for the data of Figure 2B.

Disubstituted Compounds. Considering the K_i values obtained for monosubstituted 6-anilinouracils, we predicted that maximum inhibitor potency would be achieved with a combination of a large, hydrophobic meta substituent and a small, hydrophobic para substituent, e.g., Me. This prediction was verified by the observation (cf. Table I) that the most potent compounds, **34** and **37**, combined a large meta group (I, CH_2Cl) and a *p*-methyl group. No correlation was found between log K_i and π for 3,4-disubstituted compounds, a result which probably reflects the differing requirements of lipophilicity and group size for substituents in the meta and para positions.

We observed that 6-(3′,5′-dimethylanilino)uracil (**40**), a compound containing substituents in both meta positions, was much weaker (K_i = 124 μM) than the 3′-Me derivative (**4**; K_i = 42.8 μM). The relative weakness of this derivative suggests that the drug binding site of the enzyme is asymmetric, reacting favorably with substituents on one side of the phenyl ring and repelling substituents—perhaps sterically—on the other side. The concept of an asymmetric site also was consistent with results of comparison of the potencies of other disubstituted compounds—for example, the potency of the 2′,3′-dimethyl derivative (**25**; K_i = 223 μM) vs. that of the 2′,5′-dimethyl derivative (**27**; K_i = 1360 μM). *o*-Methyls interfere with binding in both cases, but to a greater degree when the second methyl group is on the opposite side of the ring. The compound in which the substituents are on the same side of the ring (**25**) was fully six times more potent than **27**, in which the substituents are located on opposite sides.

The inhibitory activities of α- and β-naphthylaminouracils provided further support for the concept of asymmetry of the binding site; the β isomer (**43**, K_i = 6.8 μM), which mimics a 3′,4′-disubstituted anilinouracil, was considerably more potent than the α isomer (**42**, K_i = 82.6 μM), which mimics the structure of a 2′,3′-disubstituted anilinouracil.

6-(Alkylamino)uracils. Earlier studies[2] indicated that replacement of an aromatic ring with saturated (cycloalkyl) rings did not yield pol III specific inhibitors. For example, 6-(cyclohexylamino)- and 6-[(cyclohexylmethyl)amino]-uracils were inactive. We thought, however, that a simple alkyl group might be capable of binding to the enzyme by virtue of its isosterism with part of the phenyl ring of a

(9) S. H. Unger and C. Hansch, *Progr. Phys. Org. Chem.*, **12**, 92–98 (1976).

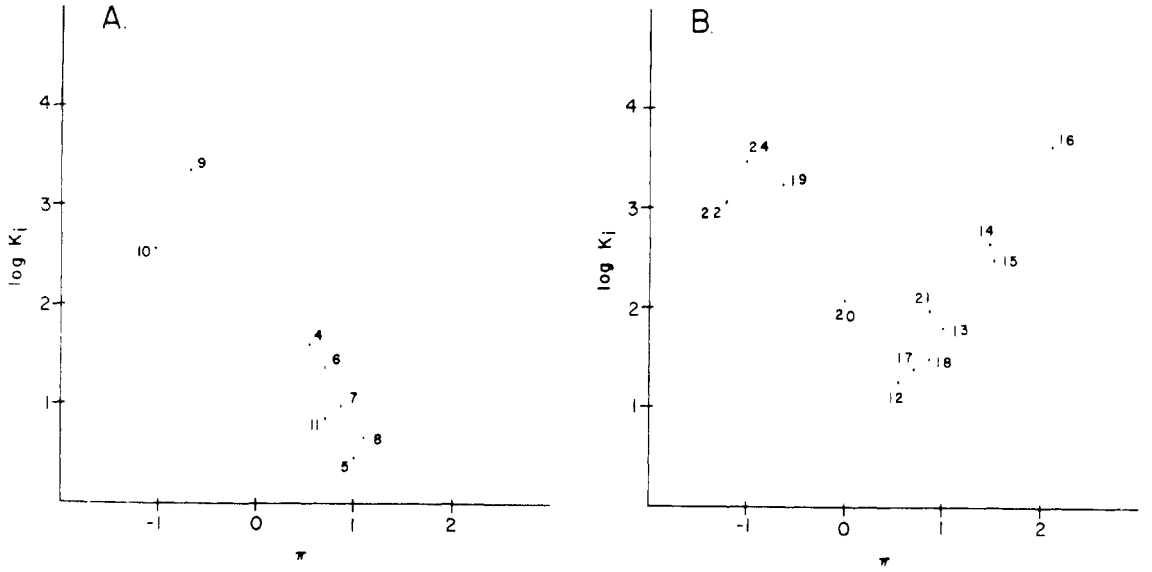


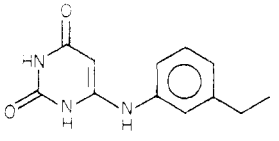
Figure 2. Correlation between substituent hydrophobic constants (π) and log K_i for monosubstituted 6-anilinouracils against *B. subtilis* pol III: (A) meta-substituted compounds; (B) para-substituted compounds. Values of π were obtained from ref 8, except π_{CH_2Cl} = 0.64 (C. Hansch, personal communication).

Table II. Activity of 6-(Alkylamino)uracils against *B. subtilis* DNA Polymerase III

no.	alkyl group	K_i, μM
44	$-CH_2CH_3$	inact[a]
45	$-(CH_2)_2CH_3$	inact
46	$-CH_2CH{=}CH_2$	22 400[b]
47	$-(CH_2)_3CH_3$	5 370
48	$-(CH_2)_4CH_3$	335
49	$-(CH_2)_2CH(CH_3)_2$	2 400
50	$-(CH_2)_5CH_3$	370
51	$-(CH_2)_5OH$	13 500[b]
52	$-(CH_2)_9CH_3$	inact

[a] Compounds producing <10% inhibition at 1000 μM were considered inactive. [b] Estimated.

6-anilinouracil; a likely candidate was the *n*-pentyl derivative, the structure of which is compared schematically below with that of 6-(*m*-ethylanilino)uracil (**5**). The *n*-



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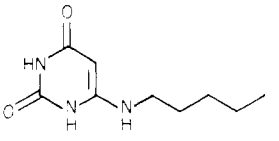

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pentyl (**48**) and *n*-hexyl (**50**) compounds were active inhibitors: they were the most potent of the series of rather weak 6-(alkylamino)uracils listed in Table II. Small alkyl chains (C_2–C_3) in compounds **44**–**46** apparently did not interact appreciably with the enzyme, whereas the C_{10} chain of **52** was clearly to large to fit the binding site. A branched C_5 chain like that of **49** conferred only very weak activity, probably as a consequence of its inability to achieve a "planar" conformation.

Activities of 6-Anilinouracils on a Mutant DNA Polymerase III. Pol III/*azp-12*, an enzyme derived[3] from an inhibitor-resistant strain of *B. subtilis*, is specifically resistant to inhibitors bearing an OH or NH_2 group in the phenyl ring of phenylhydrazino and benzylamino compounds and normally sensitive to inhibitors bearing H, alkyl, or halo substituents.[1,2] The effects of anilino inhibitors on the *azp-12* enzyme were considerably more varied than expected. The data of Table III indicate that pol III/*azp-12* exhibited resistance to certain compounds, normal sensitivity to some, and clear *hypersensitivity* to others. The inhibitors in Table III are arranged in order

Table III. Comparison of Inhibitory Activities of 6-Anilinouracils on Mutant and Wild-Type Polymerases

		K_i, μM		
no.	Ph substituent(s)	pol III/*azp-12*	WT pol III	deg of resistance[a]
9	3′-OH	inact	2630	
10	3′-CH_2OH	5130	413	12.4
31	3′-Me-4′-NH_2	158	19	8.3
5	3′-Et	18.6	2.9	6.4
22	4′-NH_2	3900	640	5.1
8	3′-I	20.4	4.7	4.3
4	3′-Me	178	42.8	4.2
12	4′-Me	69	17.5	3.9
28	3′,4′-Me_2	3.5	0.9	3.9
38	3′-NH_2-4′-Me	298	122	2.4
19	4′-OH	3800	1770	2.2
7	3′-Br	21.9	10.3	2.1
6	3′-Cl	41.7	25	1.7
20	4′-OMe	120	120	1.0
30	3′-Me-4′-Br	0.85	1	0.85
24	4′-CH_2OH	1950	3200	0.6
17	4′-Cl	14.1	25	0.56
16	4′-*n*-Bu	2190	4960	0.4
13	4′-Et	20	65.7	0.3
18	4′-Br	4	31.6	0.13
14	4′-*n*-Pr	54	452	0.12
15	4′-*i*-Pr	22	342	0.065

[a] $K_i(azp\text{-}12)/K_i$(WT).

of decreasing degree of resistance of the mutant enzyme, $K_i(azp\text{-}12)/K_i$(WT). Resistance of pol III/*azp-12* was clearly not limited to compounds with an OH or NH_2 group (**9**, **10**, and **22**) but occurred with all compounds bearing a meta substituent, even those containing hydrophobic groups (**5**, **8**, **4**, and **28**). In contrast, the mutant enzyme was distinctly hypersensitive to compounds with hydrophobic para substituents (**13**–**18**) and even to a small degree to **24**, which contains a *p*-CH_2OH group.

Substituent effects on binding to pol III/*azp-12* differed quantitatively from those described above for the wild-type enzyme. A plot of π vs. log $K_i(azp\text{-}12)$ for meta-substituted compounds (Figure 3A) yielded a correlation similar to that found for the wild-type enzyme (cf. Figure 2A) but indicating a smaller contribution of π to binding. Figure 3B deplicts a plot of substituent π values vs. log $K_i(azp\text{-}12)$ for para-substituted compounds and shows a distinct lack of correlation compared with that found for the wild-type

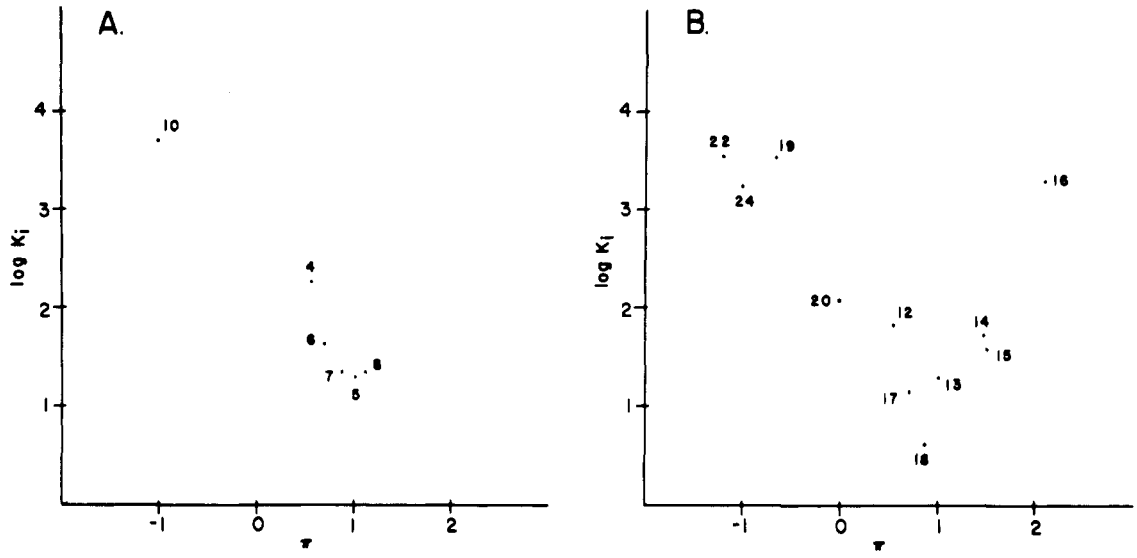


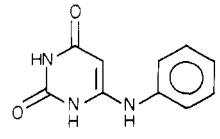
Figure 3. Correlation between substituent hydrophobic constants (π) and log K_i for monosubstituted 6-anilinouracils against pol III/*azp-12*: (A) meta-substituted compounds; (B) para-substituted compounds. Values of π were obtained from ref 8.

enzyme (cf. Figure 2B). In general, the mutant enzyme accommodated large para and small meta substituents, a behavior completely opposite to that of the wild-type enzyme, which accommodated small para and large meta substituents.

Of particular interest were the effects of OH and CH_2OH groups. The results in Table III indicate that when these groups were in the meta position (**9** and **10**) the mutant enzyme was strongly resistant, whereas inhibitors with these groups in the para position (**19** and **24**) only differed about twofold in K_i with respect to both enzymes. The results of Table III also show, for reasons that are not clear, that the *azp-12* enzyme was somewhat resistant to *all* compounds containing the NH_2 group (**31**, **22**, and **38**).

Discussion

Utility of Anilinouracils in Examination of Structure–Activity Relationships and Enzyme Structure. The systematic evaluation of the effects of 6-anilinouracils on pol III provide a view of substituent effects which is considerably more accurate than that obtained previously with phenylhydrazino and benzylamino inhibitors. The utility of the anilino compounds has resulted primarily from the relatively constant spatial relationship of the phenyl ring and the enzyme binding site—a constancy which cannot be assigned to compounds with more flexible side chains. We propose that the phenyl ring of 6-anilinouracils occupies the conformation shown in panel C of Figure 1, in which the N^6-phenyl bond is syn to the C_5–C_6 bond of the pyrimidine ring and in which the phenyl and pyrimidine rings are mutually perpendicular. The syn relationship of the N^6-phenyl and C_5–C_6 bonds follows from the involvement of the 6-N–H in hydrogen bonding to cytosine in the template[10] (panel A, Figure 1). A coplanar arrangement of rings as in **53**, although structurally



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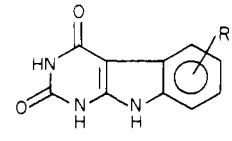

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possible, probably does not represent the active inhibitor conformation, because a series of planar analogues of **53**, 9*H*-pyrimido[4,5-*b*]indole-2,4-diones[11] (**54**), does not inhibit DNA polymerase III (unpublished observations).

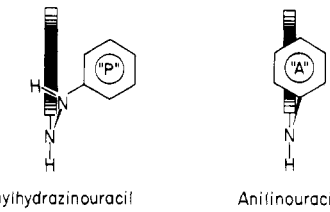

Figure 4. Structures of 6-(phenylhydrazino)uracil and 6-anilinouracil viewed perpendicular to the plane of the phenyl rings, "P" and "A", respectively.

The graphs of Figure 2 show that, while enzyme binding of meta-substituted compounds is directly related to the hydrophobic character of the substituent, the situation for para-substituted compounds is more complex. For the latter compounds, it is likely that increasing group size limits enzyme binding, even of groups with high π values. We are currently attempting to evaluate the roles of π and group size via the use of Hansch-type calculations.

The anilino compounds hold much promise as a basis for the synthesis of an inhibitor capable of binding irreversibly to the polymerase. The meta position is a particularly attractive site for the introduction of a reactive group, in view of its greater tolerance of larger groups compared to the para position.

Further Delineation of the Inhibitor Binding Site of Pol III. One of the most important consequences of the discovery that 6-anilinouracils are active, dGTP-specific inhibitors of *B. subtilis* DNA polymerase III is their contribution to our concept of the drug binding site of the enzyme. We find it reasonable to assume that phenylhydrazino and benzylamino inhibitors act in a conformation which places the phenyl ring in or near the same plane as that of anilino compounds. Structures of 6-anilinouracil and 6-(phenylhydrazino)uracil are compared in Figure 4 from a perspective perpendicular to the plane of the respective phenyl rings. Manipulation of space-filling models demonstrates that when the phenyl rings are coplanar, that of the phenylhydrazino compound ("P" of Figure 4) is displaced to the right (or left) by a ring diameter from that

(10) J. Mackenzie, M. Neville, G. E. Wright, and N. C. Brown, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 512 (1973).

(11) G. E. Wright, *J. Heterocycl. Chem.*, **13**, 539 (1976).

of the anilino compound ("A" of Figure 4). This assumption permits a simple explanation of why 6-anilinouracil itself is inactive and why large, hydrophobic meta substituents impart considerable potency to these compounds. The enzyme binding site must overlap the "P" domain to a greater degree than the "A" domain. Substituents on 6-anilinouracil which project into the enzyme binding site are required, therefore, to provide hydrophobic binding strength sufficient to elicit inhibitory activity.

The "A" and "P" concept also accommodates the behavior of the mutant *azp-12* enzyme toward meta- and para-substituted anilinouracils (Table III). For example, pol III/*azp-12* is resistant to the *m*-CH_2OH derivative (**10**) but normally sensitive to the *p*-CH_2OH isomer (**24**); the OH group of **10** lies much closer to the position occupied by the OH of 6-[(*p*-hydroxyphenyl)hydrazino]uracil [H_2·HPUra], the inhibitor used to generate the mutant enzyme, than does the OH of **24**. Thus, the meta substituents of anilinouracils are selectively subjected to the region of the mutation conferring resistance to H_2·HPUra.

The *pol C* mutation, *azp-12*, specifies a minor change in enzyme structure—a change near the active, substrate binding site which does not alter the enzyme's affinity for substrates or its catalytic activity.[3] The nature of the mutation was hypothesized[12] to be the loss of a hydrophilic component, thus generating an enzyme resistant to *p*-OH and NH_2 phenylhydrazino and benzylamino inhibitors. The present results indicating resistance to meta hydrophobic substituents (cf. Table III) tend to discredit this hypothesis. Clearly, the results indicate that it is not the nature of the substituents per se but their position and size which determine resistance or hypersensitivity of the *azp-12* enzyme to 6-anilinouracils. A more comprehensive understanding of the drug binding site of pol III will require the development of specific inhibitors capable of covalently binding the site for direct physical analysis.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were done by HetChem Co., Harrisonville, MO, and by Schwarzkopf Microanalytical Laboratory, Woodside, NY; all agreed to ±0.4% of calculated values unless noted in Table IV. Nuclear magnetic resonance spectra were obtained with a Perkin-Elmer R-12B/TT7 Fourier transform instrument; proton spectra obtained in Me_2SO-d_6 were consistent with the proposed structures.

Synthesis of 6-anilinouracils and 6-(alkylamino)uracils employed 6-chlorouracil and the appropriate amine in refluxing glyme as described previously.[2] Physical data on new compounds are presented in Table IV. Compounds **1–4**, **6**, **12**, **16**, **25–29**, **42**, and **43** have been reported.[13] Compounds **17** and **18** were gifts of the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute; compounds **50** and **52** were gifts of Imperial Chemical Industries Pharmaceuticals Division, Macclesfield, England.

Table IV. 6-Anilinouracils and 6-(Alkylamino)uracils

no.	yield, %[a]	crystn solvent	mp, °C	formula[b]
5	94	EtOH	297–300	$C_{12}H_{13}N_3O_2$
7	70	50% HAc	319–321	$C_{10}H_8N_3O_2Br$
8	44	HAc	316–318	$C_{10}H_8N_3O_2I$
9	66	25% DMF	300–301	$C_{10}H_9N_3O_3 \cdot 0.5H_2O$
10	62	H_2O	267–270	$C_{11}H_{11}N_3O_3$
11	81[c]	50% HAc	>360	$C_{11}H_{10}N_3O_2Cl$
13	81	50% HAc	330–331	$C_{12}H_{13}N_3O_3$
14	62	75% HAc	325–328	$C_{13}H_{15}N_3O_2$
15	75	75% HAc	288–290	$C_{13}H_{15}N_3O_2$
19	93	50% DMF	338–340	$C_{10}H_9N_3O_3$
20	94	90% DMF	325–327	$C_{11}H_{11}N_3N_3$
21	33	50% HAc	327–330	$C_{11}H_8N_3O_2F_3 \cdot 0.5H_2O$[d]
22	95[e]	25% glyme	324–327	
23	83	90% Me_2SO	346–347	$C_{12}H_{12}N_4O_3 \cdot 0.5H_2O$
24	75	50% Me_2SO	270–274	
30	47	HAc	305–307	$C_{11}H_{10}N_3O_2Br \cdot 0.5H_2O$
31	69	30% DMF	298–301	$C_{11}H_{12}N_4O_2 \cdot H_2O$
32	60[f]	g	316–319	$C_{13}H_{14}N_4O_3 \cdot 0.5H_2O$
33	56	80% HAc	334–336	$C_{11}H_{10}N_3O_2Cl$
34	67	75% HAc	334–335	$C_{11}H_{10}N_3O_2I$
35	44	50% HAc	334–335	$C_{10}H_7N_3O_2Cl_2$
36	62	H_2O	296–298	$C_{12}H_{13}N_3O_3$
37	80[h]	50% HAc	>350	$C_{12}H_{12}N_3O_2Cl \cdot 0.5H_2O$[i]
38	75	H_2O	301–303	$C_{11}H_{12}N_4O_2 \cdot H_2O$
39	62[j]	g	311–315	$C_{13}H_{14}N_4O_3$
40	75	50% HAc	312–315	$C_{12}H_{13}N_3O_2 \cdot 0.5H_2O$
41	38	40% EtOH	308–311	$C_{13}H_{14}N_3O_2$
44	46	H_2O	303–305	$C_6H_9N_3O_2$
45	83	H_2O	278–281	$C_7H_{11}N_3O_2 \cdot H_2O$
46	80	H_2O	254–255	$C_7H_9N_3O_2$
47	83	75% EtOH	272–274	$C_8H_{13}N_3O_2$
48	79	EtOH	272–273	$C_9H_{15}N_3O_2$
49	79	glyme	289–291	$C_9H_{15}N_3O_2$
51	50	H_2O	236–237	$C_9H_{15}N_3O_3$

[a] See Experimental Section for general method of synthesis. [b] C, H, N analyses; compounds **22** and **24** were very hygroscopic and did not give satisfactory analyses. [c] Obtained by dissolving compound **10** in concentrated HCl for 24 h. [d] N: calcd, 15.00; found, 14.54. [e] Obtained by alkaline hydrolysis of compound **23**. [f] Obtained by acetylation of compound **31**. [g] Precipitated by HCl from a solution in NaOH. [h] Obtained by dissolving compound **36** in concentrated HCl for 24 h. [i] N: calcd, 15.30; found, 14.75. [j] Obtained by acetylation of compound **38**.

The preparation of wild-type and *azp-12* DNA polymerase III from *B. subtilis* and the method by which they were assayed have been described.[2,3] Inhibitor assays were performed in the absence of the competing nucleotide, dGTP, to give a direct measure of K_i, the inhibitor concentration yielding 50% inhibition of enzyme activity; the details of inhibitor assays are described elsewhere.[14]

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