Synthesis, Cell Growth Inhibition, and Antitumor Screening of 2-(*p*-*n*-Butylanilino)purines and Their Nucleoside Analogues

George E. Wright,* Lech W. Dudycz, Zygmunt Kazimierczuk,† Neal C. Brown, and Naseema N. Khan

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.
Received April 28, 1986

Derivatives of N^2-(*p*-*n*-butylphenyl)guanine (BuPG) and 2-(*p*-*n*-butylanilino)adenine (BuAA) were synthesized and tested as inhibitors of mammalian DNA polymerase α, cell growth, and macromolecule synthesis. 2-(*p*-*n*-Butylanilino)-6-chloropurine (BuACl) served as a useful intermediate to prepare a series of 6-substituted analogues. BuACl, as its sodium salt, reacted with 2-deoxy-3,5-di-*p*-toluoyl-β-D-ribofuranosyl chloride in acetonitrile to give 64% of the corresponding 9-β nucleoside (blocked BuAdCl) and only 14% of the 7-β isomer. Deblocking and substitution of chlorine in BuAdCl generated a series of 2-(*p*-*n*-butylanilino)-9-(2-deoxy-β-D-ribofuranosyl)purine derivatives. Reaction of the sodium salt of BuACl with (2-acetoxyethoxy)methyl bromide also afforded, after deblocking and substitution of the 6-chloro group, a series of 2-(*p*-*n*-butylanilino)-9-[(2-hydroxyethoxy)methyl]purines. The bases synthesized were inhibitors of DNA polymerase α isolated from Chinese hamster ovary cells, the most potent compounds being 6-methoxy and 6-methylthio derivatives of 2-(*p*-*n*-butylanilino)purine. When tested for their ability to inhibit [^{3}H]thymidine incorporation into DNA in HeLa cell cultures and the growth of exponentially growing HeLa cells, 9-(2-deoxy-β-D-ribofuranosyl) derivatives had greater potency than their base counterparts, but "adenine" analogues, such as 2-(*p*-*n*-butylanilino)-2′-deoxyadenosine (BuAdA, IC_{50} = 1 μM), were considerably more potent than N^2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine (BuPdG, IC_{50} = 25 μM). Derivatives bearing the 9-[(2-hydroxyethoxy)methyl] group were nearly as potent inhibitors of [^{3}H]thymidine incorporation in these experiments as the corresponding deoxyribonucleosides. Base and deoxynucleoside derivatives also inhibited cellular RNA synthesis, and several compounds, at high concentrations, inhibited protein synthesis. BuPG, BuAA, and four deoxyribonucleoside derivatives of 2-(*p*-*n*-butylanilino)purines were tested against P-388 lymphocytic leukemia in mice. None of the compounds increased the survival time of test animals, but two of them, BuAdA and its 6-desamino derivative BuAdP, were lethal at the highest concentration used (400 mg/kg).

N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine 5′-triphosphate (BuPdGTP) and 2-(*p*-*n*-butylanilino)-2′-deoxyadenosine 5′-triphosphate (BuAdATP) are potent and selective inhibitors of DNA polymerase α, the replicative polymerase from mammalian cells.[1] These compounds act at the nanomolar level by competing with the analogous purine deoxyribonucleoside 5′-triphosphates. Like other highly ionized compounds these nucleotides do not possess significant cytotoxicity against mammalian cells in culture. The bases and nucleosides from which these inhibitors were derived, however, are cytotoxic. N^2-(*p*-*n*-Butylphenyl)guanine (BuPG),[2] for example, is an inhibitor of HeLa DNA polymerase α and the growth of HeLa cells in culture[3] and had a differential effect on the growth of various human normal and transformed cell lines in culture.[4] We thought that other purine analogues fitted with the DNA polymerase α specific 2-(*p*-*n*-butylanilino) group may display cytotoxicity and have potential for development as anticancer agents. In one approach to the design of selective cell growth inhibitors, we have prepared several 6-substituted purines to evaluate as polymerase inhibitors and as cell growth inhibitors. We also considered the possibility that nucleoside forms of 2-(*p*-*n*-butylanilino)-purines could be "activated" through phosphorylation by cellular kinases and generate in situ potent, nucleotide drug forms. Lastly, the current interest in modified nucleosides that can be phosphorylated by virus-specific kinases, e.g., bases containing "acyclonucleoside" side chains like (2-hydroxyethoxy)methyl, led us to prepare several analogous 2-(*p*-*n*-butylanilino)purine derivatives for preliminary cytotoxicity evaluation. This paper reports the synthesis of base, 2-deoxyribonucleoside, ribonucleoside, and "acyclonucleoside" analogues of 2-(*p*-*n*-butylanilino)purines, their properties toward DNA polymerase α and cell growth, and the results of antitumor testing of representative compounds in this series.

The *p*-*n*-butylphenyl group on the 2-amino group of guanine and the *p*-*n*-butylanilino group on position 2 of adenine afforded potent inhibitors with selectivity for mammalian DNA polymerase α, but no action against DNA polymerases β or γ. Inhibition of DNA polymerase α from HeLa,[3] calf thymus,[4] and Chinese hamster ovary (CHO) cells[1] by BuPG is competitive with dGTP, while inhibition of the enzyme from CHO cells[2] and HeLa cells (unpublished results) by BuAA is competitive with dATP. Competition by specific purine nucleotides is consistent with the inhibitor mechanism involving base pairing with complementary pyrimidine residues in the DNA template. We first sought to identify features of base structure that would determine inhibitory potency and mechanism prior to examining effects of compounds on mammalian cells, which require DNA polymerase α for growth.

Chemistry

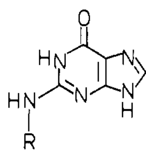
Both 2-chloro- and 2-bromohypoxanthines react with anilines in the presence of the aniline hydrochloride to give N^2-arylguanines.[3,5] N^2-(*p*-*n*-Butylphenyl)guanine (BuPG, **1**), and other analogues for this work, e.g., **2** and **3**, were made in good yields from 2-bromohypoxanthine. The prototype adenine analogue, BuAA (**5**), was prepared indirectly, by amination with methanolic ammonia at 150 °C of the 6-chloropurine derivative, BuACl (**4**). The 6-chloro derivative, obtained by chlorination of BuPG, proved to be a pivotal compound in this work for several reasons. First, **4** was found to be an active DNA polymerase inhibitor itself; second, it provided a convenient starting material for the synthesis of other 6-substituted purine bases, viz., the 6-amino (**5**), 6-H (**6**), and 6-methoxy (**8**) 2-(butylanilino)purines. Finally, BuACl is a valuable

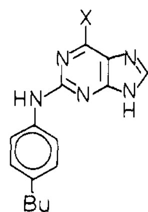
† Present address: Department of Biophysics, University of Warsaw.

(1) Khan, N. N.; Wright, G. E.; Dudycz, L. W.; Brown, N. C. *Nucleic Acids Res.* 1984, *12*, 3695; 1985, *13*, 6331.
(2) Acronyms used for all compounds referred to in this paper are collected in Table II.
(3) Wright, G. E.; Brown, V. M.; Baril, E. F.; Brown, N. C. *Nucleic Acids Res.* 1982, *10*, 4431.
(4) Rochowska, M.; Siedlecki, J.; Skurzak, H.; Wright, G.; Zmudzka, B. *Biochim. Biophys. Acta* 1982, *699*, 67.
(5) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* 1984, *27*, 175.

0022-2623/87/1830-0109$01.50/0 © 1986 American Chemical Society

starting material for the stereoselective preparation of β anomers of 2-deoxyribofuranosyl derivatives.



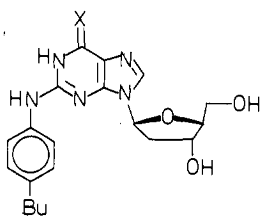


1, R = C_6H_4-4-(*n*-Bu)

2, R = C_6H_3-3,4-$(CH_2)_3$

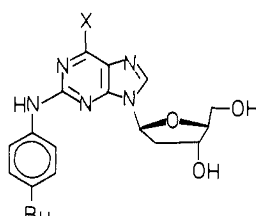
3, R = C_6H_3-3-Et-4-Me

4, X = Cl **5**, X = NH_2 **6**, X = H **8**, X = OMe **9**, X = SMe



10, X = O

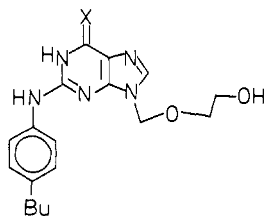
20, X = S



17, X = Cl

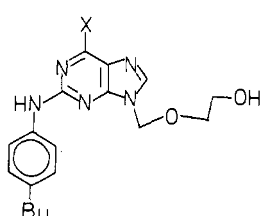
18, X = NH_2

19, X = H



25, X = O

29, X = S



23, X = Cl **27**, X = NH_2 **28**, X = H **30**, X = OMe

The 9-β-(2-deoxyribofuranosyl) derivative of BuPG, BuPdG (**10**), was previously prepared by direct glycosylation, affording **10** in about 18% yield, and by reduction of the corresponding 9-β-ribofuranosyl derivative **13** in 35% yield.[5] Low yields and laborious separation procedures prompted us to attempt a sodium salt glycosylation procedure, recently reported[6] to produce high yields (60–71%) of 9-β-(2-deoxyribofuranosyl) derivatives of 6-chloropurines. When a solution of **4** in acetonitrile was treated with sodium hydride and 2-deoxy-3,5-di-*p*-toluoyl-β-D-ribofuranosyl chloride, two nucleoside products were obtained. Chromatography on silica gel gave a compound identified as the 9-β isomer (**15**) in 64% yield and a minor component, the 7-β isomer (**16**), in 14% yield. The ^{1}H NMR spectra at 250 MHz suggested that both products were β anomers, e.g., H_1 triplets at δ 6.43 and 6.74 for **15** and **16**, respectively. The identity of **15** as the desired 9-β isomer was proved through its hydrolysis (and simultaneously deblocking) by 2-mercaptoethanol and sodium ethoxide to give a compound identical with authentic BuPdG (**10**).[5] Similarly, hydrolysis of **16** gave 7-β-BuPdG (**11**; results not shown).

Deblocking of **15** with sodium methoxide in methanol at room temperature gave BuAdCl (**17**). Like its base analogue **4**, this deoxyribofuranosyl compound could be converted unequivocally to several 6-substituted deoxyribonucleosides. Ammonoloysis of **17** with ammonia in methanol at 120 °C gave the deoxyadenosine derivative BuAdA (**18**) in 81% yield. Reduction of **17** with hydrogen and palladium on carbon gave the 6-unsubstituted nucleoside BuAdP (**19**) in 77% yield, while reaction with sodium hydrosulfide afforded 87% of the 6-thioxodeoxyguanosine analogue BuPdTG (**20**).

Table I. Inhibition of Chinese Hamster Ovary DNA Polymerase α by 2-Anilinopurines

no.	acronym	K_i,[a] μM	competitor[b]
1	BuPG	12	dGTP
2	TMPG	44	dGTP
3	EMPG	1760	dGTP
4	BuACl	2.6	dATP
5	BuAA	14.4	dATP
6	BuAP	5	dATP
7	BuPTG	36	dGTP
8	BuAOMe	1.4	dATP
9	BuASMe	1.0	dATP

[a] Concentration of compound to give half-maximal inhibition of enzyme activity in the absence of competitive substrate (truncated assay; see Experimental Section). [b] Added substrate required to antagonize inhibitory effect.

We also used the sodium salt method to prepare acyclonucleoside analogues. The sodium salt of compound **4** gave a mixture of 9- and 7-[(2-acetoxyethoxy)methyl] derivatives, **21** and **22**, upon treatment with (2-acetoxyethoxy)methyl bromide. Regioselectivity was less than in the case of the glycosylation reaction above: the 9-isomer was obtained in 44% yield and the 7-isomer in 22% yield. Structural assignments of **21** and **22** as 9- and 7-substituted isomers, respectively, are based primarily on the difference in ^{1}H NMR chemical shifts of 8-H and NCH_2O resonances in these and related compounds. In the 6-chloro blocked (**21** and **22**) and deblocked "acyclo" derivatives (**23** and **24**), 8-H and NCH_2O resonances appear upfield in the 9-isomers relative to those of the 7-isomers, a consistent finding in pairs of 9- and 7-[(2-hydroxyethoxy)methyl][7] and 9- and 7-[(dihydroxypropoxy)methyl]purine analogues.[8] This difference is also apparent in the relevant chemical shifts of the BuPG derivatives **25** and **26**, obtained after hydrolysis of **21** and **22**, respectively (see Experimental Section). UV spectra were less useful for these structural assignments a priori because the differences between λ_{max} of 9- and and 7-substituted isomers of 2-(*p*-*n*-butylanilino)purines is small relative to those of guanines and because their relative magnitudes are opposite. For example, λ_{max} (pH 13) of **25** is 282 nm and that of **26** is 271 nm. (Compare with comparable λ_{max} values for guanosine (261 nm) and 7-(β-D-ribofuranosyl)guanine (283 nm).) In general, we have observed that UV spectra of nucleoside derivatives of BuPG follow a similar pattern: the 2′-deoxyribonucleosides **10** and **11** have λ_{max} (pH 13) values of 278 and 266 nm, respectively,[5] and the ribonucleosides **13** and **14** have λ_{max} (pH 13) values of 281 and 273 nm, respectively (unpublished results). Thus, the use of **21** as a common precursor to the guanine (**25**), adenine (**27**), 6-H (**28**), 6-thioxo (**29**), and 6-methoxy (**30**) analogues of 2-(*p*-*n*-butylanilino)purines assures that all are the 9-[(2-hydroxyethoxy)methyl] isomers. Each of these 6-substituted "acyclonucleosides" was obtained in high yield (see Experimental Section).

(6) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379.

(7) Parkin, A.; Harndon, M. R. *J. Heterocycl. Chem.* **1982**, *19*, 33.

(8) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.

Results

Inhibition of DNA Polymerase α. We have employed DNA polymerase α (pol α) isolated from CHO cells as the model enzyme for determining the structural basis for 2-anilinopurine inhibition of pol α. Inhibitory potencies and competitive substrates for inhibition of CHO pol α by the series of base analogues of 2-anilinopurines **1–9** are presented in Table I. The assay involved activated calf thymus DNA as template primer and measured the incorporation of [^{3}H]TMP into acid-insoluble product (see Experimental Section). Modifications in the phenyl ring substituents of N^2-phenylguanines caused profound changes in potency; for example, compound **3** was about 150-fold less potent than BuPG (**1**), whereas a related compound bearing a 3,4-trimethylene group (**2**) was only 3-fold less active. Other modifications in the N^2-phenyl group consistently caused a decrease in inhibitory activity relative to **1** (unpublished results).

Although weaker in potency, BuPG derivatives such as **2** and **3** are still competitive with dGTP. The 6-thioxoguanine derivative **7** was 3-fold less potent than **1**, perhaps reflecting a decrease in efficiency of Watson–Crick-like hydrogen bonding with cytosine residues in the DNA template. BuAA (**5**), the prototype adenine analogue, was roughly equipotent with **1** and, as expected from previous work,[1] was competitive with dATP. Surprisingly, the other 6-substituted derivatives (**4**, **6**, **8**, and **9**) were *more* potent than **5** and, although they lack a hydrogen bond donating group at C-6, were competitive exclusively with dATP (see Table I). The most potent 6-substituted analogue, BuASMe (**9**), was at least 10-fold more potent than BuAA and still behaved mechanistically as a "dATP analogue". These results are fully in accord with experimental[9] and theoretical[10] evidence that the related O^6-methylguanine both hydrogen bonds with thymine residues in DNA and acts as a 2′-deoxyadenosine residue in DNA templates. By analogy, 2-(butylanilino)purines with nontautomeric groups at position 6 are apparently able to base pair with thymines in a DNA template strand and retain affinity, even enhanced affinity, to the DNA polymerase α which they inhibit.

Effects on Cell Growth and Macromolecule Synthesis. As a potential indicator of cytotoxicity, the compounds synthesized for this study were tested for their capacity to inhibit replicative DNA synthesis by cells in culture. For these experiments, cultures of exponentially growing HeLa S_3 cells were treated with compounds, and after 3 h, [^{3}H]thymidine was added to the cultures. After 30 min, the cells were analyzed for content of alkali-stable, acid-precipitable [^{3}H]DNA (see Experimental Section). The results of Table II show that all compounds tested inhibited DNA synthesis but by very different degrees. N^2-Phenylguanine analogues without the *p*-*n*-butyl substituent showed both less (**3**) and greater activity (**2**) than BuPG itself. Although TMPG (**2**) was somewhat weaker as a polymerase inhibitor (see Table I), it is marginally more potent in cell cultures, perhaps suggesting a permeability barrier to **1** or significant binding of **1** by proteins in the culture medium.

All of the base-modified analogues of BuPG (**4–9**) were modestly more active than BuPG as inhibitors of [^{3}H]thymidine incorporation by HeLa cells. For example, BuAA (**5**) was about 3-fold more potent than **1** in this assay, but the most potent polymerase inhibitor, BuASMe (**9**), was only twice as potent as **1**.

Table II. Effects of 2-Anilinopurines and Related Nucleoside Analogues on DNA Synthesis by HeLa Cells in Culture[a]

no.	acronym	% inhibn of [^{3}H]thymidine incorporation by 100 μM compds	inhibn of [^{3}H]thymidine incorporation: IC_{50}, μM
1	BuPG	63	69
2	TMPG	79	52
3	EMPG	44	nd[c]
4	BuACl	99	35
5	BuAA	92	23
6	BuAP	78	47
7	BuPTG	94	40
8	BuAOMe	80	30
9	BuASMe	72	32
10	BuPdG	86	37
11	7-β-BuPdG	53[b]	nd
12	7-α-BuPdG	46[b]	nd
13	BuPGr	75[b]	nd
14	7-β-BuPGr	38[b]	nd
17	BuAdCl	97	11
18	BuAdA	96	1
19	BuAdP	97	4.8
20	BuPTdG	63	nd
23	acBuACl	99	9
25	acBuPG	81	38
27	acBuAA	97	8.5
28	acBuAP	97	4.8
29	acBuPTG	87	nd
30	acBuAOMe	80	nd

[a] Compounds dissolved in Me_2SO or Me_2SO alone were added to exponentially growing HeLa cells in suspension culture. After 3 h, [*methyl*-^{3}H]thymidine was added to give 1 μCi/mL. After 30 min, the cultures were processed for DNA content (see Experimental Section). Control incorporation corresponded to 6900 cpm [^{3}H]-DNA/1 mL of culture. [b] Compounds tested at 200 μM. [c] nd, not done.

Among the 2-deoxyribofuranosyl derivatives of BuPG, the 9-β isomer BuPdG (**10**) showed enhanced activity (IC_{50} = 37 μM) and the 7-β and 7-α isomers, **11** and **12**, were considerably less active (estimated IC_{50} = ca. 200 μM) than BuPG itself. Interestingly, the 9-β-ribofuranosyl derivative of BuPG, **13**, at 200 μM caused 75% inhibition of thymidine incorporation, while the 7-β ribonucleoside (**14**) had only modest activity. Collectively, the results indicate the substitution of BuPG at the 9- and 7-positions with 2-deoxyribofuranosyl or ribofuranosyl groups did not cause significant increases in inhibitory potency in these experiments. These results argue against either active uptake of the nucleoside forms by cells or activation by phosphorylation to more potent nucleotide forms (see Discussion).

Alteration of BuPdG at the 6-position did produce significant changes in inhibition of HeLa DNA synthesis. The 6-thioxo derivative **20** was less potent than **10**, but the deoxyadenosine analogue BuAdA (**18**), with an observed ID_{50} of 1 μM, was 37 times more potent than **10**. Indeed, the other 6-substituted deoxyribonucleosides were also more potent than BuPdG, the 6-chloro (**17**) and 6-H (**19**) nucleosides being, respectively, 3- and 8-fold more potent than **10**.

Analogous 2-(*p*-*n*-butylanilino)-6-substituted-purines fitted with the (2-hydroxyethoxy)methyl ("acyclo") group at the 9-position also showed enhanced activity against [^{3}H]thymidine incorporation by HeLa cells in comparison with the corresponding base analogues (Table II). The activities of the guanine analogue acBuPG (**25**) and the 6-chloro (**23**) and 6-H (**28**) derivatives were virtually indistinguishable from those of the corresponding 9-(2-deoxyribofuranosyl) analogues. In contrast, the 6-amino

(9) Kierdaszuk, B.; Stolarski, R.; Shugar, D. *Eur. J. Biochem.* **1983**, *130*, 559.
(10) Pohorille, A.; Lowe, G. H. *Biophys. Chem.* **1985**, *22*, 37.

Table III. Effects of Selected Compounds on Growth and Macromolecule Synthesis by HeLa Cells[a]

compd	cell growth inhibn: IC_{50}, μM	[^{3}H]thymidine incorporation: IC_{50}, μM	% inhibn of [^{3}H]leucine incorporation by 100 μM compds
1 (BuPG)	65	69	13
5 (BuAA)	40	23	47
10 (BuPdG)	25	37	7
17 (BuAdCl)	10.3	11	87
18 (BuAdA)	1.4	1	56
19 (BuAdP)	5	4.8	58

[a] Growth inhibition was determined from cell counts after exposure to compounds or solvent (Me_2SO) after 72 h in culture. Measurements of [*methyl*-^{3}H]thymidine in DNA were done as described for the results in Table II. Determination of [^{3}H]leucine incorporation into protein involved incubation of exponentially growing HeLa cells for 3 h in the presence of 1 μCi/mL [4,5-$^3H_2$]-leucine (see Experimental Section).

compound **27**, although a little more potent than the base BuAA, was 8-fold *less* potent than the 9-(2-deoxyribofuranosyl) derivative BuAdA (**18**).

In order to correlate inhibition of DNA synthesis with cell-growth inhibition, to evaluate the selectivity of DNA replication as the target of the cytotoxic effect, and to select compounds for antitumor assays, certain analogues were studied for their ability to inhibit HeLa growth in culture. Table III summarizes growth inhibition and DNA- and protein-synthesis inhibition by several key compounds in this series. In each case IC_{50} values for cell growth and [^{3}H]thymidine incorporation inhibition were virtually identical, indicating that inhibition of DNA synthesis correlates roughly with cytotoxicity. Several of the compounds, most notably those that were *not* guanine analogues, also inhibited protein synthesis by HeLa cells in culture, although only at much higher concentrations than those required to inhibit DNA synthesis. Table III shows that inhibition of [^{3}H]leucine incorporation into protein by BuAA (**5**) and by the 6-substituted deoxyribofuranosyl derivatives **17–19** was significant at 100 μM, a concentration where BuPG and BuPdG had little effect.

The results shown in Table III suggested that inhibition of replicative DNA synthesis alone could account for cytotoxicity of this series of compounds. Our original reports concerning the cellular target of "butylphenyl" polymerase inhibitors demonstrated that BuPG (**1**) and its pyrimidine ancestor, 6-(*p*-*n*-butylanilino)uracil (BuAU), selectively inhibited DNA synthesis by HeLa cells in culture, having no observable effect on RNA or protein synthesis.[3,11] We tested several of the new analogues reported here as well as BuPG for their effects on [^{3}H]uridine incorporation into nucleic acids by HeLa cells. The results of Table IV indicate that BuPG and BuAA (**5**) and their 9-(2-deoxyribofuranosyl) derivatives inhibitied [^{3}H]uridine incorporation into *both* RNA and DNA with equal potency.

The assay for RNA in the presence of [^{3}H]uridine is based on the difference between radioactivity found in total acid-precipitable nucleic acids (a small but measurable amount is found in DNA) and that fraction remaining in a sample incubated with NaOH to hydrolyze RNA. To ascertain if the procedure was, in fact, reliably measuring RNA synthesis, we tested aphidicolin, a selective DNA synthesis inhibitor,[12] under the same conditions. At 10 μM, aphidicolin suppressed incorporation of label by 84% into

Table IV. Effect of Compounds on [^{3}H]Uridine Incorporation by HeLa Cells[a]

compd	% inhibition into DNA	% inhibition into RNA
1 (BuPG), 100 μM	76	76
5 (BuAA), 100 μM	93	87
10 (BuPdG), 100 μM	91	91
18 (BuAdA), 10 μM	81	73
aphidicolin, 10 μM	84	0

[a] Compounds dissolved in Me_2SO or Me_2SO alone were added to exponentially growing HeLa cells in suspension culture. After 3 h, [5,6-$^3H_2$]uridine was added to give 1 μCi/mL. After 45 min, the cultures were processed for determination of DNA and RNA (see Experimental Section). Control incorporation corresponded to 3500 cpm [^{3}H]DNA and 14950 cpm [^{3}H]RNA per 1 mL of culture.

only alkali-stable DNA and had no effect on alkali-labile RNA (Table IV).

To determine the significance of the observation that BuPG inhibited both cellular DNA and RNA synthesis in these experiments, we tested effects of duration of exposure of HeLa cells to BuPG (1–3 h) and duration of exposure to [^{3}H]uridine (1–45 min) on RNA and DNA synthesis. The results (not shown) confirmed that 100 μM BuPG, under any combination of these conditions, inhibited both RNA and DNA synthesis equally, whereas aphidicolin (10 μM) remained absolutely selective as an inhibitor of DNA synthesis. Finally, experiments were conducted with synchronized HeLa cell cultures in S phase in which [^{3}H]uridine incorporation into DNA was nearly as extensive as that into RNA. Inhibition of *both* RNA and DNA synthesis following continuous exposure of synchronized cells to 0.5 μCi/mL of [^{3}H]uridine was observed for BuPG (**1**), BuAA (**5**), and their 9-(2-deoxyribofuranosyl) derivatives **10** and **18**, respectively, whereas aphidicolin affected incorporation of label only into DNA (results not shown).

Antitumor Testing. The six compounds shown in Table III, **1**, **5**, **10**, **17–19**, were tested for in vivo antitumor activity in the P-388 lymphocytic leukemia model in mice according to National Cancer Institute protocols.[13] None of the compounds at doses as high as 400 mg/kg demonstrated antitumor activity in this system compared to the positive control compound, 5-fluorouracil.

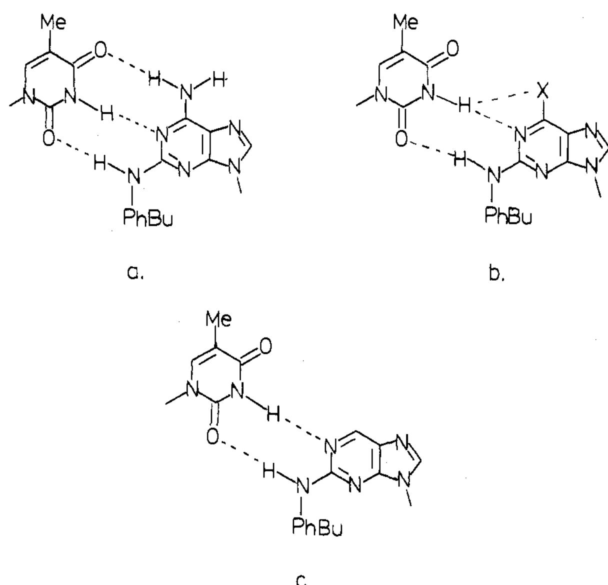
Except for **18** and **19**, the compounds were generally not toxic to mice as evidenced by only modest changes in average body weights after 5 days of treatment with **1**, **5**, **10**, and **17**. However, **18** and **19** at 400 mg/kg were lethal to the test animals. Compound **18** (BuAdA), in fact, after a single dose of 400 mg/kg and **19** (BuAdP) after two such doses killed all the test animals in each group. The lethality of these compounds may be related to their generally more potent cytotoxic effects and, as suggested from the results of Tables III and IV, other sites of action in addition to DNA replication.

Discussion

N^2-Substituted guanines represent a novel class of inhibitors of replicative DNA polymerases. The substituent on the 2-amino group of guanine apparently dictates selectivity of the compound by providing affinity for a site adjacent to the active site of a sensitive DNA polymerase. N^2-(*p*-*n*-Butylphenyl)guanine (**1**) was originally designed as a selective inhibitor of mammalian DNA polymerase α, whereas N^2-(3,4-trimethylenephenyl)guanine (TMPG, **2**) and N^2-(3-ethyl-4-methylphenyl)guanine (EMPG, **3**) more strongly inhibit DNA polymerase III from *Bacillus subtilis*

(11) Wright, G. E.; Brown, N. C.; Baril, E. F. *Nucleic Acids Res.* **1980**, *8*, 99.
(12) Ikegami, S.; Taguchi, T.; Ohashi, M.; Oguro, M.; Nagano, H.; Mano, Y. *Nature (London)* **1978**, *275*, 458.
(13) In Vivo Cancer Models, NIH Publication No. 84-2635, Feb. 1984, p 27.

Chart I



(ref 3 and unpublished results).

When attached to an appropriate base, the *p-n*-butylphenyl substituent, as demonstrated in this and related papers,[1,3,4] causes inhibition of DNA polymerase α from a variety of mammalian cells. Our purpose in this work was to probe the inhibitor binding site on a pol α with specifically modified inhibitors, with a view toward designing one or more compounds that would be strongly cytotoxic and have potential for displaying anticancer activity.

The results of testing of base analogues of the prototype compound BuPG (**1**) as pol α inhibitors indicate that there are two broad classes of inhibitors: those like **1** and its 6-thioxo analogue **7** that are competitive with the substrate dGTP, and those with nontautomeric substituents, e.g., the adenine analogue **5** and compounds **4**, **6**, **8**, and **9**, that are competitive with dATP. This classification results from the required interaction of these compounds with the DNA template in the reaction, in addition to their interaction with the enzyme. It is well-established that inhibitors must form Watson–Crick-like hydrogen bonds with either cytosine or thymine residues on single-stranded DNA, presumably at the primer terminus of template–primer DNA.[14,15] A postulated structure for one complex, that between a BuAA and a thymine residue, is shown in Chart I as structure a. The activity of compounds that bear nontautomeric substituents at position 6 implies that these inhibitors are able to use the 2-NH group to form a hydrogen bond with the thymine O^2 (Chart I, structure a), and this interaction must compensate for the loss of hydrogen-bond donation from the 6-NH_2 group to the more orthodox thymine O^4. In this context, the calculated configuration of the related *syn*-O^6-methylguanine (O^6-MeG)–thymine (T) base pair shows that, in addition to the O^2(T)···NH_2(G) hydrogen bond, the 3-NH of T may interact simultaneously with the N-3 and O^6 acceptor atoms.[10] By analogy, such a structure (Chart I, structure b, X = OMe) may explain the capacity of the 6-methoxy inhibitor **8** to act as a dATP analogue in inhibition of pol α. For the 6-H compound **6**, which lacks a substituent at C-6, potency as a pol α inhibitor and reversibility by dATP suggest that it interacts as depicted in structure c (Chart I) with thymine. The observation that the 6-methylthio derivative **9** is the most potent, dATP-competitive inhibitor of pol α within this series could be related to a greater interaction energy between **9** and thymine in a configuration that optimizes 3-NH(T)···S(BuASMe) hydrogen bonding.

There is virtually no information available on the biochemical effects on N^2 substitution of guanine or guanine nucleosides. The present work, in particular, provides no evidence that 2-(*p-n*-butylanilino)purine derivatives are substrates of, for example, nucleoside kinases. No significant enhancement of inhibitory activity on cell growth or DNA synthesis by ribonucleoside or deoxyribonucleoside derivatives compared to bases was observed (Tables II and III). If nucleosides had been concentrated by cells or if nucleotides had been formed, such as the 5′-triphosphates known to be exceedingly potent pol α inhibitors,[1] we would have expected greatly enhanced inhibitory effects in cell cultures compared with those on isolated DNA polymerase α. Although it is possible that the nucleosides are degraded to the corresponding bases to produce cytotoxic effects, it should be noted that the nucleoside forms of BuPG are themselves reversible inhibitors of DNA polymerase α. For example, BuPdG (**10**) was slightly more potent than BuPG as an inhibitor of HeLa DNA polymerase α, but the 7-β and 7-α isomers **11** and **12** were about 10-fold weaker inhibitors than **10**.[5]

Among the "dATP" analogues, the relatively greater potencies of the deoxyribofuranosyl derivatives compared to those of BuPG can be partially explained by their greater potencies as polymerase inhibitors. However, the adenine derivative BuAdA (**18**), a derivative of the weakest polymerase inhibitor at the base level (see Table I), was the most potent cell-growth inhibitor in the series (Table III). The reason for this is not clear, although preliminary experiments suggest that cytotoxic effects may be nonselective or at least involve other targets in addition to DNA polymerase α. For example, BuPG (**1**) and BuPdG (**10**) at 100 μM had little effect on protein synthesis by HeLa cells in culture, but the same concentration of BuAA (**5**) and the nucleosides BuAdCl (**17**), BuAdA (**18**), and BuAdP (**19**) caused significant inhibition of protein synthesis (Table III). In this regard, BuAdCl (**17**), although 10-fold less potent than BuAdA (**18**) as a cell-growth inhibitor, was more potent as a protein-synthesis inhibitor. Tests of cellular RNA synthesis suggested that compounds in this series are equally effective inhibitors of both RNA and DNA synthesis, in contrast to our earlier reports about this class of compounds that demonstrated selective inhibition of cellular DNA synthesis.[3,11] The mechanism(s) by which the highly cytotoxic nucleosides (**17–19**) inhibit cell growth and macromolecule synthesis is being studied.

The presence of the 9-[(2-hydroxyethoxy)methyl] group on 2-(*p-n*-butylanilino)purines had little effect on their ability to inhibit [^{3}H]thymidine incorporation by HeLa cells (Table II), a result that argues against any metabolism of such "acyclonucleosides" by cellular enzymes. Rather, the differences in potency roughly parallel those of the corresponding bases and deoxyribofuranosyl derivatives (see above). Indeed, at the level of purified DNA polymerase α, the acyclo compounds inhibited the enzyme with potencies similar to those of the bases (results not shown). Preliminary experiments also indicate that none of the acyclo compounds (**23–30**) inhibits the growth of the herpes viruses, HSV-1 and -2 (P. Medveczky and G. E. Wright, unpublished results) measured by DNA dot–blot hybridization assays.

(14) Mackenzie, J. M.; Neville, M. M.; Wright, G. E.; Brown, N. C. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 512.
(15) Wright, G. E.; Brown, N. C. *Biochim. Biophys. Acta* **1976**, *432*, 37.

Although the results of screening of selected 2-(*p*-*n*-butylanilino)purine analogues did not show protection from P-388 lymphocytic leukemia in mice, it is intriguing that the two toxic compounds, **18** and **19**, also are the most potent inhibitors of HeLa growth and DNA synthesis (Table III). We do not know if their lethality to mice is a direct result of their effect on cellular nucleic acid metabolism or if other targets, e.g., protein synthesis as suggested in the results of Table III, may be involved.

The Nature of the Cellular Target for BuPG and Related Compounds. We have consistently observed in this and related work[3,4] that the potencies of *guanine*-based derivatives, e.g., BuPG (**1**), BuPdG (**10**), BuPGr (**13**), as inhibitors of mammalian cell growth and DNA synthesis are lower than their potencies as DNA polymerase α inhibitors. Although cellular-permeability barriers, inactivation, and/or strong protein binding may limit intracellular concentrations of these compounds, it is also possible that the target enzyme as it participates in DNA replication is inherently less sensitive to the inhibitors than the isolated and purified DNA polymerase α. This latter possibility has been suggested by recent findings with permeabilized mammalian cells in culture, in which replicative and repair DNA synthesis can be observed in situ by measuring incorporation of labeled dNTPs directly into DNA.[16] In such experiments, BuPdGTP and BuAdATP, nucleotide derivatives that inhibit purified pol α with K_i values of 1–10 nM, are much less potent as inhibitors of "pol α" catalyzed replication and repair (IC_{50} of BuPdGTP = ca. 10 μM) in permeabilized human diploid fibroblast cells (S. L. Dresler, personal communication). In addition, BuPG and BuPdG at 100 μM had little effect on either process. In contrast, aphidicolin, another pol α inhibitor, did not differ in inhibitory potency between purified DNA polymerase α and in situ DNA replication when studied under the same conditions.[16] These results suggest, at a minimum, that pol α exists in vivo in a replication complex with characteristics that make it relatively BuPG/BuPdGTP-insensitive. Assuming that pol α is indeed the major target for cytotoxic activity of these compounds, the biologically active form of DNA polymerase α may be more sensitive to N^2-substituted guanines and related inhibitors that have substituents other than *p*-*n*-butylphenyl. The finding that a less potent pol α inhibitor, TMPG (**2**) (Table I), was more potent than BuPG as a DNA synthesis inhibitor in HeLa cell cultures (Table II) is consistent with this hypothesis.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by the Microanalysis Laboratory, University of Massachusetts, Amherst; experimental analyses were within ±0.4% of calculated values unless noted. NMR spectra were obtained at 250 MHz with a Bruker WM250 instrument; chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane. Proton resonances characteristic of the *p*-*n*-butylphenyl group were observed for all relevant compounds, but are not reported. In proton spectra of the 2-deoxy-β-D-ribofuranosyl groups of compounds **17**–**20**, only the positions of 1′-H are reported; first-order analysis showed other sugar proton chemical shifts and coupling constants in these compounds virtually identical with those reported for **10**.[5] UV spectra were obtained with a Beckman Model 25 spectrophotometer. Labeled compounds were obtained from New England Nuclear. Aphidicolin was a gift from Dr. J. Douros, Division of Cancer Treatment, National Cancer Institute.

***N*²-(3-Ethyl-4-methylphenyl)guanine (3).** A solution of 2-bromohypoxanthine (0.5 g, 2.32 mmol), 3-ethyl-4-methylaniline and its hydrochloride (2.32 mmol each) in 2-methoxyethanol (6 mL), and water (3 mL) was heated at reflux for 12 h. The cooled reaction mixture was diluted with water (75 mL), and the crude product was isolated by filtration. Crystallization from DMF–H_2O (1:1) gave 0.34 g (54%) of **3**: mp 227–230 °C; NMR (Me_2SO-d_6) δ 10.5 (s, 1 H, 1-H), 8.20 (s, 1 H, 2-NH), 7.83 (s, 1 H, 8-H), 7.39 (d, 1 H, Ph6-H), 7.33 (s, 1 H, Ph2-H), 7.10 (d, 1 H, Ph5-H), 2.5 (CH_2, obscured by solvent), 2.22 (s, 3 H, PhMe), 1.17 (t, 3 H, CH_3). Anal. ($C_{14}H_{15}N_5O\cdot0.67H_2O$) C, N; H: calcd, 5.85; found, 5.40.

2-(*p*-*n*-Butylanilino)-6-chloropurine (4). A solution of **1** (3.5 g, 12.4 mmol) in phosphoryl chloride (35 mL) containing *N*,*N*-dimethylaniline (4 mL) was heated at reflux for 10 min. The reaction mixture was poured slowly into ice–water (600 mL). After 2 h the solution was brought to pH 3 with sodium acetate, and the yellow solid was collected by filtration. Crystallization from 50% aqueous ethanol gave 3.6 g (96%) of **4**: mp 174–175 °C; NMR (Me_2SO-d_6) δ 9.70 (s, 1 H, 2-NH), 8.27 (s, 1 H, 8-H). Anal. ($C_{15}H_{16}N_5Cl$) C, H, N.

2-(*p*-*n*-Butylanilino)adenine (5). A solution of **4** (3.2 g, 10.7 mmol) in methanol saturated with ammonia (250 mL) was heated at 150 °C during 16 h. The solution was concentrated to 50 mL, decolorized with carbon, filtered, and acidified with acetic acid. The precipitate was collected and purified on a silica gel column. Elution with 5% methanol in chloroform gave, upon concentration of the eluate, 1.97 g (65%) of **5** as a colorless crystals: mp 252–255 °C. The product could also be crystallized from aqueous methanol: NMR (Me_2SO-d_6) δ 12.3 (s, 1 H, 9-H), 8.58 (s, 1 H, 2-NH), 7.78 (s, 1 H, 8-H), 6.73 (s, 2 H, 2-NH_2). Anal. ($C_{15}H_{18}N_6$) C, H, N.

2-(*p*-*n*-Butylanilino)purine (6). A mixture of compound **4** (1 g, 3.3 mmol), potassium carbonate (0.6 g), and 10% Pd/C (0.6 g) was shaken under a hydrogen atmosphere (50 psi) for 10 h. The reaction mixture was filtered through Celite and concentrated to about 2 mL. This solution was added dropwise to 60 mL of stirred water. The solid precipitate was isolated and crystallized from methanol to give **6** (0.68 g, 77%) as a colorless solid: mp 207–208 °C; NMR (Me_2SO-d_6) δ 9.33 (s, 1 H, 2-NH), 8.78 (s, 1 H, 6-H), 8.20 (s, 1 H, 8-H). Anal. ($C_{15}H_{17}N_5\cdot0.25H_2O$) C, H, N.

2-(*p*-*n*-Butylphenyl)-6-thioguanine (7). A suspension of **1** (3.4 g, 12 mmol) and phosphorus pentasulfide (10 g) in pyridine (130 mL) was heated at reflux for 3 h. The dark solution was poured into water (800 mL), and the resulting precipitate was purified twice by dissolving in 1 N sodium hydroxide and precipitating the product with acetic acid. The yield of nearly colorless **7** was 2.5 g (70%): mp >310 °C; NMR (Me_2SO-d_6) δ 11.92 (s, 1 H, 1-H), 8.73 (s, 1 H, 2-NH), 8.10 (s, 1 H, 8-H). Anal. ($C_{15}H_{17}N_5S$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-methoxypurine (8). A solution of **4** (302 mg, 1 mmol) in 10 mL of 1 N sodium methoxide in methanol was heated at reflux. After 24 h, 3 mL of 1 N sodium methoxide in methanol was added, and reflux was continued for 24 h. The reaction mixture was acidified by the addition of glacial acetic acid (1 mL) and poured into 50 mL of H_2O. The precipitate was filtered to give 255 mg (86%) of nearly pure **8**. Crystallization from methanol gave pale cream colored **8**: mp 189–192 °C; NMR (Me_2SO-d_6) δ 9.12 (s, 1 H, 2-NH), 7.95 (s, 1 H, 8-H), 4.05 (s, 3 H, OCH_3). Anal. ($C_{16}H_{19}N_5O\cdot0.25MeOH$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-(methylthio)purine (9). A solution of **7** (0.31 g, 1.04 mmol) in a mixture of 0.1 N sodium hydroxide (10 mL) and ethanol (10 mL) was treated with iodomethane (73 μL, 1.16 mmol) in ethanol (2 mL). After 0.5 h the precipitated product (0.315 g, 97%) was isolated and crystallized from aqueous ethanol to give **9**: mp 193–195 °C; NMR (Me_2SO-d_6) δ 12.8 (s, 1 H, 9-H), 9.25 (s, 1 H, 2-NH), 8.04 (s, 1 H, 8-H), 2.66 (s, 3 H, SCH_3). Anal. ($C_{16}H_{19}N_5S\cdot0.5H_2O$) H, N; C: calcd, 59.60; found, 59.12.

2-(*p*-*n*-Butylanilino)-6-chloro-9-(2-deoxy-3,5-di-*p*-toluoyl-β-D-ribofuranosyl)purine (15) and Its 7-Isomer (16). A mixture of **4** (2.7 g, 9 mmol) and sodium hydride (50% suspension in mineral oil, 0.47 g, 9.8 mmol) in acetonitrile (75 mL) was stirred at room temperature for 20 min. 2-Deoxy-3,5-di-*p*-toluoyl-β-D-ribofuranosyl chloride (3.6 g, 9.3 mmol) was added in small portions during 20 min, and stirring was continued for 1 h. The mixture was diluted with chloroform (100 mL) and filtered through Celite. The filtrate was evaporated to dryness and the residue chromatographed on a silica gel column (4 × 40

(16) Dresler, S. L. *J. Biol. Chem.* **1984**, *259*, 13947.

cm). Elution with a gradient of toluene–5% acetone in toluene (4 L) gave two main products. The first was crystallized from ethanol to give 3.75 g (64%) of the 9-isomer (15) as colorless crystals: mp 143–144 °C; NMR ($CDCl_3$) δ 6.43 (t, 1 H, 1′-H). Anal. ($C_{36}H_{36}N_5O_5Cl$) C, H, N. The second product, 0.82 g (14%) of the 7-isomer (16), was isolated as a foam: mp 129–132 °C; NMR (Me_2SO-d_6) δ 6.74 (t, 1 H, 1′-H). Anal. ($C_{36}H_{36}N_5O_5Cl$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-chloro-9-(2-deoxy-β-D-ribofuranosyl)purine (17). A stirred solution of 15 (4 g, 6.12 mmol) in methanol (200 mL) was treated with a solution of 1 N sodium methoxide in methanol (12 mL). After 24 h the solution was evaporated to dryness, and the residue was chromatographed on a silica gel column (4 × 30 cm). Elution with 10% methanol in chloroform (2 L) gave the product, which was crystallized from aqueous ethanol to give 1.95 g (76%) of 17: mp 159–161 °C; NMR (Me_2SO-d_6) δ 9.66 (s, 1 H, 2-NH), 8.42 (s, 1 H, 8-H), 6.36 (t, 1 H, 1′-H). Anal. ($C_{20}H_{24}N_5O_3Cl$) C, H, N.

Selective Hydrolysis of 17. A solution of the 6-chloro nucleoside 17 (0.84 g, 2 mmol) in ethanol (40 mL) was treated with 0.7 mL (9.9 mmol) of 2-mercaptoethanol and a solution of 1 N sodium methoxide in methanol (3 mL). After being heated at reflux for 72 h, the solution was diluted with water (50 mL). Acidification of the solution to pH 5 with acetic acid precipitated a colorless solid (0.76 g, 95%), which was chromatographically and spectrally identical with authentic **10**.

2-(*p*-*n*-Butylanilino)-2′-deoxyadenosine (18). A mixture of 17 (0.7 g, 1.67 mmol) in 25% methanolic ammonia (16 mL) was heated in a sealed bomb at 120 °C for 5 h. After evaporation to dryness, the residue was chromatographed on a silica gel column (2.5 × 30 cm). Elution with a gradient of chloroform–10% methanol in chloroform gave the product, which was crystallized from methanol to give 0.54 g (81%) of **18** as a colorless solid: mp 167–169 °C; NMR (Me_2SO-d_6) δ 8.62 (s, 1 H, 2-NH), 8.05 (s, 1 H, 8-H), 6.31 (t, 1 H, 1′-H). Anal. ($C_{20}H_{26}N_6O_3$) C, H; N: calcd, 21.11, found, 20.35.

2-(*p*-*n*-Butylanilino)-9-(2-deoxy-β-D-ribofuranosyl)purine (19). A mixture of 17 (0.4 g, 0.96 mmol), potassium carbonate (0.2 g), and 10% Pd/C (0.35 g) in 90% ethanol (50 mL) was shaken under a hydrogen atmosphere (50 psi) for 4 h. The mixture was filtered through Celite, the filtrate evaporated to dryness, and the residue chromatographed on a silica gel column (2 × 10 cm). The product was eluted with 10% methanol in chloroform and crystallized from aqueous ethanol, yielding 0.28 g (77%) of **19**: mp 125–127 °C; NMR (Me_2SO-d_6) δ 9.48 (s, 1 H, 2-NH), 8.79 (s, 1 H, 6-H), 8.41 (s, 1 H, 8-H), 6.38 (t, 1 H, 1′-H). Anal. ($C_{20}H_{25}N_5O_3 \cdot H_2O$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-thioxo-9-(2-deoxy-β-D-ribofuranosyl)purine (20). Sodium hydrosulfide hydrate (0.4 g) was added to a solution of 17 (0.4 g, 0.96 mmol) in ethanol (25 mL). After 30 min at reflux the solution was treated with an additional 0.4 g of sodium hydrosulfide hydrate. After 1 h at reflux the mixture was diluted with water and the solution brought to pH 5 with acetic acid. The pale yellow solid was filtered and crystallized from aqueous ethanol to give 0.35 g (87%) of **20**: mp 145–148 °C; NMR (Me_2SO-d_6) δ 8.17 (s, 1 H, 8-H), 6.23 (t, 1 H, 1′-H). Anal. ($C_{20}H_{25}N_5O_3S \cdot H_2O$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-chloro-9-[(2-acetoxyethoxy)methyl]purine (21) and Its 7-Isomer (22). A suspension of 4 (1.55 g, 5.15 mmol) and sodium hydride (50% in mineral oil; 0.26 g, 5.4 mmol) in dry acetonitrile (110 mL) was stirred at room temperature for 30 min. (2-Acetoxyethoxy)methyl bromide (1.08 g, 5.5 mmol) was added, and stirring was continued for 20 min. The reaction mixture was filtered through Celite and concentrated, and the residue was dissolved in chloroform (200 mL). The organic solution was washed with water (4 × 100 mL), dried (sodium sulfate), and evaporated to dryness. The residue was chromatographed on a silica gel column (2 × 25 cm) with 1% isopropyl alcohol in chloroform. The first product was crystallized from ethanol to give 0.94 g (44%) of the 9-isomer (**21**): mp 123–125 °C; NMR ($CDCl_3$) δ 7.94 (s, 1 H, 8-H), 5.58 (s, 2 H, NCH_2O), 4.22 (t, 2 H, $COOCH_2$), 3.78 (t, 2 H, CCH_2O), 2.03 (s, 3 H, CH_3CO). Anal. ($C_{20}H_{24}N_5O_3Cl$) C, H, N. The second eluate was crystallized from aqueous ethanol, giving 0.47 g (22%) of 7-isomer (**22**): mp 111–112 °C; NMR ($CDCl_3$) δ 8.13 (s, 1 H, 8-H), 5.72 (s, 2 H, NCH_2O), 4.22 (t, 2 H, $COOCH_2$), 3.71 (t, 2 H, CCH_2O), 2.03 (s, 3 H, CH_3CO). Anal. ($C_{20}H_{24}N_5O_3Cl$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-chloro-9-[(2-hydroxyethoxy)methyl]purine (23). A solution of **21** (0.245 g, 0.58 mmol) in ethanol (15 mL) and concentrated ammonium hydroxide (15 mL) was heated at 40 °C for 6 h. After evaporation to dryness the residue was purified by chromatography on a silica gel column (2 × 20 cm). The product, eluted with 20% isopropyl alcohol in chloroform, was crystallized from ethanol–hexane to give **23** as fine crystals (0.18 g, 82%): mp 139–140 °C; UV λ_{max} (methanol) 275 nm (ε 28 100); NMR (Me_2SO-d_6) δ 9.89 (s, 1 H, 2-NH), 8.40 (s, 1 H, 8-H), 5.59 (s, 2 H, NCH_2O), 3.54 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{22}N_4O_2Cl$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-chloro-7-[(2-hydroxyethoxy)methyl]purine (24). A solution of **22** (0.35 g, 0.84 mmol) in methanol saturated with ammonia at 0 °C (20 mL) was stored at room temperature for 48 h. The solution was evaporated to dryness, and the residue was crystallized from 50% aqueous ethanol to give 0.232 g (74%) of **24**: mp 96–99 °C; UV λ_{max} (methanol) 271 nm (ε 32 400); NMR (Me_2SO-d_6) δ 9.67 (s, 1 H, 2-NH), 8.68 (s, 1 H, 8-H), 5.73 (s, 2 H, NCH_2O), 3.50 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{22}N_4O_2Cl$) C, H, N.

N^2-(*p*-*n*-Butylphenyl)-9-[(2-hydroxyethoxy)methyl]guanine (25). A solution of **21** (0.42 g, 1 mmol) in ethanol (45 mL) was treated with 2-mercaptoethanol (0.3 mL, 4.3 mmol) and sodium ethoxide (3 mL of a 1 M solution in ethanol), and the solution was heated at reflux. After 24 h acetic acid (0.5 mL) was added and the solution was concentrated to 5 mL. Water (5 mL) was added, and the precipitate was isolated by filtration and crystallized from water to give 0.3 g (85%) of **25**: mp 276–277 °C; UV λ_{max} (methanol) 275 nm (ε 23 300); NMR (Me_2SO-d_6) δ 10.5 (s, 1 H, 1-NH), 8.73 (s, 1 H, 2-NH), 7.93 (s, 1 H, 8-H), 5.46 (s, 2 H, NCH_2O), 3.54 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{23}N_5O_3$) C, H, N.

N^2-(*p*-*n*-Butylphenyl)-7-[(2-hydroxyethoxy)methyl]guanine (26). A solution of **22** (0.34 g, 0.8 mmol) in ethanol (40 mL) was treated with 2-mercaptoethanol (0.25 mL, 3.6 mmol) and sodium ethoxide (2.5 mL of a 1 M solution in ethanol), and the solution was heated at reflux for 2.5 h. Acetic acid (0.5 mL) was added and the solution was concentrated to half-volume. Water (15 mL) was added, and the gelatinous precipitate was collected by filtration and crystallized from ethanol, yielding 0.18 g (63%) of **26**, softens at 190 °C and then decomposes: UV λ_{max} (methanol) 266 nm (ε 22 700); NMR (Me_2SO-d_6) δ 10.7 (s, 1 H, 1-NH), 8.45 (s, 1 H, 2-NH), 8.20 (s, 1 H, 8-H), 5.64 (s, 2 H, NCH_2O), 3.49 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{23}N_5O_3$) H, N; C: calcd, 60.50; found, 59.91.

2-(*p*-*n*-Butylanilino)-9-[(2-hydroxyethoxy)methyl]adenine (27). A solution of **21** (0.5 g, 1.2 mmol) in 20% methanolic ammonia (7 mL) was heated in a bomb at 120 °C for 4 h. The solution was evaporated to dryness and the residue crystallized from aqueous ethanol to yield 0.25 g (58%) of **27**: mp 187–189 °C; NMR (Me_2SO-d_6) δ 8.77 (s, 1 H, 2-NH), 7.96 (s, 1 H, 8-H), 5.47 (s, 2 H, NCH_2O), 3.52 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{24}N_6O_2 \cdot 0.5H_2O$) C, H, N.

2-(*p*-*n*-Butylanilino)-9-[(2-hydroxyethoxy)methyl]purine (28). A mixture of **21** (0.42 g, 1 mmol), potassium carbonate (0.28 g), and 10% Pd/C (0.35 g) in 90% ethanol (35 mL) was shaken under a hydrogen atmosphere (50 psi) for 12 h. The mixture was diluted with ethanol (50 mL) and filtered through Celite. The filtrate was evaporated to dryness, annd the residue was dissolved in ethanol (30 mL) containing concentrated ammonium hydroxide (2 mL). After being allowed to stand for 18 h at room temperature, the solution was poured into water (60 mL) and the precipitate was isolated. Crystallization from aqueous ethanol gave 0.28 g (81%) of **28**: mp 139 °C; NMR (Me_2SO-d_6) δ 9.55 (s, 1 H, 2–NH), 8.82 (s, 1 H, 6-H), 8.34 (s, 1 H, 8-H), 5.59 (s, 2 H, NCH_2O), 3.53 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{23}N_5O_2$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-thioxo-9-[(2-hydroxyethoxy)methyl]purine (29). A solution of **21** (0.42 g, 1 mmol) and sodium hydrosulfide hydrate (0.3 g) in ethanol (25 mL) was heated at reflux for 30 min. Additional sodium hydrosulfide (0.4 g) was added and heating continued for 30 min. The reaction solution was added dropwise to water (50 mL) and the solution was brought to pH 5 with acetic acid. The precipitate was isolated and dissolved in ethanol (20 mL) containing concentrated ammonium hydroxide (5 mL). The solution was diluted with water (50 mL), heated, and acidified to pH 5 with acetic acid and allowed to cool

to room temperature. The light yellow product was collected by filtration and crystallized from aqueous ethanol to give 0.325 g (87%) of **29**: mp 210–212 °C; NMR (Me_2SO-d_6) δ 12.08 (s, 1 H, 1-NH), 9.14 (s, 1 H, 2-NH), 8.28 (s, 1 H, 8-H), 5.66 (s, 2 H NCH_2O), 3.69 (s, 4 H, $(CH_2)_2$). Anal. ($C_{18}H_{23}N_5O_2S$) C, H, N.

2-(*p*-*n*-Butylanilino)-6-methoxy-9-[(2-hydroxyethoxy)-methyl]purine (30). A solution of **21** (0.42 g, 1 mmol) and 1 M sodium methoxide in methanol (3 mL) in methanol (20 mL) was heated at reflux for 2 h. The solution was diluted with water (50 mL) and brought to pH 5 with acetic acid. The collected precipitate was crystallized from aqueous ethanol, giving 0.35 g (94%) of **30**: mp 140–141 °C; NMR (Me_2SO-d_6) δ 9.36 (s, 1 H, 2-NH), 8.14 (s, 1 H, 8-H), 5.54 (s, 2 H, NCH_2O), 4.08 (s, 3 H, OCH_3), 3.52 (s, 4 H, $(CH_2)_2$). Anal. ($C_{19}H_{25}N_5O_3 \cdot 0.5H_2O$) C, H, N.

Growth of HeLa Cells. HeLa S_3 cells were a gift from Dr. E. Baril. They were grown in suspension culture at densities of ca. (3–7) × 10^5 cells/mL in Joklik's modified Eagle's medium with 3.5% each of calf and fetal bovine serum.[17] Cell counts were determined by hemocytometer or with a Coulter counter. For certain experiments cells were synchronized by the double hydroxyurea block technique[18] using 0.5 mM hydroxyurea at each block and an initial cell density of (2–3) × 10^5 cells/mL.

Growth Inhibition. Exponentially growing HeLa cells (ca. 2.5 × 10^4 cells/mL) in medium were distributed into spinner flasks and incubated for 3 h at 37 °C. Inhibitors dissolved in Me_2SO or an equivalent volume of Me_2SO were added, and an aliquot of cells was removed for counting (t = 0). Aliquots were removed at 24, 48, and/or 72 h and counted in a Coulter counter. Percent inhibition of cell growth was calculated as previously described.[4]

DNA Polymerase α. This enzyme was isolated from Chinese hamster ovary (CHO) cells and assayed as described.[19] Inhibitor assays were done with activated DNA in the absence of expected competitor nucleotides (dGTP or dATP), and under these truncated conditions, K_i values for inhibitors were measured directly as the concentration giving half-maximal inhibition of enzyme activity.

Macromolecule Synthesis by HeLa Cells. DNA. A suspension of exponentially growing HeLa cells (7 × 10^5 cells/mL) was distributed in 3-mL aliquots to sterile, capped glass tubes. Drug solutions in Me_2SO or Me_2SO for controls were added, and the cell suspensions were incubated at 37 °C in a shaking bath. After 3 h a solution of [*methyl*-^{3}H]thymidine was added to each tube to give a specific activity of 1 μCi/mL. After 30 min incorporation was quenched by the addition of 3 mL of ice-cold phosphate-buffered saline (pH 7), and the mixture was centrifuged at 2000 rpm for 10 min. The supernatant was aspirated and 1 mL of 1 M sodium hydroxide was added. After incubation at 65 °C for 1 h, 1 mL of ice-cold 20% trichloroacetic acid (TCA) containing 20 mM sodium pyrophosphate was added, and after chilling in ice for 15 min, the solution was filtered through GF/C disks (Whatman). The filters were washed with 1% TCA and ethanol, dried, and counted.

Protein. Exponentially growing HeLa cells (7 × 10^5 cells/mL) in suspension were treated with 1 μCi/mL [4,5-^{3}H]leucine and distributed in 2-mL aliquots in sterile glass tubes. Me_2SO or drug solutions in Me_2SO were added, and the suspensions were incubated in a shaking bath at 37 °C for 3 h. Cold solutions of bovine serum albumin and 25% TCA (200 μL) were added to each tube, the suspensions were centrifuged at 2000 rpm for 10 min, and the precipitates were washed three times with 0.5 mL of 0.1 N HCl and dissolved in 0.5 mL of 1 N NaOH. The solutions were heated for 30 min at 60 °C, neutralized with 10 N HCl (50 μL), diluted with 4 mL of Aqueous Counting Scintillator (A.C.S., Amersham), and counted.

RNA. Aliquots (0.5 mL) of suspensions of exponentially growing HeLa cells (7 × 10^5 cells/mL) in sterile glass, capped tubes were treated with inhibitor solutions in Me_2SO or equal volumes of Me_2SO. After 3 h at 37 °C [5,6-$^3H_2$]uridine was added to each culture to give 1 μCi/mL. After incubation for 45 min at 37 °C, aliquots were worked up for determination of (1) DNA alone and (2) DNA and RNA. (1) NaOH (1 M, 0.5 mL) was added to each tube, and the solutions were incubated at 60 °C for 1 h. One milliliter of cold 20% TCA containing 20 mM sodium pyrophosphate was added to each tube, and the solutions were chilled for 15 min. Workup and counting were done as described above for thymidine incorporation. (2) Total nucleic acids were determined by adding 0.5 mL of cold 10% TCA containing 10 mM sodium pyrophosphate to each tube, chilling for 15 min, centrifuging, and working up as described above for thymidine incorporation.

Antitumor Assays. Drug tests in vivo against the P-388 lymphocytic leukemia in mice were done by EG and G Bogden Laboratories, Worcester, MA. Test compounds and 5-fluorouracil dispersed in Klucel were administered intraperitoneally, and the results were evaluated according to the NCI protocol.[13]

Acknowledgment. The technical assistance of Homayoun Nazarian and James Mitchener is greatly appreciated. NMR data at 250 MHz were obtained at the Worcester Consortium NMR Facility at Clark University, supported by the National Science Foundation (Grant DMR 8108697). This work was supported by grants from the National Institutes of Health (GM21747) and from the Milheim Foundation for Cancer Research.

Registry No. 1, 83173-14-2; **3**, 104715-65-3; **4**, 104715-66-4; **5**, 99304-81-1; **6**, 104715-67-5; **7**, 104715-68-6; **8**, 104715-69-7; **9**, 104715-70-0; **10**, 87781-96-2; **15**, 104715-71-1; **16**, 104715-72-2; **17**, 104715-73-3; **18**, 99304-82-2; **19**, 104715-74-4; **20**, 104715-75-5; 21, 104715-76-6; **22**, 104715-77-7; **23**, 104715-78-8; **24**, 104715-79-9; **25**, 104715-80-2; **26**, 104715-81-3; **27**, 104715-82-4; **28**, 104715-83-5; **29**, 104715-84-6; **30**, 104715-85-7; 2-bromohypoxanthine, 87781-93-9; 3-ethyl-4-methylaniline, 104715-64-2; 3-ethyl-4-methylaniline hydrochloride, 87986-23-0; 2-deoxy-3,5-di-*p*-toluoyl-β-D-ribofuranosyl chloride, 52304-86-6; (2-acetoxyethoxy)methyl bromide, 81777-40-4.

Supplementary Material Available: Table summarizing chemotherapy response of P-388 lymphocytic leukemia to 2-(*p*-*n*-butylanilino)purines (1 page). Ordering information is given on any current masthead page.

(17) Baril, E.; Mitchener, J.; Lee, L.; Baril, B. *Nucleic Acids Res.* **1977**, *4*, 2641.
(18) Walters, R. A.; Tobey, R. A.; Hildebrand, C. E. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 212.
(19) Khan, N. N.; Brown, N. C. *Mol. Cell. Biochem.* **1985**, *68*, 169.