Inhibitors of *Bacillus subtilis* DNA Polymerase III. Influence of Modifications in the Pyrimidine Ring of Anilino- and (Benzylamino)pyrimidines

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Substituent effects governing inhibition of DNA polymerase III from *Bacillus subtilis* were examined in several series of N^6-substituted 6-aminopyrimidines. The presence of alkyl groups as large as *n*-butyl in the 3-position of 6-(5-indanylamino)uracil had no effect on inhibitor–enzyme binding. Substituents in the 4-position of a series of 2-amino-6-(benzylamino)pyrimidines had complex effects: alkoxy and phenoxy derivatives were less active than the parent 4-oxo (isocytosine) compound, but alkylphenoxy and halophenoxy derivatives were more active than the 4-phenoxy compound itself, suggesting that hydrophobic binding can occur between 4-substitutents and the enzyme surface and that space between the pyrimidine ring and pol III may represent the active site of the enzyme. Replacement of 5-H by methyl and ethyl groups drastically decreased inhibitory activity of 6-(benzylamino)- and 6-*p*-toluidinouracils, but 5-bromo and 5-iodo analogues were equipotent with the parent compounds. These results indicate that the phenyl rings of these compounds must exist in conformations in which they are perpendicular to the pyrimidine ring plane and that charge-transfer stabilization of such "active conformations" may compensate for steric barriers from 5-halo groups in the inhibitor–enzyme complex.

The mechanism by which certain antibacterial N^6-substituted 6-aminopyrimidines inhibit DNA polymerase III (pol III) from Gram-positive bacteria involves Watson–Crick-like hydrogen bonding to pyrimidine bases in the DNA template[1] and binding of the N^6-substituent to a hydrophobic site on the enzyme.[2-4] Uracil derivatives such as **1**, **3**, and **4** (Table I) hydrogen bond with cytosine residues, and their action is competitive with 2′-deoxyguanosine 5′-triphosphate (dGTP); isocytosines such as **2** and **5** bind with thymine residues and are competitive with 2′-deoxyadenosine 5′-triphosphate (dATP). The N^6-substituents as represented in compounds **1–5** provide the major site of inhibitor–enzyme binding, and inhibition of enzyme activity ultimately results from the formation of a DNA–inhibitor–enzyme complex.[5,6]

The nature of the "inhibitor binding site" of DNA polymerase III that binds the N^6-substituent of these compounds has been characterized by qualitative[2-4] and quantitative[7] structure–activity relationships. Limited information is available, however, about inhibitory effects of substituents at positions in the pyrimidine ring other than the 6-position. 2-Thiouracil derivatives had much weaker activity than the corresponding uracils,[6] and a 1-methyl analogue of 6-(phenylhydrazino)uracil was inactive, a likely consequence of its inability to pair with cytosine.[2]

Although the nature of the "inhibitor binding site" of the enzyme has been characterized by the results of structure–activity studies with substituted 6-anilinouracils,[4] the relationship between that site and the substrate binding (active) site of pol III has yet to be established. Comparison between the structures of an inhibitor and its competitive substrate when bound to a pyrimidine base at the primer terminus of template DNA has led to several hypotheses regarding inhibitor conformation and the spatial orientation of inhibitor and substrate binding sites.

Table I. Representative Inhibitors of *B. subtilis* DNA Polymerase III

no.	compd	K_i,[a] μM	competitive with	ref
1	6-(benzylamino)uracil	7.5	dGTP	3
2	6-(benzylamino)isocytosine	33	dATP	this work
3	6-*p*-toluidinouracil	17	dGTP	4
4	6-(5-indanylamino)uracil	0.4	dGTP	4
5	6-(5-indanylamino)isocytosine	2	dATP	this work

[a] Inhibitor concentration giving 50% inhibition of enzyme activity under truncated assay conditions (see Results section).

It has been proposed[4] that the inhibitor, when bound to the enzyme, assumes an active conformation in which the N^6-aryl group is in a plane perpendicular to that of the pyrimidine ring, both to permit hydrogen bonding with the template base and to project the phenyl ring into the inhibitor binding site. Further, if enzyme is bound to the DNA–inhibitor complex in the same way that it is bound to the DNA–substrate complex, the 4-oxo group of inhibitors may project into the active, substrate binding site of the enzyme. The work presented here was undertaken in an effort to test these hypotheses.

Results

Enzyme Assay. The DNA polymerase assay used in these studies is a truncated reaction in which the competing nucleotide (dGTP or dATP, depending on the class of inhibitor) is deleted.[6] This assay is useful in providing a direct measure of K_i, the inhibitor concentration producing 50% inhibition of enzyme activity. The K_i determined by this method has been shown to be identical with the inhibitor–enzyme binding constant, K_{cx}, which describes the inhibitor concentration that produces 50% complex formation.[5,6] Thus, this relationship allows K_i values to be related approximately to substituent-induced free energy changes in inhibitor–enzyme binding, $\Delta(\Delta G) = -RT \ln (K_{i1}/K_{i2})$.

3-Substituted Derivatives. Substituent effects on pol III inhibition associated with the 3-position of the pyrimidine ring were investigated with use of 6-(5-indanylamino)uracil (**4**) as the reference compound. Derivatives of **4** bearing 3-methyl, ethyl, *n*-propyl, and *n*-butyl groups, **6–9**, respectively, were essentially equipotent with the parent compound (K_i = 0.3–0.4 μM). We conclude from these data that substituents in the 3-position do not contact enzyme and that the 3-NH group of uracil derivatives

(1) Mackenzie, J. M.; Neville, M. M.; Wright, G. E.; Brown, N. C. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 512.
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(3) Brown, N. C.; Gambino, J.; Wright, G. E. *J. Med. Chem.* **1977**, *20*, 1186.
(4) Wright, G. E.; Brown, N. C. *J. Med. Chem.* **1980**, *23*, 34.
(5) Clements, J.; D'Ambrosio, J.; Brown, N. C. *J. Biol. Chem.* **1975**, *250*, 522.
(6) Wright, G. E.; Brown, N. C. *Biochim. Biophys. Acta* **1976**, *432*, 37.
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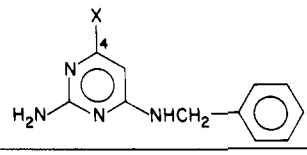
Table II. K_i Values for Inhibition of DNA Polymerase III by 2-Amino-4-substituted-6-(benzylamino)pyrimidines



no.	4-substituent	K_i,[a] μM
2	O[b]	33
10	Cl	17
11	S[b]	27
12	Me	1230
13	NH_2	3090
14	OMe	204
15	OEt	199
16	O-*n*-Pr	161
17	O-c-C_6H_{11}	794
18	O-C_6H_5	542
19	O-(4-Me-C_6H_4)	242
20	O-(3-Me-C_6H_4)	164
21	O-(3,4-Me_2-C_6H_3)	154
22	O-(3-Et-C_6H_4)	213
23	O-(4-Et-C_6H_4)	130
24	O-(4-Cl-C_6H_4)	79
25	O-(4-Br-C_6H_4)	85
26	O-(4-HNHCOMe-C_6H_4)	460
27	O-(4-*n*-Pr-C_6H_4)	251
28	O-(4-CH_2OH-C_6H_4)	1410
29	SMe	316
30	$SCH_2C_6H_5$	inact
31	SCH_2-(4-Cl-C_6H_5)	inact

[a] Inhibitor concentration giving 50% inhibition of enzyme activity assayed in the absence of dATP. [b] Oxo and thio forms with tautomeric H on N-3.

does not react with the enzyme or participate in the inhibition mechanism.

4-Substituted Derivatives. Variation of substituents in the 4-position of a 2-oxopyrimidine inhibitor introduces uncertainty into the interpretation of inhibition data. Nontautomeric groups in the 4-position may lead to altered tautomerism of the 1-NH group and produce a pyridine-like 1-N that would be expected to destroy the capacity of the compound to form hydrogen bonds with a cytosine base in the template. For example, compounds with no substituent at a site analogous to position "4" such as 4-(phenylhydrazino)-2-pyrimidone and 4-(benzylamino)-2-pyrimidone are inactive as inhibitors of pol III, although the corresponding "4-oxo" (uracil) compounds are potent inhibitors. NMR studies have shown[8] that the mobile H in 4-(phenylhydrazino)-2-pyrimidone is *not* on the ring nitrogen that should hydrogen bond with cytosine, and the benzylamino derivative appears to behave in the same manner (unpublished results). Also, a derivative containing the amino group at position "4" was but weakly inhibitory to pol III (K_i = 370 μM) relative to the uracil analogue (K_i = 0.5 μM).[2]

2-Aminopyrimidine derivatives, in contrast to 2-oxopyrimidines, should retain the capacity to form hydrogen bonds with thymine bases in the template regardless of the tautomeric properties of the substituent at the 4-position. On the basis of this reasoning and the observations (see above) that the 3-NH group does not influence inhibition, we investigated structure–activity relationships involving 4-substituents in a series of 2-amino-6-(benzylamino)pyrimidines. The benzyl group in this series represented a convenient group to introduce synthetically and a good determinant for expressing inhibitory activity in the prototype compound (see Table I).

(8) Wright, G. E. *J. Heterocycl. Chem.* **1984**, *20*, 1037.

Table III. Comparison between K_i and pK_a of Selected 2-Amino-6-(benzylamino)pyrimidines

no.	4-substituent	pK_a[a]	K_i,[b] μM
2	O	3.22	33
11	S	3.33	27
10	Cl	3.56	17
12	Me	7.98	1230
13	NH_2	6.97	3090
14	OMe	5.76	204
19	O-(4-Me-C_6H_4)	5.82	242
20	O-(3-Me-C_6H_4)	5.27	164
24	O-(4-Cl-C_6H_4)	4.77	79

[a] See Experimental Section. [b] From Table II.

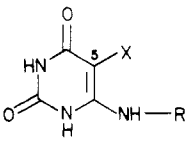
Data for inhibition of DNA polymerase III by a series of 2-amino-4-substituted-6-(benzylamino)pyrimidines are presented in Table II. Several generalizations can be made regarding the influence of 4-substituents. The 4-thio derivative (**11**) is equipotent with the prototype 4-oxo (isocytosine) analogue, **2**. Replacement of the tautomeric oxo and thio groups by chloro gave an even more potent compound (**10**), although in **10** (and in the remainder of the compounds in Table II) there is no mobile H on N-3.

Replacement of 4-oxo by electron-releasing methyl and amino groups led to drastic decreases in activity: the 4-methyl (**12**) and 4-amino (**13**) derivatives were about 40- and 100-fold less potent than **2**, respectively. A direct effect of these groups to decrease enzyme–inhibitor binding was considered unlikely because larger groups such as OMe (**14**) and SMe (**29**) did not decrease activity to such extents. However, an expected effect of the electron-releasing methyl and amino groups would be to increase basicity of compounds such as **12** and **13**. Consequently, pK_a values for selected 2-amino-6-(benzylamino)pyrimidines were determined spectrophotometrically and are presented in Table III along with the K_i values for inhibition of pol III by the compounds. It is clear that the most basic compounds, **12** and **13**, are those with least inhibitory activity against pol III. The compounds with lowest pK_a values (**2**, **10**, and **11**) are those with the greatest inhibitory activity, while a group of 4-ether derivatives that are less inhibitory have intermediate pK_a values. The pH of the pol III assay solution is 7.6, and **12** and **13** would be expected to be significantly protonated under these conditions. If the cationic inhibitor species, especially that resulting from protonation at N-1, are incapable of hydrogen bonding with thymines in the DNA template, the weak activity of **12** and **13** may be ascribed, at least in part, to increased pK_a rather than solely to a direct influence of the substituents on enzyme binding.

Methylation of the prototype 4-oxo and 4-thio compounds decreased inhibitory activity of the resulting ethers, **14** and **29**, by 6- and 12-fold, respectively. These results may reflect the loss of or interference with a hydrogen-bonding interaction involving the 4-substituent and the enzyme. That these results do not reflect solely a steric effect, at least in the 4-oxy series, is indicated by the observation that the activities of the ethyl and *n*-propyl ether derivatives, **15** and **16**, do not differ significantly from that of the methoxy compound (**14**).

The 4-cyclohexoxy (**17**) and 4-phenoxy (**18**) derivatives were much weaker inhibitors than **14**, and weaker yet than the simple alkoxy compounds. However, substituted phenoxy analogues, e.g., compounds **19**–**28**, were often more active than the 4-phenoxy compound itself. In general, small hydrophobic groups in the meta and para positions of the phenoxy ring gave compounds equivalent to or better than **14** as inhibitors. Indeed, the *p*-chloro (**24**) and *p*-bromo (**25**) analogues were only 2–3-fold less active

Table IV. K_i Values for Pol III Inhibition by 5-Substituted Uracils



compd (6-R)	5-substituent	K_i,[a] μM
6-(benzylamino)uracils		
1	H	7.5
32	Me	436
33	Et	2820
34	aza[b]	20
35	Br	9
36	I	9
37	N=O	inact
6-*p*-toluidinouracils		
3	H	17
38	Me	1580
39	Et	inact
40	aza[b]	70
41	Br	14
42	N=O	inact

[a] Inhibitor concentration giving 50% inhibition of enzyme activity assayed in the absence of dGTP. [b] 5-CH replaced by N.

than the prototype 4-oxo compound **2**. These results are considered in greater detail in the Discussion section.

The presence of the benzylthio or (*p*-chlorobenzyl)thio groups at position 4 yielded inactive compounds (**30** and **31**).

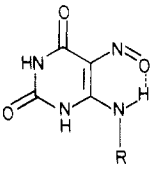
5-Substituted Derivatives. The effects of 5-substituents on inhibition of pol III were studied in two series of compounds. The first series employed the relatively flexible 6-(benzylamino)uracil (**1**) as the prototype, and the second series employed the more rigid 6-*p*-toluidinouracil, **3**. The data of Table IV clearly show the abrupt decrease in inhibitory activity in the first series upon replacement of 5-H by Me and Et. The thymine analogue **32** is about 60-fold less active than **1** and the 5-ethyl derivative **33** nearly 400-fold. These results are consistent with the postulate that the phenyl ring plane of compounds such as **1** must be perpendicular to the pyrimidine ring plane to retain inhibitory activity; this active conformation would be profoundly interrupted by the presence of bulky groups at the 5-position. The effects of these substituents on the second, more rigid 6-*p*-toluidinouracil series are even more dramatic, the 5-methyl analogue (**38**) being nearly 100-fold less active than **3** and the 5-ethyl analogue (**39**) being inactive.

Surprisingly, 5-halo derivatives (**35**, **36**, **41**) were equipotent with the prototype compounds as inhibitors of pol III (Table IV), implying that they may adopt the same active conformation as the corresponding uracils. This could occur by charge-transfer interaction between the halogen atom and phenyl ring in a manner characteristic of such complexes,[9] a process that could overcome the steric repulsion that occurs in the case of "inert" 5-substituents such as Me and Et.

Replacement of C-5 by N gave 5-aza compounds (*s*-triazines) whose inhibitory activities, as depicted for the 6-(benzylamino) (**34**) and 6-*p*-toluidino (**40**) derivatives in Table IV, were consistently 2–3-fold lower than those of the corresponding uracils. This modest change could be a consequence of the electronic effect of the 5-aza group on inhibitor–cytosine binding or to the possible existence of barbituric acid like structures proposed for similar aminotriazinediones.[10]

(9) Hassel, O. *J. Mol. Phys.* **1958**, *1*, 241.

5-Nitroso compounds, such as **37** and **42**, were inactive as inhibitors of DNA polymerase III, probably because these compounds exist as intramolecularly hydrogen-bonded conformers.[11] The NMR chemical shifts of the 6-NH protons of **37** and **42** in Me_2SO-d_6 were found at δ 13.23 and 14.70, respectively, significantly downfield from those of the corresponding 5-H compounds, i.e., δ 6.58 for **1** and δ 8.07 for **3**. These downfield shifts are suggestive of strong intramolecular hydrogen bonding involving the nitroso group, a process that would force these compounds to adopt structures such as that depicted here:



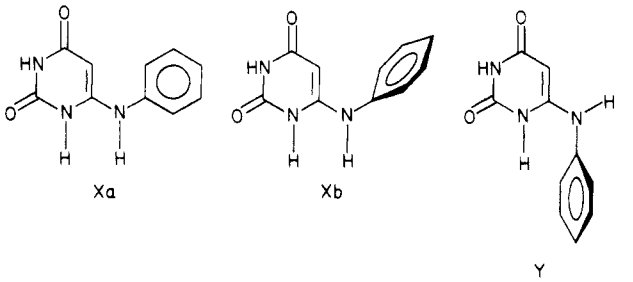
37, R = CH_2Ph
42, R = *p*-MePh

It is not possible for such a structure to hydrogen bond with cytosine in a DNA template nor would the phenyl rings of **37** and **42** be able to bind with the polymerase.

Discussion

Collectively, the results presented in this paper provide good evidence for the specific conformational requirements of N^6-substituents among inhibitors of DNA polymerase III and for the existence, in the inhibitor–enzyme–template complex, of space adjacent to the 4-position of the pyrimidine ring that may represent the surface of the enzyme active site. These two features of inhibitors will be considered in turn.

The model of inhibitor action involving Watson–Crick-like hydrogen bonding with template pyrimidine bases essentially dictates both a syn relationship between the 6-NH bond and the pyrimidine 1-N and coplanarity of 6-N–H with the pyrimidine ring. Apparently, the absence of substituents in the 5-position of the pyrimidine ring (other than H) allows conformers such as X to predominate over conformer Y, at least in the inhibitor–enzyme–template complex. The phenyl ring of 6-anilinopyrimidines, therefore, likely exists as depicted in structures Xa and Xb. (Analogous structures may also be constructed



Xa Xb Y

for 6-benzylamino pyrimidines.) Model building certainly favors structure Xb in which the rings are mutually perpendicular, a prediction supported by the observation that pyrimido[4,5-*b*]indole-2,4-diones, compounds that resemble Xa, do not inhibit pol III.[3] The effects of 5-alkyl substituents in depressing inhibitory activity (cf. compounds **32**, **33**, **38**, **39** in Table IV) are fully in accord with these expectations. The fact that 5-halo derivatives (**35**, **36**, **41**) do not differ in inhibitory activity from their 5-H counterparts seems anomalous, Br and I being substituents

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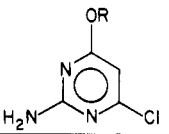
(11) Fuchs, H.; Gottlieb, M.; Pfleiderer, W. *Ber.* **1978**, *111*, 982.

Table V. Synthesis and Characterization of 2-Amino-4-oxy-6-chloropyrimidines[a]



R	yield, %	cryst solvent	mp, °C	formula	anal.
n-Pr	84	EtOH	64–66	$C_7H_{10}N_3OCl$	C, H, N
c-C_6H_{11}	32	5% EtOH	140	$C_{10}H_{14}N_3OCl$	C, H, N
C_6H_5	56	EtOH	178–180	$C_{10}H_8N_3OCl{\cdot}0.25H_2O$	C, H, N
C_6H_4-4-Me	66	5% EtOH	220–221	$C_{11}H_{10}N_3OCl{\cdot}0.5H_2O$	C, H, N
C_6H_4-3-Me	45	5% EtOH	136–138	$C_{11}H_{10}N_3OCl{\cdot}0.25H_2O$	C, H, N
C_6H_3-3,4-diMe	64	5% EtOH	189–190	$C_{12}H_{12}N_3OCl{\cdot}0.25H_2O$	C, H, N
C_6H_4-4-Cl	64	5% EtOH	201–203	$C_{10}H_7N_3OCl_2{\cdot}0.5H_2O$	C, H, N
C_6H_4-4-$NHCOCH_3$	62	5% EtOH	265–267	$C_{12}H_{11}N_4O_2Cl{\cdot}0.5H_2O$	C, H, N
C_6H_4-4-Et	56	aq EtOH	190–191	$C_{12}H_{12}N_3OCl$	C, H, N
C_6H_4-4-Br	25	5% EtOH	179–181	$C_{10}H_7N_3OBrCl$	C, H, N
C_6H_4-3-Et	77	EtOH	156–158	$C_{12}H_{12}N_3OCl$	C, H, N
C_6H_4-4-*n*-Pr	65	$CHCl_3$	179–181	$C_{13}H_{14}N_3OCl{\cdot}0.125H_2O$	C, H, N
C_6H_4-4-CH_2OH	27	5% EtOH	183–185	$C_{11}H_{10}N_3OCl$	C, H, N

[a] The general reaction procedures are described in the Experimental Section.

large enough to destabilize a structure such as Xb. It is attractive to postulate that a charge-transfer interaction stabilizes structure Xb when the 5-substituent is Br and I, but there is no experimental evidence for this. Ultraviolet spectra show no essential difference between 5-methyl and 5-halo compounds (for example, the absorption maxima for **32** and **35** are both at 278 nm), nor do they reveal long-wavelength maxima commensurate with a charge-transfer interaction. Proton NMR experiments, in fact, clearly indicate that not only 5-alkyl but 5-halo inhibitors probably do not exist as structures such as X in Me_2SO-d_6 solution. The proton resonances of 6-(benzylamino)uracil that are expected to be involved in the pairing interaction with the DNA template, viz., 1-H and 6-NH, selectively undergo downfield shifts of 0.94 and 0.80 ppm, respectively, in the presence of a 7-fold excess of 1-methylcytosine. The corresponding resonances of 6-(benzylamino)thymine (**32**) and 5-iodo-6-(benzylamino)-uracil (**36**) do not shift more than 0.08 ppm under the same conditions, indicating that they do not interact appreciably in Watson–Crick fashion with cytosine;[1,6] i.e., they probably do not adopt a syn 6-NH/1-N relationship (cf. structures Xa and Xb) in solution. One can only conclude that the energies of interaction of the phenyl rings with enzyme and of charge-transfer stabilization are sufficient to overcome an initial steric barrier to the adoption of structure Xb for 5-halo compounds but not for 5-alkyl compounds that lack a stabilizing force for structure Xb.

The role of the 4-oxo group of isocytosine inhibitors such as **2** and **5** and of uracil inhibitors such as **1**, **3**, and **4** does not appear to be directly related to binding with DNA polymerase III. Rather, we conclude that significant space, likely occupied by ordered water molecules, intervenes between the 4-oxo group and the active site and that the 4-oxo group is probably hydrogen bonded to one or more of these waters. The results presented in Table II clearly indicate that considerable free space exists between the 4-position of the pyrimidine ring and the enzyme surface in the pol III–inhibitor–DNA template complex. This conclusion is supported indirectly by our studies of an analogous series of inhibitors of the replicative DNA polymerase α from mammalian cells. The prototype compound in this series, 6-(*p*-*n*-butylanilino)uracil, inhibited pol α from a variety of sources by a mechanism apparently identical with that observed for pol III inhibitors.[12] On the basis of the postulate that these inhibitors bind near the active sites of the polymerases, we prepared active purine derivatives, i.e., N^2-substituted guanines bearing substituents that determined selectivity for pol III or pol α.[13] In the pol α-specific series, we prepared N^2-(*p*-*n*-butylphenyl)guanine and its corresponding 2′-deoxyribonucleoside 5′-triphosphate. This latter substrate analogue, BuPdGTP, was an exceedingly potent inhibitor of pol α (K_i = ca. 0.001 μM),[14] a result consistent with the likelihood that it binds the substrate binding (active) site of pol α. A similar deoxyribonucleotide, for example, that derived from the pol III inhibitor N^2-(5-indanyl)guanine,[13] is expected to be a potent inhibitor of DNA polymerase III. We are beginning the synthesis and evaluation of such a nucleotide as a molecular probe of DNA polymerase III.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were done by Imperial Chemical Industries, Ltd., Pharmaceuticals Division (England), and are accurate to within 0.5% of calculated values unless otherwise noted. Proton nuclear magnetic resonance spectra were obtained at 60 MHz with a Perkin-Elmer R-12B instrument equipped with a Nicolet TT7 Fourier transform accessory or at 250 MHz with a Bruker WM 250 instrument; spectra obtained in Me_2SO-d_6 solutions were consistent with proposed structures. Ultraviolet spectra were determined with a Beckman Model 25 spectrophotometer. Thin-layer chromatographic analyses were performed on glass plates coated with silica gel K5F of 250-μm thickness for analytical work and 1000-μm thickness for preparative work (Whatman); R_f values obtained on an analytical scale were reproducible in preparative-scale separations. The details of enzyme isolation and assays are described elsewhere.[6]

The following compounds were prepared by reported methods: 2-amino-4,6-dichloropyrimidine,[15] 2-amino-4-methoxy-6-chloro- and 2-amino-4-ethoxy-6-chloropyrimidines,[16] 5-alkyl-6-chlorouracils,[17] 6-chloro-*s*-triazine-2,4-dione,[18] and compounds **1**, **3**, and **4**,[19] **10**, **11**, and **29**,[20] **13**,[21] **37**,[22] and **42**.[23] 3-Alkyl-6-chlorouracils

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(17) Langley, B. British Patent 845 378.

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Table VI. Synthesis and Characterization of Inhibitors[a]

compd	yield, %	cryst solvent	mp, °C	formula	anal.
2	62	H_2O	221–223	$C_{11}H_{12}N_4O_2$	C, H, N
5	53	EtOH	242–245	$C_{13}H_{14}N_4O \cdot 0.5EtOH$	C, H, N
6	61	50% HOAc	270–273	$C_{14}H_{15}N_3O_2$	C, H, N
7	89	EtOH	245–248	$C_{15}H_{17}N_3O_2$	C, H, N
8	25	EtOH	259–260	$C_{16}H_{19}N_3O_2$	C, H, N
9	82	80% HOAc	242–245	$C_{17}H_{21}N_3O_2 \cdot 0.125H_2O$	C, H, N
12	64	H_2O	154–155	$C_{12}H_{14}N_4$	C, H, N
14	26	5% EtOH	106–108	$C_{12}H_{14}N_4O \cdot 0.33EtOH$	C, H, N
15	35	5% EtOH	112–114	$C_{13}H_{16}N_4O \cdot 0.25H_2O$	C, H, N
16	43	5% EtOH	127–129	$C_{14}H_{18}N_4O \cdot 0.5EtOH$	C, H, N
17	48	5% EtOH	129–130	$C_{17}H_{22}N_4O \cdot 0.25H_2O$	C, H, N
18	43	5% EtOH	96–98	$C_{17}H_{16}N_4O \cdot 0.33H_2O$	C, H, N
19	36	5% EtOH	144–146	$C_{18}H_{18}N_4O \cdot 0.33H_2O$	C, H, N
20	64	5% EtOH	98–100	$C_{18}H_{18}N_4O \cdot 0.5H_2O$	C, H, N
21	42	5% EtOH	149–151	$C_{19}H_{20}N_4O \cdot 0.5H_2O$	C, H, N
22	45	5% EtOH	85–87	$C_{19}H_{20}N_4O$	C, H, N
23	46	5% EtOH	126–128	$C_{19}H_{20}N_4O \cdot 0.25H_2O$	C, H, N
24	74	5% EtOH	108–110	$C_{17}H_{15}N_4OCl \cdot 0.5H_2O$	C, H, N
25	47	5% EtOH	75–77	$C_{17}H_{15}N_4OBr$[b]	C, H, N
26	35	5% EtOH	74–76	$C_{19}H_{19}N_5O_2 \cdot 0.5EtOH \cdot 0.5H_2O$	C, H, N
27	51	5% EtOH	98–99	$C_{20}H_{22}N_4O \cdot 0.5H_2O$	C, H, N
28	40	$CHCl_3$	145–146	$C_{18}H_{18}N_4O_2$	C, H, N
30	48	Me_2CO/pet. ether	108–110	$C_{18}H_{18}N_4S \cdot 0.25H_2O$	C, H, N
31	35	Me_2CO/heptane	115–116	$C_{18}H_{17}N_4SCl$	C, H, N
32	18	75% HOAc	275–278	$C_{12}H_{13}N_3O_2$	C, H, N
33	17	EtOH	241–243	$C_{13}H_{15}N_3O_2$	C, H, N
34	42	50% HOAc	303–305	$C_{10}H_{10}N_4O_2$	C, H, N
35	24	50% HOAc	198–200	$C_{11}H_{10}N_3O_2Br$	C, H, N
36	10	dioxane	217–219	$C_{11}H_{10}N_3O_2I$	C, H, N
38	42	aq HOAc	271–273	$C_{12}H_{13}N_3O_2$	C, H, N
39	39	EtOH	245–247	$C_{13}H_{15}N_3O_2 \cdot 0.125EtOH$	C, H, N
40	33	HOAc	331–334	$C_{10}H_{10}N_4O_2$	C, H, N
41	43	EtOH	208–210	$C_{11}H_{10}N_3O_2Br \cdot 0.5EtOH$	C, H, N

[a] The general reaction procedures are described in the Experimental Section. [b] N: calcd, 15.09; found, 16.8 (two analyses).

were a gift from Imperial Chemical Industries, Ltd.

The general synthetic procedures for intermediates and inhibitors reported below are supplemented by details in Tables V and VI, respectively.

3-Alkyl-6-(5-indanylamino)uracils. 5-Indanylamine was heated at reflux in 2-methoxyethanol with the appropriate 3-alkyl-6-chlorouracil for 16 h. Dilution of the reaction mixture with water gave the products (**6–9**) whose yields and properties are described in Table VI.

2-Amino-4-oxy-6-chloropyrimidines. These intermediates were prepared by reaction between 2-amino-4,6-dichloropyrimidine and a salt of the appropriate alcohol or phenol. For lower boiling alcohols, the dichloropyrimidine was heated at reflux in a solution containing 1.1 mol equiv of sodium alkoxide in the alcohol. The cyclohexyloxy derivative was prepared similarly except that the reactants were heated at reflux in toluene. Aryloxy derivatives were made by reaction of the dichloropyrimidine with the potassium aryl oxide in anhydrous ethanol at reflux for 2 h. Yields and physical data for new compounds are presented in Table V.

2-Amino-4-oxy-6-(benzylamino)pyrimidines. These compounds were synthesized by heating the appropriate 2-amino-4-oxy-6-chloropyrimidine with 1.1 mol equiv of benzylamine for 8 h in refluxing 2-methoxyethanol. The major side product in these reactions was usually 2-amino-4,6-di(benzylamino)pyrimidine. After removal of the solvent in vacuo, the products were isolated by preparative TLC, and their characteristics are summarized in Table VI.

4-(Benzylthio)pyrimidines. Solutions of **11** in 1 N potassium hydroxide were stirred for 1.5 h with benzyl chloride and *p*-chlorobenzyl chloride (1.1 mol equiv). The precipitates were isolated by filtration and crystallized to give **31** and **32**, respectively, as described in Table VI.

5-Alkyluracil Derivatives. The 5-alkyl-6-(benzylamino)-uracils (**32**, **33**) and 5-alkyl-6-*p*-toluidinouracils (**38**, **39**) were prepared by heating a mixture of the 5-alkyl-6-chlorouracil and the amine in 2-methoxyethanol for 16 and 4 h, respectively. Physical data are presented in Table VI.

5-Bromo-6-(benzylamino)uracil (35). Bromine (324 mg, 2.0 mmol) was added to a stirred suspension of **1** (400 mg, 1.8 mmol) in glacial acetic acid (6 mL). After all the material had dissolved, water (ca. 20 mL) was added, and the precipitated solid was isolated by filtration. **5-Bromo-6-*p*-toluidino)uracil (41)** was prepared similarly, except that the product spontaneously crystallized from the reaction mixture. Details can be found in Table VI.

5-Iodo-6-(benzylamino)uracil (36). 6-(Benzylamino)uracil (300 mg, 1.38 mmol) was suspended in 1 N sodium hydroxide solution (34 mL). Iodine (351 mg, 1.38 mmol) was added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered, and the filtrate was brought to pH 5 with acetic acid to precipitate the product (Table VI).

5-Azauracils. 6-Chloro-*s*-triazine-2,4-dione was heated at reflux for 6 h with excess benzylamine and with *p*-toluidine to give, after dilution of the reaction mixtures with water and collection of products, compounds **34** and **40**, respectively (Table VI).

Determination of pK_a Values of 4-Substituted 2-Amino-6-(benzylamino)pyrimidines. The pK_a values listed in Table III were determined in aqueous solutions by using a spectrophotometric method. Stock solutions of 100 μM were prepared in 1% ethanol in water; the presence of ethanol was required to dissolve the compound initially. These stock solutions were diluted 2:1 into aqueous buffers. The buffers, acids, and bases used included 1.0 N HCl, 0.01 N sodium acetate, 0.0067 M Na_2HPO_4–KH_2PO_4, and 0.01 N KOH. These were prepared just before use from 1.0 N or 0.067 M stock solutions that were mixed to the approximate desired pH and diluted with freshly degassed distilled water. UV spectra were recorded at 25 °C in the 200–350-nm range. Absorbances were read at a selected analytical wavelength (determined from the initial scans) and pK_a values calculated from the average of three absorbance readings taken in solutions of close-range pH.

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Registry No. 1, 5759-80-8; 2, 60308-49-8; 5, 100763-38-0; 6, 100763-39-1; 7, 100763-40-4; 8, 100763-41-5; 9, 100763-82-4; 10, 91066-67-0; 11, 6944-08-7; 12, 100763-42-6; 13, 91333-15-2; 14, 100763-43-7; 15, 100763-44-8; 16, 100763-45-9; 17, 100763-46-0; 18, 100763-47-1; 19, 100763-48-2; 20, 100763-49-3; 21, 100763-50-6; 22, 100763-51-7; 23, 100763-52-8; 24, 100763-53-9; 25, 100763-54-0; 26, 100763-55-1; 27, 100763-56-2; 28, 100763-57-3; 29, 91769-84-5; 30, 100763-58-4; 31, 100763-59-5; 32, 24722-42-7; 33, 100763-60-8; 34, 100763-61-9; 35, 28484-80-2; 36, 100763-62-0; 37, 5770-18-3; 38, 100763-63-1; 39, 100763-64-2; 40, 100763-65-3; 41, 100763-66-4; 42, 7155-22-8; NaOPr, 6819-41-6; NaO-(c-C_6H_{11}), 22096-22-6; KoPh, 100-67-4; p-KOC_6H_4Me, 1192-96-7; m-KOC_6H_4Me, 36294-16-3; KOC_6H_3-3,4-diMe, 40590-38-3; p-KOC_6H_4Cl, 1121-74-0; p-KOC_6H_4NHAc, 35719-43-8; p-KOC_6H_4Et, 75121-14-1; p-KOC_6H_4Br, 3046-26-2; m-KOC_6H_4Et, 75121-13-0; p-KOC_6H_4Pr, 100763-67-5; p-$KOC_6H_4CH_2OH$, 100763-68-6; $PhCH_2NH_2$, 100-46-9; p-$NH_2C_6H_4Me$, 106-49-0; 2-amino-4-propoxy-6-chloropyrimidine, 100763-69-7; 2-amino-4-cyclohexyloxy-6-chloropyrimidine, 100763-70-0; 2-amino-4-phenoxy-6-chloropyrimidine, 100763-71-1; 2-amino-4-(p-tolyloxy)-6-chloropyrimidine, 100763-72-2; 2-amino-4-(m-tolyloxy)-6-chloropyrimidine, 100763-73-3; 2-amino-4-(3,4-xylyloxy)-6-chloropyrimidine, 100763-74-4; 2-amino-4-(p-chlorophenoxy)-6-chloropyrimidine, 100763-75-5; 2-amino-4-(p-acetamidophenoxy)-6-chloropyrimidine, 100763-76-6; 2-amino-4-(p-ethylphenoxy)-6-chloropyrimidine, 100763-77-7; 2-amino-4-(p-bromophenoxy)-6-chloropyrimidine, 100763-78-8; 2-amino-4-(m-ethylphenoxy)-6-chloropyrimidine, 100763-79-9; 2-amino-4-(p-propylphenoxy)-6-chloropyrimidine, 100763-80-2; 2-amino-4-(p-hydroxymethylphenoxy)-6-chloropyrimdine, 100763-81-3; 5-indanylamine, 24425-40-9; 2-amino-4,6-dichloropyrimidine, 56-05-3; 5-methyl-6-chlorouracil, 1627-28-7; 5-ethyl-6-chlorouracil, 20295-24-3; 6-chloro-s-triazine-2,4-dione, 69125-10-6; DNA polymerase, 9012-90-2.

Synthesis and Biological Activity of Several Amino Nucleoside–Platinum(II) Complexes

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Several platinum(II) complexes of 3′,5′-diamino-3′,5′-dideoxythymidine (compound **1**), 5′-amino-5′-deoxythymidine (compound **2**), and 3′-amino-3′-deoxythymidine (compound **3**) and the respective 2′-deoxyuridine amino nucleoside complexes, **4–6**, have been synthesized. Whereas compounds **1**, **2**, and **4–6** had no inhibitory effect on the replication of murine L1210 cells in cell culture, compound **3** [$(3'\text{-AdThd})_2PtCl_2$] inhibited these cells with an ED_{50} of 0.8 μM. Incubation of L1210 cells with 10–20 μM compound **3** for 2 h produced less than 18% inhibition of RNA, DNA, or protein synthesis, which is of questionable significance. However a 16-h incubation resulted in an increased uptake of labeled thymidine into DNA (77%), labeled uridine into RNA (17%), and labeled amino acids into protein (100%). These unexpected results indicate that inhibition of macromolecules may not be involved in the inhibition of the replication of L1210 cells. The increased incorporation of labeled metabolites into macromolecules may be related to the increase in cell volume after a 2-h incubation of L1210 cells with compound **3** plus a marked increase after 2 h in the proportion of cells in their S phase. Compound **3** appears to delay the progression of cells through their cell cycle. A marked inhibitory effect on the transport of methionine or aminoisobutyric acid into L1210 cells was found with compound **3**, which was slightly greater than that produced with cisplatin. Compound **3** had a dose-dependent effect on the survival of mice bearing the L1210 ascites neoplasm, with a T/C × 100 of 175 at a dose of 320 mg/kg. Investigation of the kinetics of decomposition in aqueous systems demonstrated that the primary UV-absorbing decomposition product is 3′-amino-3′-deoxythymidine and that only a limited amount of the compound is formed (<8%). Although 3′-amino-3′-deoxythymidine could account for a part of the inhibition of the replication of L1210 cells in culture, it cannot account for the inhibition of amino acid transport by compound **3**, the platinum complex of 3′-amino-3′-deoxythymidine. Compound **3** has been shown to limit part of the amino acid uptake into L1210 cells in a similar manner to cisplatin.

Amino analogues of thymidine are of interest because of their biological activities. The 5′-amino analogue of thymidine was first synthesized by Horwitz et al.[4] and found to have good antiviral activity against the replication of herpes simplex virus both in vitro[5–8] and in vivo.[8] The corresponding 3′-amino analogue of thymidine was synthesized by both Miller and Fox[9] and Horwitz et al.[10] and found to have potent inhibitory activity against the replication of both murine Sarcoma-180 and L1210 cells in vitro[7,11] and in vivo.[12] The 3′,5′-diamino analogue of

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