KOH at room temperature for 4 h. Usual workup of the reaction and crystallization of the product furnished **6** almost quantitatively.

3-[4-(Acetyloxy)phenyl]-1,2-diphenyl-2-propen-1-one (10). This was prepared by acetylation of the phenol **9** wth acetic anhydride in the presence of excess of pyridine. Usual workup of reaction and crystallization of the product furnished **10**.

1,3-Bis[4-(acetyloxy)phenyl]-2-phenyl-2-propen-1-one (13). This was prepared by acetylation of the mixture of phenols **12a** and **12b** with acetic anhydride in the presence of pyridine, followed by crystallization of the reaction product.

Biology. Materials. [2,3,6,7-^{3}H]Estradiol ([^{3}H]E_2, 100 Ci mmol^{-1}) was purchased from New England Nuclear Corp. and was assessed as >95% radiochemically pure by use of a Panax radio TLC scanner. Unlabeled estradiol (E_2) was obtained from Steraloids Inc., activated charcoal, Norit A, from Sigma Chemicals, and Dextran T-70 from Pharmacia Fine Chemicals. All other chemicals and reagents were of analytical or scintillation grade.

Female mice (21–23-days old, 8–12 g of body weight) of Swiss strain and rats (21–23-days old, 25–40 g of body weight) of Charles Foster strain were obtained from the CDRI rodent colony. For receptor binding experiments the mice were primed subcutaneously with 1 μg of E_2 each, 24 h prior to use, to increase the yield of the receptor protein in their uteri.

Preparation and Handling of the Test Solutions. Owing to the susceptibility of the TAPs to isomerization, particularly on light exposure, their stock solutions in DMF–buffer (1:1, v/v) for RBA assays and in propylene glycol–saline (1:1, v/v) for the bioassays were prepared without undue warming and with minimal light exposure. The solutions were, as far as possible, kept refrigerated and protected from light during the course of their use. Under these conditions nearly all the compounds retained their geometrical identities as assessed by periodic TLC examination.

Receptor Binding Experiments. Receptor binding procedures were essentially the same as reported earlier.[26] Briefly, the competition experiments with uterine cytosols were performed at 4 °C, 18-h incubation, and with triplicate tubes for each competitor concentration. Each incubate (260 μL) in TEA buffer (Tris-HCl, 10 mmol; EDTA, 1.65 mmol; NaN_3, 0.02%; pH 7.4) was 0.4 equiv in uteri, 7% in DMF, 2×10^{-9} M in [^{3}H]E_2, and $\sim 10^{-4}$ to 10^{-9} in the competitors. For the separation of free from bound [^{3}H]E_2, each incubate was treated at 4 °C for 15 min with a 100-μL aliquot of charcoal–dextran slurry (2.5% and 0.25%, w/v, respectively) in TEA buffer. Radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer in 10 mL of methanol–toluene–dioxane mixture (1.5:2.5:2.5, v/v), containing 0.5% PPO, 0.01% POPOP, and 9% naphthalene.

Each experiment was performed in duplicate and the RBA values were always within 10% of each other.

Bioassays. For uterotrophic assay, various doses of the test compounds in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v) were injected subcutaneously to the animals in groups of 6–10 on three consecutive days, while the control group received similar injections of the vehicle alone. The animals were autopsied 24 h after the last injection, and their uterine weights were recorded.

Antiuterotrophic assays were performed similarly, but in this case the animals were coadministered 1 μg of E_2 each in the same vehicle but at a different site. The control group in this case received 1 μg of E_2 plus the vehicle alone, also at two different sites.

Acknowledgment. We are grateful to Dr. A. H. Todd of ICI Ltd., U.K., for supplying a gift sample of tamoxifen.

Registry No. **1a**, 7474-65-9; **1b**, 7512-67-6; **2a**, 94348-26-2; **2b**, 94348-27-3; **3a**, 63645-39-6; **3b**, 94348-28-4; **4a**, 94348-29-5; **4b**, 94348-30-8; **5a**, 94348-31-9; **5b**, 94348-32-0; **6**, 94369-84-3; **7a**, 94369-85-4; **7b**, 94369-86-5; **8a**, 34236-65-2; **8b**, 34236-57-2; **9**, 94348-22-8; **10**, 94348-23-9; **11a**, 94348-33-1; **11b**, 94348-34-2; **12a**, 94348-35-3; **12b**, 94348-36-4; **13**, 94348-24-0; **14a**, 94348-37-5; **14b**, 94348-38-6; **15**, 94348-25-1; 2-[4-(acetyloxy)phenyl]-1-phenylethanone, 94348-39-7; 2-(4-hydroxyphenyl)-1-phenylethanone, 6420-90-2; β-pyrrolidinoethyl chloride hydrochloride, 7250-67-1.

Supplementary Material Available: ^{1}H NMR and mass spectral data of all the compounds (3 pages). Ordering information is given on any current masthead page.

5-Cinnamoyl-6-aminouracil Derivatives as Novel Anticancer Agents. Synthesis, Biological Evaluation, and Structure–Activity Relationships

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A biological evaluation in the series of 5-cinnamoyl-6-aminouracils has been undertaken. These compounds have been found to be in an extended planar conformation fitting well with a possible stacking interaction between the nucleic bases of DNA; thus an eventual anticancer activity by intercalation could be hoped. 1,3-Dimethyl-5-cinnamoyl-6-aminouracil was found to be active when administered ip against ip-implanted P388 leukemia in vivo (percent T/C = 124). Two other compounds, 1,3-dimethyl-5-cinnamoyl-6-[(2-morpholinoethyl)amino]uracil and 1,3-dimethyl-5-cinnamoyl-6-[(2-piperidinoethyl)amino]uracil, bearing a hydrophilic side chain on the 6-amino group, have exhibited cytoxic activity in vitro against L1210 leukemia. Structure–activity relationships have been determined from these results and from studies of biological interactions with DNA.

Uracil derivatives play an important role in the field of biology as fundamental constituents of nucleic acids. In medicinal chemistry, 1,3-dimethyl-6-aminouracil (**1**) is well-known as a starting compound for the synthesis of a number of xanthines related to theophyllin.[1] In our hands, from this molecule **1**, new condensed heterocycles have been synthetized, which often exhibited interesting pharmacological activity.[2–4] In the course of those studies, we have pointed out that 1,3-dimethyl-6-aminouracil derivatives substituted at the 5-position by a carbonyl group were conformationally stabilized by a strong hydrogen bond between the CO group and one of the protons of the 6-amino groups.[5] The latter concept led us to the design

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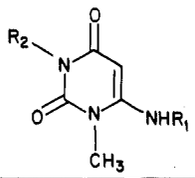
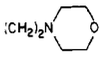
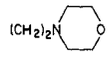
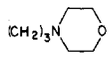
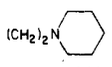
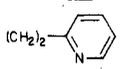
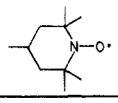
[§] Laboratoire de Biochimie.

(1) Traube, W. *Ber.* **1900**, *33*, 3035.
(2) Bernier, J. L.; Lefebvre, A.; Lespagnol, C. *Eur. J. Med. Chem.* **1977**, *12*, 239.
(3) Bernier, J. L.; Lefebvre, A.; Lespagnol, C. *Eur. J. Med. Chem.* **1977**, *12*, 341.
(4) Bernier, J. L.; Hénichart, J. P.; Warin, V.; Baert, F. *J. Pharm. Sci.* **1980**, *69*, 1343.
(5) Bernier, J. L.; Lefebvre, A.; Hénichart, J. P.; Houssin, R.; Lespagnol, C. *Bull. Soc. Chim. Fr.* **1976**, *3*, 617.

0022-2623/85/1828-0497$01.50/0

Table I



compd	R_1	R_2	% yield	mp, °C	formula	m/e (M^+)	IR ($\nu_{C=O}$), cm^{-1}
1	H	CH_3	83	295	$C_6H_9N_3O_2$	155	1700
2	H	H	81	290	$C_5H_7N_3O_2$	141	1700
3	H		68	216	$C_{11}H_{18}N_4O_3$	254	1680
4		CH_3	80	128	$C_{12}H_{20}N_4O_3$	268	1700
5		CH_3	77	124	$C_{13}H_{22}N_4O_3$	282	1700
6		CH_3	71	116	$C_{12}H_{20}N_4O_2$	252	1705
7		CH_3	68	110	$C_{13}H_{22}N_2O_2$	266	1705
8		CH_3	78	168	$C_{13}H_{17}N_4O_2$	261	1710
9	$(CH_2)_2N(CH_3)_2$	CH_3	87	101	$C_{10}H_{18}N_4O_2$	226	1705
10	$(CH_2)_3N(CH_3)_2$	CH_3	83	102	$C_{11}H_{20}N_4O_2$	240	1700
11		CH_3	76	248	$C_{15}H_{25}N_4O_3$	309	1700

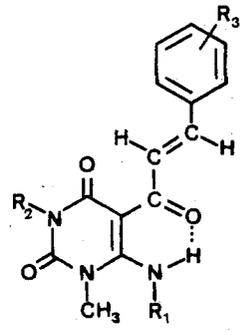
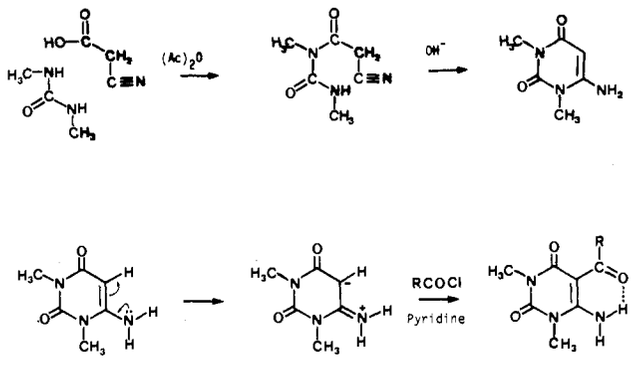


Figure 1. View of 16 drawn by PLUTO. Crystal data are as follows: M_r ($C_{21}H_{27}N_4O_4Cl$) = 434.9 orthorhombic space group *Pcba*, a = 24.635 (9) Å, b = 14,463 (3) Å, c = 12.147 (6) Å, $\alpha = \beta = \gamma = 90.0°$, V = 4327.92 Å^3, Z = 8, D(calcd) = 1.32, D(measd) = 1.33 mg m^{-3}, (Cu Kα) = 1.5418 Å, and R = 0.049 for 1423 considered as observed $F > 6\sigma$ (F).

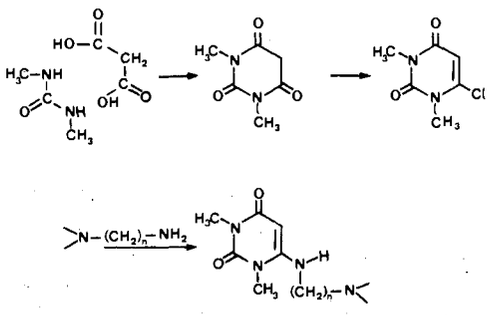
of new polycyclic molecules, by the introduction of a cinnamoyl group at the 5-position of 1,3-dimethyl-6-aminouracil derivatives. Such a compound would possess a polycyclic planar structure with a uracil moiety exhibiting a possible affinity for the DNA and with an extended aromatic moiety corresponding to the cinnamoyl group. The whole molecule if planar could give a complete overlap of the DNA base pairs and thus could be considered as a good candidate for intercalation as other well-known polycyclic antitumor drugs such as actinomycin or daunorubicin. On the basis of this model, we have introduced hydrophilic or lipophilic groups either to enhance water solubility or to provide a range of hydrophilic–lipophilic balance (see Figure 1). Furthermore, a molecule (**15**) was substituted at the 5-position by a carboxyphenethyl group in order to check the importance of the planarity on the activity.

Chemistry. Compound **1** was prepared by condensation of dimethylurea with cyanoacetic acid, followed by cyclization in alcaline medium (Scheme I). Compounds **4–11** (see Table I) were obtained from 1,3-dimethyl-6-chloro-

Scheme I



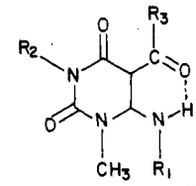
Scheme II

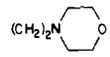
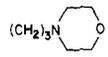
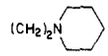
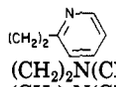
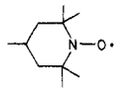
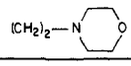


uracil and corresponding amines in the presence of sodium carbonate in ethanolic solution (Scheme II). Compound **3** was obtained by the action of (chloroethyl)morpholine on the 3-methyl-6-aminouracil in the presence of sodium ethylate. Compounds **12–25** (see Table II) were prepared by electrophilic attack of acyl chlorides on these uracils (Scheme I).

Physical Properties. The planarity of the molecules was ascertained either in solution by ^{1}H NMR or in the solid state by X-ray crystallography. In solution, ^{1}H NMR results showed that 1,3-dimethyl-5-acyl-6-aminouracils

Table II



compd	R_1	R_2	R_3	% yield	mp, °C	formula	m/e (M^+)	IR ($\nu_{C=O}$), cm^{-1}
12	H	CH_3	$CH{=}CHC_6H_5$	88	245	$C_{15}H_{15}N_3O_3$	285	1730
13	H	CH_3	$CH{=}CHC_6H_4OCH_3$	93	240	$C_{16}H_{17}N_3O_4$	315	1730
14	H	CH_3	$CH{=}CHC_6H_2(OCH_3)_3$	83	242	$C_{16}H_{17}N_3O_6$	375	1710
15	H	CH_3	$CH_2CH_2C_6H_5$	79	178	$C_{15}H_{17}N_3O_3$	287	1710
16		CH_3	$CH{=}CHC_6H_5$	83	202	$C_{21}H_{26}N_4O_4{\cdot}HCl$	398	1720
17		CH_3	$CH{=}CHC_6H_5$	76	164	$C_{22}H_{28}N_4O_4{\cdot}HCl$	412	1720
18		CH_3	$CH{=}CHC_6H_5$	74	214	$C_{21}H_{26}N_4O_3{\cdot}HCl$	382	1720
19		CH_3	$CH{=}CHC_6H_5$	66	220	$C_{22}H_{28}N_4O_3{\cdot}HCl$	396	1720
20		CH_3	$CH{=}CHC_6H_5$	77	210	$C_{22}H_{23}N_4O_3{\cdot}HCl$	391	1730
21	$(CH_2)_2N(CH_3)_2$	CH_3	$CH{=}CHC_6H_5$	81	207	$C_{19}H_{24}N_4O_3{\cdot}HCl$	356	1730
22	$(CH_2)_3N(CH_3)_2$	CH_3	$CH{=}CHC_6H_5$	75	198	$C_{20}H_{26}N_4O_3{\cdot}HCl$	370	1730
23		CH_3	$CH{=}CHC_6H_5$	69	236	$C_{24}H_{31}N_4O_4$	439	1730
24	H	H	$CH{=}CHC_6H_5$	87	290	$C_{14}H_{13}N_3O_3$	271	1730
25	H		$CH{=}CHC_6H_5$	77	245	$C_{20}H_{24}N_4O_4{\cdot}HCl$	384	1720

(12–15) were stabilized by the establishment of a hydrogen bond between the 6-amino and the 5-carbonyl groups. The free rotation of the amino group was hindered by this bond, and consequently the resonances corresponding to these two protons occurred at different chemical shifts, at 11 ppm for the proton engaged in the hydrogen bond vs. 8.5 ppm for the other. The downfield shift of the former was a reflection of the deshielding effect of the close carbonyl. The hydrogen bond could be evaluated by heating the solution at 120 °C where both protons appeared as a single peak at 9.5 ppm.

In the solid state, X-ray crystallography of compound **12** confirmed the existence of a stabilized structure[6] in the series. The least-squares plane calculated through the phenyl ring and the uracil ring indicated that the molecule was almost planar with an extended structure (overall length of 9.71 Å), which could account for an extensive stacking interaction between the nucleic bases of the DNA. Intramolecular interactions appeared to be stabilizing forces in this conjugated system since the 5-carbonyl group participated in a strong hydrogen bond with the 6-amino group (1.876 Å).

Compounds **16–22** are 6-amino-substituted molecules in which NMR spectra could not point out the possible implication of the single 6-amino proton in the stabilization of the molecule. X-ray crystallography of compound **16** has been realized (Figure 2).

This molecule exhibited an extented structure with a small dihedral angle of 7° between the two rings. This disruption of the planarity could be attributed to a repulsion between the bulkyl 6-(morpholinoethyl)amino side chain and the *N'*-methyl group. Consequently the 6-amino proton was not linked via a hydrogen bond with the oxygen atom of the 5-carbonyl group as for previous compounds (**12–15**). Furthermore, the X-ray structure of **16** established that a chlorine atom was linked to the diamino-chain NH protons by two hydrogen bonds involving two electron lone pairs of this atom. The stabilization of compound **16** in a quasi-planar form compatible with a possible inter-

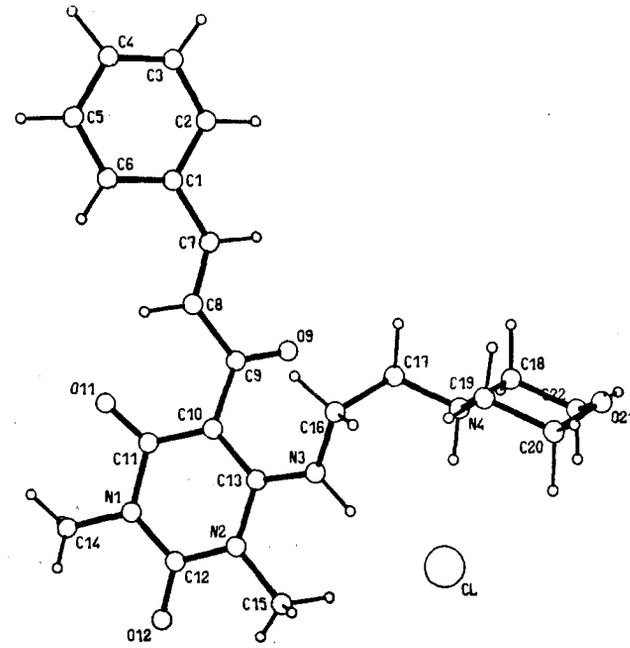


Figure 2. Dependence of ESR spectra of AATEMPO on the presence of calf thymus DNA: (A) spectrum of the freely rotating radical, (B) immobilized radical in the presence of DNA, (C) results of two superimposed spectra of A (free) and B (bound) types.

(6) Warin, V.; Foulon, M.; Baert, F.; Bernier, J. L.; Hénichart, J. P. *Acta Crystallogr., Sect B* **1980**, *36*, 1721.

Table III. Δt_m and K_d Values of Some 5-Cinnamoyluracils

	12	13	14	15	16	17	19	25
Δt_m,[a] °C	6	0.5	0	0	1.5	1	1.5	8
K_d[b] ($\times 10^{-4}$ M)	1				5.5		5	0.5

[a] 10^{-3} M calf thymus DNA was used in the presence of 0.5×10^{-3} M solutions of uracils. [b] K_d were evaluated by EPR on uracils exhibiting a significant Δt_m.

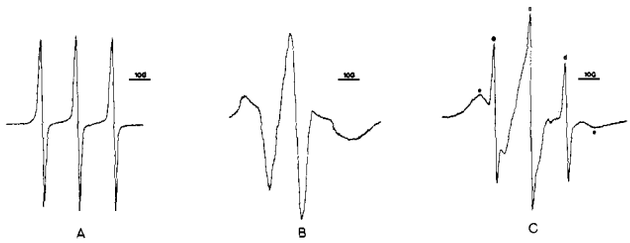


Figure 3. Hypothetical position of 5-cinnamoyl-6-aminouracils in the double helix.

action with DNA could be very likely attributed to an extension of the cinnamoyl π cloud to the uracil ring.

Biology. Interaction with DNA. UV Spectroscopy: Δt_m. 5-Cinnamoyluracils absorbed in the same region as the DNA (three peaks at 251, 256, 263 nm) so it was difficult to determine a hypochromicity and a bathochromic effect in the presence of DNA. Instead, we chose to study the interaction of these drugs with the double helix by Δt_m technique. It has been shown that an effect typical of DNA-complexing chemotherapeutic drugs was to stabilize double-helical DNA against heat denaturation. Thus, Δt_m of calf thymus DNA has been evaluated in the absence (67.5 ± 0.5 °C) and in the presence of a series of uracils as shown in Table III.

The better stabilization of the double helix was obtained for compounds **12** and **25**. Noteworthy was the fact that reduction of the double bond (**15**) and substitution on the phenyl ring (**13**, **14**) suppressed the interaction with DNA. Products substituted by hydrophilic side chains on the 6-amino groups seemed to interact less tightly (**16**, **17**, **19**).

ESR Spectroscopy. A spin-labeled molecule (**23**) was synthetized in order to check the intercalation of 5-cinnamoyluracils by ESR. The vital properties of a spin-label lies in the fact that the shape of its electron spin resonance spectrum is sensitive to the polarity of the environment and to the type of motion.[7] When a stable paramagnetic nitroxide is chemically attached to a biological molecule a "spy" is formed whose spectrum reveals certain properties of the labeled site.[8] Intercalation of spin-labeled drugs in DNA can be studied with use of these properties.[9,10] The spin-label–DNA complex in solution was ultrafiltered twice until most of the filtrate was expelled and then, after redissolution of the paste, analyzed by ESR. The observed signal of **23** was composed of three sharp peaks of equal intensity and about a 14-G separation, indicating that the nitroxide was freely rotating. Thus, the probe exhibited an affinity for DNA since it was not expelled by ultrafiltration but was not deeply buried in the double helix since the signal was characteristic of unbound species (A, Figure 3).

Table IV. Antitumor Screening Results of 12 (NSC 290115)

no.	dose, mg/kg	MST days (% T/C)	av wt change	survivors day 5
12	200	116	-0.4	6/6
	100	113	-0.5	5/6
	50	124	-2.6	6/6
	25.0	120	-1.7	6/6
	12.5	124	-2.1	6/6

In the same conditions, spin-labeled 9-aminoacridine (AATEMPO) gave a spectrum of bound species (B, Figure 3) and in solution without any ultrafiltration AATEMPO–DNA complex exhibited a ESR spectrum which corresponds to the superimposition of preceding tracings (C, Figure 3). According to these properties, we have developed a technique permitting the calculation of AATEMPO binding parameters (K_d) to DNA[9,10] and of other intercalating drugs by competition by using either the Scatchard plot[11] or Lineweaver–Burk plot.[12] The equilibrium dissociation constant found for AATEMPO was $K_d = 2 \times 10^{-5}$M and by competition the K_d of some characteristic uracils was determined. Results scheduled in Table III showed that the K_d values of this family of uracils was lower than those of acridines,[9] but the interaction with DNA was, one more time, demonstrated.

In Vitro Cytostatic Activity. Inhibition of growth of cultured L1210 cells has been tested on most of the compounds excluding some (**12–15**) for which solubility in water was too low to permit a good diffusion in the culture medium. In the tested compounds, two were found to be active: **16** ($ID_{50} = 5 \times 10^{-6}$ M, $LD_{50} = 3 \times 10^{-6}$ M) and **19** ($ID_{50} = 7 \times 10^{-6}$ M, $LD_{50} = 10^{15}$ M). Compound **25** revealed an equal cytotoxicity and lethality (10^{-5} M).

In Vivo Antitumor Activity. All the compounds were tested as inhibitors of the P388 leukemia by the NCI (Bethesda or Brussel) according to standard methods. Only compound **12** (Table IV) passed the criteria of the NCI for activity.

Discussion

No really strong antitumor activity was exhibited. The first conceived molecule (**12**) was found to be the only one product active in vivo, while other more hydrophilic molecules (**16**, **19**) exhibited an activity in vitro. It should be noted that the former molecule **12** could not be studied in vitro since it crystallized in the culture medium. The lack of activity of the latter molecules in vivo could be explained by their metabolism and disposition. The crucial difference between these two sorts of compounds lies also in their structure as determined by X-ray. Compound **12** was well stabilized in a planar structure by a strong hydrogen bond while compounds **16** and **19**, owing to the bulky side chain borne by the 6-amino group, were less planar and exhibited a lower affinity for DNA ($K_d = 5 \times 10^{-4}$ M, Δt_m = 1–2 °C).

It could be postulated that compound **12** would act by intercalation in the DNA, while compounds **16** and **19** would only insert their cinnamoyl moiety between the base pairs of the double helix. ESR spectrum of compound **23** indicated that the 6-amino group was very likely located in the major groove. Δt_m and K_d values of **25** showed that this compound gave the best stabilizing effect on DNA. In the light of these results, the position of the planar

(7) For a review, see: "Spin-labelling. Theory and Application"; Berlinger, L. J., Ed.; Academic Press: New York, 1976, Vol. 1; 1979, Vol. 2.
(8) Hsia, J. C.; Piette, L. H. *Arch. Biochem. Biophys.* **1969**, *129*, 296.
(9) Bernier, J. L.; Hénichart, J. P.; Catteau, J. P. *Anal. Biochem.* 1981, *117*, 12.
(10) Hénichart, J. P., Bernier, J. L.; Catteau, J. P. *Hoppe-Zeyler's Z. Physiol. Chem.* **1982**, *363*, 835.
(11) Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660.
(12) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
(13) Papesch, V.; Schroeder, E. F. *J. org. Chem.* **1951**, *13*, 1879.
(14) Blicke, F. F.; Godt, M. C., Jr. *J. Am. Chem. Soc.* **1954**, *76*, 2798.
(15) Pfleiderer, W.; Schündehütte, K. H. *Justus Liebigs Ann. Chem* **1958**, *612*, 158.

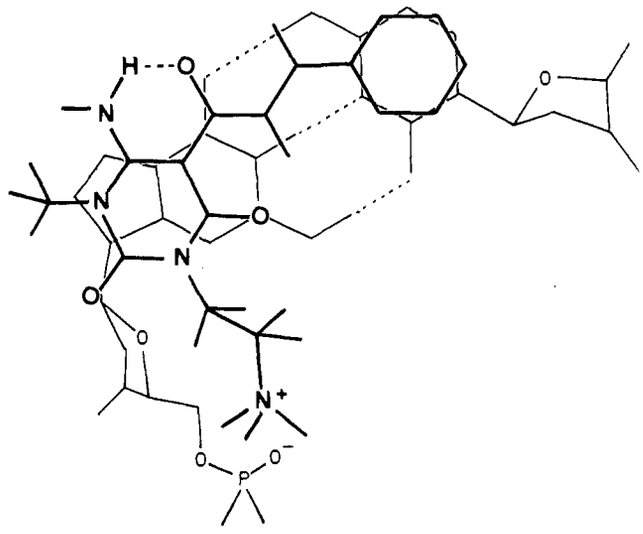


Figure 4.

5-cinnamoyl-6-aminouracils in the double helix could be determined: the cinnamoyl moiety was deeply buried, while on the uracil ring, the 6-amino group was oriented toward the major groove and the N_3 position was pointed toward a phosphate of the helix, favoring a saline bond in the case of compound **25** (Figure 4).

In conclusion, we can presume that intercalation in the DNA is one of the major reasons of potential antitumor properties of compounds in this series of 6-amino-5-cinnamoyluracils (**12**, **16**, **19**), but other factors are implied in the activity since compound **25**, in spite of its good fitting in the double helix, gave disappointing results in the different antitumor tests. In other well-studied series of antitumor drugs, it has been demonstrated that DNA binding is not the only determinant of activity. A dramatic example are the isomeric compounds *m*-AMSA, the clinically useful derivative amsacrine,[16] and o-AMSA in which the methoxy group is on the ortho instead of the meta position. o-AMSA is completely inactive in L1210 tests[17] even though it intercalates as *m*-AMSA into superhelical DNA.[18] It has been recently reported that the difference in the DNA cutting potencies should be responsible for the lack of antitumor activities of o-AMSA, acridine, and 9-aminoacridine.[19] It is conceivable that such a difference could be involved in the present series of 5-cinnamoyl-6-aminouracil. More generally, it is now admitted that if the intercalation is the primary process for the activity of many antitumor drugs, other phenomena are implied and must be taken into account, such as metabolism, disposition of the molecules, and free-radical production.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus. IR, NMR, and mass spectra, which were in agreement with the structure cited, were respectively recorded on a Beckman Acculab I, a Brucker WP 80 SY at 80 MHz, and a Ribermag R10-10 quadrupole mass spectrometer combined with a Riber 400 data system, using direct insertion of samples. Crystallographic data were collected on a Philips PW 1100 difractometer with graphite-monochromated Cu Kα radiation. DNA melting curves were measured by using a Uvikon Kontron 810/820 spectrophotometer coupled to a Uvikon Recorder 21 and a Uvikon Thermoprinter 48. Samples were placed in a thermostated cell holder (10-mm path length). The cuvette was heated by circulating water from a Haake unit set. The temperature inside the cuvette was monitered by using a thermocouple in contact with the solution. The absorbance at 260 nm was measured over the range 50–95 °C with a heating rate of 1 °C/min. The "melting" temperature t_m was taken to be the midpoint of the hyperchromic transition. ESR measurements were recorded on a Varian E109 X-band spectrometer with a E 238 cavity operating in the TM_{110} mode. A 100-KHz high-frequency modulation was used with a 20-mW microwave power. The sample solutions were disposed into a flat quartz cell. Combustion analyses were performed on a Perkin-Elmer CHN 240 apparatus. All new compounds exhibited consistent spectral data and elemental analyses within ±0.4%.

Chemistry. Preparation of 1-Methyl-3-substituted-6-aminouracils 1–3 (Table I). Compounds **1** and **2** were prepared as previously described[13,14] (see Scheme I) and 1-methyl-3-(2-morpholinoethyl)-6-aminouracil (**3**) was obtained from **2** (14.1 g, 0.1 mol) after treatment in absolute ethanol by an equimolar amount of (choroethyl)morpholine (18.6 g, 0.1 mol) in the presence of sodium ethylate (4.6 g, 0.2 mol). A sodium chloride precipitate was eliminated and the ethanolic solution evaporated in vacuo. The crude residue was crystallized in acetone: IR 1680 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25 (s, 3 H, CH_3), 2.0–4.4 (m, CH_2), 4.65 (s, 1 H, C_5H), 6.80 (s, 2 H, NH_2); MS, *m/e* 254 (M^+). Anal. ($C_{11}H_{18}N_4O_3$) C, H, N.

Preparation of 1,3-Dimethyl-6-(substituted-amino)uracils 4–11 (Table I). An ethanolic solution of 1,3-dimethyl-6-chlorouracil[15] (17.6 g, 0.1 mol) was treated with an equimolar amount of *N*-aminoalkylamine in the presence of sodium carbonate (0.1 mol). The mixture was stirred and heated under reflux for 24 h. After elimination of the NaCl precipitate, the filtrate was concentrated in vacuo to dryness. The residual solid was taken up with acetone and recrystallized twice from this solvent to give white crystals.

1,3-Dimethyl-6-[(2-morpholinoethyl)amino]uracil (4): IR 1700 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.1, 3.3 (s, 2 × 3 H, NCH_3), 4.8 (s, 1 H, C_5H, 7.0 (t, 1 H, J = 6.6 Hz, NH), 3.1–4.2 (m, morpholinoethyl CH_2); MS 268 (M^+). Anal. ($C_{12}H_{20}N_4O_3$) C, H, N.

1,3-Dimethyl-6-[(3-morpholinopropyl)amino]uracil (5): IR 1700 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.1, 3.3 (s, 2 × 3 H, NCH_3), 4.7 (s, 1 H, C_5H), 1.5–3.5 (m, pyrrolidinoethyl, CH_2), 6.8 (t, 1 H, J = 6.2 Hz, NH); MS 252 (M^+). Anal. ($C_{12}H_{20}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[(2-pyrrolidinoethyl)amino]uracil (6): IR 1705 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.1, 3.3 (s, 2 × 3 H, NCH_3), 4.7 (s, 1 H, C_5H), 1.5–3.5 (m, pyrrolidinoethyl, CH_2), 6.8 (t, 1 H, J = 6.2 Hz, NH); MS 252 (M^+). Anal. ($C_{12}H_{20}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[(2-piperidinoethyl)amino]uracil (7): IR 1705 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.1, 3.3 (s, 2 × 3 H, NCH_3), 4.55 (s, 1 H, C_5H), 1.10–3.5 (m, piperidinoethyl, CH_2), 6.5 (t, 1 H, J = 6.0 Hz, NH); MS 266 (M^+). Anal. ($C_{13}H_{22}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[(2-pyridinoethyl)amino]uracil (8): IR 1705 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.1, 3.3 (s, 2 × 3 H, NCH_3), 3.0 (t, 2 H, CH_2CH_2), 3.4 (dt, 2 H, CH_2CH_2), 6.75 (t, 1 H, NH), 4.65 (s, 1 H, C_5H), 7.25 (dd, 2 H, pyr C_4HC_5H), 7.6 (d, 1 H, J = 6 Hz, pyr C_3H), 8.45 (d, 1 H, J = 5 Hz, C_6H); MS 261 (M^+). Anal. ($C_{13}H_{17}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[[2-(dimethylamino)ethyl]amino]uracil (9): IR 1705 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 3.25, 3.35 (s, 2 × 3 H, NCH_3), 2.25 (s, 6 H, N $(CH_3)_2$)), 4.8 (s, 1 H, C_5H), 5.55 (t, 1 H, NH), 3.10 (dt, 2 H, $NHCH_2CH_2$), 2.55 (t, 2 H, CH_2CH_2); MS 226 (M^+). Anal. ($C_{10}H_{18}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[[3-(dimethylamino)propyl]amino]uracil (10): IR 1705 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 3.30, 3.35 (s, 2 × 3 H, NCH_3), 2.25 (s, 6 H, $N(CH_3)_2$), 3.20 (dt, 2 H, $NHCH_2CH_2$), 1.80 (m, 2 H, $CH_2CH_2CH_2$), 2.55 (m, 2 H, CH_2CH_2N), 8.40 (t, 1 H, NH); MS 240 (M^+). Anal. ($C_{11}H_{20}N_4O_2$) C, H, N.

1,3-Dimethyl-6-[(2,2,6,6-Tetramethyl-1-oxypiperidin-4-yl)amino]uracil (11): IR 1690 (C=O) cm^{-1}; MS 309 (M^+). Anal. ($C_{15}H_{25}N_4O_3$) C, H, N.

Preparation of 1,3-Dimethyl-5-acyl-6-aminouracils 12–15 (Table II). To a solution of 1,3-dimethyl-6-aminouracil (1; 15.5 g, 0.1 mol) in pyridine (100 mL) was added 0.1 mol of acid chloride. This mixture was stirred and heated under reflux for 2 h. The solvent was then evaporated in vacuo. The residue was washed by water to give the expected product (**12–15**) as a crude powder,

(16) Cain, B. F.; Atwell, G. J. *Eur. J. Cancer* **1974**, *10*, 539.
(17) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1975**, *18*, 1110.
(18) Waring, M. J. *Eur. J. Cancer* **1976**, *12*, 995.
(19) Wong, A.; Huang, C.; Crooke, S. T. **1984**, *23*, 2939.

which was filtered and crystallized in ethanol.

1,3-Dimethyl-5-cinnamoyl-6-aminouracil (12): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.30, 3.45 (s, 2 × 3 H, NCH_3), 8.50 (d, 1 H, J = 16 Hz, ethylenic CH), 7.65 (d, 1 H, J = 16 Hz, ethylenic CH), 7.60 (m, 5 H, arom CH), 11.52 (s, 1 H, NH bonded), 8.35 (s, 1 H, second NH proton); MS 285 (M^+). Anal. ($C_{15}H_{15}N_3O_3$) C, H, N.

1,3-Dimethyl-5-(4-methoxycinnamoyl)-6-aminouracil (13): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25, 3.40 (s, 2 × 3 H, NCH_3), 3.90 (s, 3 H, OCH_3), 8.20 (d, 1 H, J = 18 Hz ethylenic CH), 7.50 (d, 1 H, J = 10 Hz, aromatic CH), 6.95 (d, 2 H, J = 10 aromatic CH); MS 315 (M^+). Anal. ($C_{16}H_{17}N_3O_4$) C, H, N.

1,3-Dimethyl-5-(3,4,5-trimethoxycinnamoyl)-6-aminouracil (14): IR 1710 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25, 3.40 (s, 2 × 3 H, NCH_3), 3.95 (s, 9 H, OCH_3), 8.45 (d, 1 H, J = 16 Hz, ethylenic CH), 7.60 (d, 1 H, J = 16 Hz, ethylenic CH), 7.10 (s, 2 H, arom CH), 11.80 (s, 1 H, NH bonded), 8.37 (s, 1 H, other NH proton); MS 375 (M^+). Anal. ($C_{18}H_{21}N_3O_6$) C, H, N.

1,3-Dimethyl-5-[(2-phenylethyl)carbonyl]-6-aminouracil (15): IR 1710 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.16, 3.32 (s, 2 × 3 H, NCH_3), 2.70, 2.80 (t, 2 × 1 H, J = 3 Hz, CH_2), 7.36 (s, 5 H, arom CH), 11.50 (s, 1 H, NH bonded), 8.38 (s, 1 H, other NH); MS 287 (M^+). Anal. ($C_{15}H_{17}N_3O_3$) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[(aminoalkyl)amino]uracil 16–22 (Table II). To a suspension of 1,3-dimethyl-6-[(aminoalkyl)amino]uracil hydrochloride (0.10 mol) in pyridine (100 mL) was added cinnamoyl chloride (16.6 g, 0.10 mol). The mixture was heated at 115 °C for 3 h. After cooling, the resulting precipitate was filtered and washed with water to give compounds (16–22) as crude yellow powder. Recrystallization was obtained in ethanol.

1,3-Dimethyl-5-cinnamoyl-6-[(2-morpholinoethyl)amino]uracil (16): IR 1720 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25, 3.45 (s, 2 × 3 H, NCH_3), 9.75 (t, 1 H, J = 3.5 Hz, NH), 7.50, 8.25 (d, 2 × 1 H, J = 15 Hz, ethylenic CH), 7.55 (m, 5 H, arom CH), 2.8–4.0 (m, morpholinoethyl CH_2); MS 398 (M^+). Anal. ($C_{21}H_{26}N_4O_4$·HCl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[(3-morpholinopropyl)amino]uracil (17): IR 1725 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.20, 3.45 (s, 2 × 3 H, NCH_3), 11.45 (t, 1 H, J = 3 Hz, NH), 8.20, 7.50 (d, 2 × 1 H, J = 15 Hz, ethylenic CH), 7.45 (m, 5 H, arom CH), 1.8–4.2 (m, morpholinopropyl CH_2); MS 412 (M^+). Anal. ($C_{22}H_{28}N_4O_4$·CHl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[(2-pyrrolidinoethyl)amino]uracil (18): IR 1720 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.20, 3.45 (s, 2 × 3 H, NCH_3), 9.60 (t, 1 H, J = 3 Hz, NH), 8.10, 7.50 (d, 2 × 2 H, J = 16 Hz, ethylenic CH), 7.60 (m, 5 H, arom CH), 1.45–4.10 (m, pyrrolidinoethyl CH_2); MS 382 (M^+). Anal. ($C_{21}H_{26}N_4O_3$·HCl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[(2-piperidinoethyl)amino]uracil (19): IR 1720 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.10, 3.30 (s, 2 × 3 H, NCH_3), 8.10, 7.50 (d, 2 × 1 H, J = 14 Hz, ethylenic CH), 7.60 (m, 5 H, arom CH), 1.50–4.10 (m, piperidinoethyl, CH_2).

1,3-Dimethyl-5-cinnamoyl-6-[(2-pyridin-2-ylethyl)amino]uracil (20): IR 1750 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.15, 3.35 (s, 2 × 3 H, NCH_3), 9.50 (t, 1 H, NH), 8.10, 7.50 (d, 2 × 1 H, J = 16 Hz, ethylenic CH), 8.40, 7.60, 7.30 (m, 4 H, pyridinic CH), 3.0, 3.4 (m, 2 × 2 H, ethyl CH_2); MS 391 (M^+). Anal. ($C_{22}H_{23}N_4O_3$·HCl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[[2-(dimethylamino)ethyl]amino]uracil (21): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.20, 3.40 (s, 2 × 3 H, NCH_3), 2.20 (s, 6 H, $N(CH_3)_2$), 9.60 (t, 1 H, NH), 2.60, 3.20 (m, 2 × 2 H, ethyl CH_2), 7.50, 8.10 (d, 2 × 1 H, J = 16 Hz, ethylenic CH), 7.60 (m, 5 H, arom CH); MS 356 (M^+). Anal. ($C_{20}H_{24}N_4O_3$·HCl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[[3-(dimethylamino)propyl]amino]uracil (22): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25, 3.35 (s, 2 × 3 H, NCH_3), 2.25 (s, 6 H, $N(CH_3)_2$, 3.20, 2.55, 1.80 (m, 3 × 2 H, propyl CH_2), 9.80 (t, 1 H, NH), 7.50, 8.15 (d, 2 × 1 H, J = 16 Hz, ethylenic CH), 7.60 (m, 5 H, arom CH), MS 370 (M^+). Anal. ($C_{20}H_{26}N_4O_3$·HCl) C, H, N.

1,3-Dimethyl-5-cinnamoyl-6-[(2,2,4,4-tetramethyl-1-oxypiperidin-4-yl)amino]uracil (23): IR 1730 (C=O) cm^{-1}; MS 439 (M^+). Anal. ($C_{24}H_{31}N_4O_4$) C, H, N.

Preparation of 1-Methyl-3-substituted-5-cinnamoyl-6-aminouracils (24, 25) (Table II). These two compounds were prepared as described above with either **2** or **3** (as hydrochloride) as the starting material.

1-Methyl-5-cinnamoyl-6-aminouracil (24): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25 (s, 3 H, NCH_3), 10.8 (s, 1 H, CONHCO), 7.50, 8.40 (d, 2 × 1 H, J = 14 Hz, ethylenic CH), 7.60 (m, 5 H, aromatic CH), 11.80 (s, 1 H, NH bonded), other NH in the 7.20–7.80 region; MS 271 (M^+). Anal. ($C_{14}H_{13}N_3O_3$) C, H, N.

1-Methyl-3-(2-morpholinoethyl)-5-cinnamoyl-6-aminouracil (25): IR 1730 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.25 (s, 3 H, NCH_3), 7.45, 8.40 (d, 2 × 1 H, J = 14 Hz, ethylenic CH), 7.60 (m, 5 H, aromatic CH), 2.25–4.4 (m, morpholinoethyl CH_2); MS 384 (M^+). Anal. ($C_{20}H_{24}N_4O_4$·HCl) C, H, N.

Growth Inhibition of Cultured L1210 Cells. Cytotoxicity of the compounds was tested on the murine leukemia cell line L1210, in vitro. Cells were grown in culture flasks with Eagle Dulbecco medium (GIBCO) supplemented with 10% fetal calf serum (FLOW) and antibiotics. The compounds were diluted in the culture medium at the appropriate concentrations. Control and treated cells were maintained at 37 °C for 24 h in a water-saturated atmosphere containing 5% CO_2. After this period of continuous drug exposure, cells were counted in a Malassez hematimeter and the lethality was evaluated by loss of cell refringence observed with a phase-contrast microscope.

The cytotoxicity effects on cellular growth were expressed as a function of drug concentrations. For each compound, we determined (a) the inhibitory dose (ID_{50}) producing 50% inhibition of the cellular doubling time per 24 h without any significant lethality and (b) the lethal dose (LD_{50}) producing 50% of dead cells in the culture.

In Vitro Antitumor Activity. Tests were done on P388 leukemia by the NCI according to the protocol described in instruction 14: tumor inoculum P388 lymphocytic leukemia intraperitoneally into BDF_1 mice. Each mouse was inoculated once with the indicated dose and observed for 30 days. Evaluation: MST = median survival time in days; percent T/C = MST treated/MST control × 100. Criteria = percent T/C = 125 was considered as significant antitumor effect.

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Registry No. 1, 6642-31-5; **2**, 2434-53-9; **3**, 94597-42-9; **4**, 78681-06-8; **5**, 94597-43-0; **6**, 94597-44-1; **7**, 94619-57-5; **8**, 94619-58-6; **9**, 93559-40-1; **10**, 93559-41-2; 11, 94597-45-2; **12**, 74838-72-5; **13**, 93599-95-2; **14**, 93559-42-3; **15**, 61317-77-9; **16**, 78681-08-0; **16**·HCl, 93559-46-7; **17**, 94597-46-3; **17**·HCl, 93559-47-8; **18**, 94597-48-5; **18**·HCl, 94597-47-4; **19**, 94597-50-9; **19**·HCl, 94597-49-6; **20**, 94619-60-0; **20**·HCl, 94619-59-7; **21**, 94597-52-1; **21**·HCl, 94597-51-0; **22**, 94597-54-3; **22**·HCl, 94597-53-2; **23**, 94597-55-4; **24**, 94597-56-5; **25**, 94597-58-7; **25**·HCl, 94597-57-6; 1,3-dimethyl-6-chlorouracil, 6972-27-6; 2-morpholinoethylamine, 2038-03-1; 3-morpholinopropylamine, 123-00-2; 1-pyrrolidineethanamine, 7154-73-6; 1-piperidineethanamine, 27578-60-5; 2-pyridineethanamine, 2706-56-1; 2-(dimethylamino)ethylamine, 108-00-9; 3-(dimethylamino)propylamine, 109-55-7; 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy, 14691-88-4; cinnamoyl chloride, 102-92-1; 4-methoxycinnamoyol chloride, 34446-64-5; 3,4,5-trimethoxycinnamoyl chloride, 10263-19-1; 3-phenylpropionyl chloride, 645-45-4; *N*-(2-chloroethyl)morpholine, 3240-94-6.