Purine and 1-Deazapurine Ribonucleosides and Deoxyribonucleosides: Synthesis and Biological Activity

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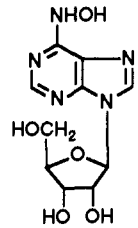
A series of 6-(hydroxylamino)purine and -1-deazapurine nucleosides were synthesized and tested for their antitumor and adenosine deaminase inhibitory activity. All the examined molecules displayed an in vitro activity comparable to that of the reference compounds 6-(hydroxylamino)-9-β-D-ribofuranosylpurine (HAPR) and ara-A, their ID_{50} ranging from 0.9 μM (16) to ~100 μM (12). The 6-hydroxylamino derivatives of 1-deazapurine 9, 12, and 17 and also the blocked compound 13 are inhibitors of ADA whereas the purine derivatives 4 and 6 and the nitro compounds 11 and 16 are resistant to the enzyme. 7-(Hydroxylamino)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (12), the less cytotoxic but the most active ADA inhibitor in the series ($K_i = 2.7 \times 10^{-7}$), greatly potentiates the antitumor activity of ara-A in vitro.

The growth-inhibitory effect and toxicity of 6-(hydroxylamino)purine and of its 9-β-D-ribofuranosyl derivative (HAPR) are well documented.[1,2] HAPR was found active



HAPR

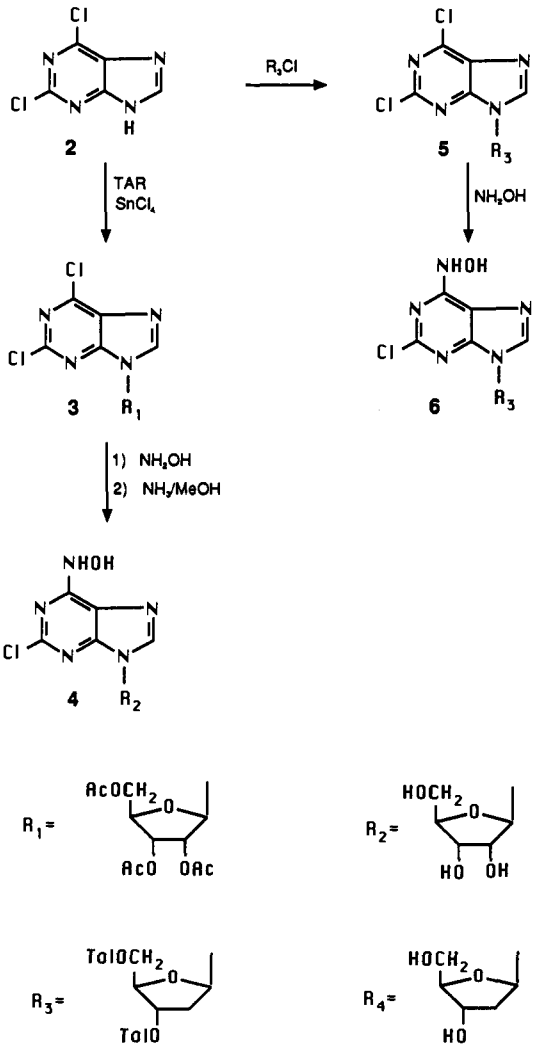
against mouse leukemia L1210, P388, P315 sensitive or resistant to mercaptopurine and against sarcoma 180 ascites in vivo. Unfortunately clinical trials in patients with acute leukemia were impaired because HAPR is readily deaminated in vivo by the enzyme adenosine deaminase (ADA) with formation of inosine and free hydroxylamine. The latter brings about formation of methemoglobin in human red cells, inducing blood cell hemolysis.

Since the presence of a chlorine atom in position 2 of adenosine makes the compound more resistant to ADA,[3,4] we prepared the 2-chloro-6-(hydroxylamino)-9-β-D-ribofuranosylpurine (4) and the corresponding 2′-deoxyribofuranosyl derivative 6 (Scheme I). Since we have previously reported that 1-deazaadenosine is an inhibitor of ADA[5] and is also endowed with antitumor activity in vitro,[6] the synthesis of N^6-hydroxylamino derivatives of 1-deazaadenosine was undertaken, leading to compounds reported in Table I.

Chemistry

The synthesis of 2-chloro-6-(hydroxylamino)-9-β-D-ribofuranosylpurine (4) was accomplished by the reactions described in Scheme I. Treatment of 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3)[7] with an excess of ethanolic hydroxylamine at reflux for 4 h followed by evaporation of the solvent afforded the protected hydroxylamine derivative which was added, without further purification, to methanolic ammonia to give the desired compound 4. The synthesis of 2-chloro-6-(hydroxylamino)-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (6) was carried out according to Scheme I. Reaction of freshly prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[8] with the sodium salt of the commercially available 2,6-dichloropurine (2),

Scheme I



generated in situ by the treatment with sodium hydride in acetonitrile, gave compound 5 and the corresponding

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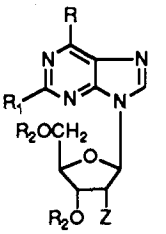
0022-2623/91/1834-2226$02.50/0

Table I. In Vitro Antitumor Activity and Inhibition of Adenosine Deaminase from Calf Intestine by Hydroxylamino and Nitro Nucleosides



compd	R	R_1	H	Z	R_2	ID_{50}, μM[b] P388[a]	ID_{50}, μM[b] L1210[a]	calf intestine: K_i, M
4	NHOH	Cl	N	OH	H	75.5	94.4	not active
6	NHOH	Cl	N	H	tol	10.4	4.9	not active
9	NHOH	H	CH	OH	H	4.9	5.3	1.2×10^{-6}
12	NHOH	H	CH	H	H	67.6	>100.0	2.7×10^{-7}
13	NHOH	H	CH	H	tol	16.9	22.5	5.3×10^{-6}
17	NHOH	Cl	CH	OH	H	60.0	91.0	6.9×10^{-5}
19	NHOH	Cl	CH	H	tol	4.3	4.8	not active
11	NO_2	H	CH	H	H	20.6	14.9	not active
16	NO_2	Cl	CH	OH	H	1.5	0.9	not active
HAPR	NHOH	H	N	OH	H	1.6	3.1	
ara-A						16.8	21.7	

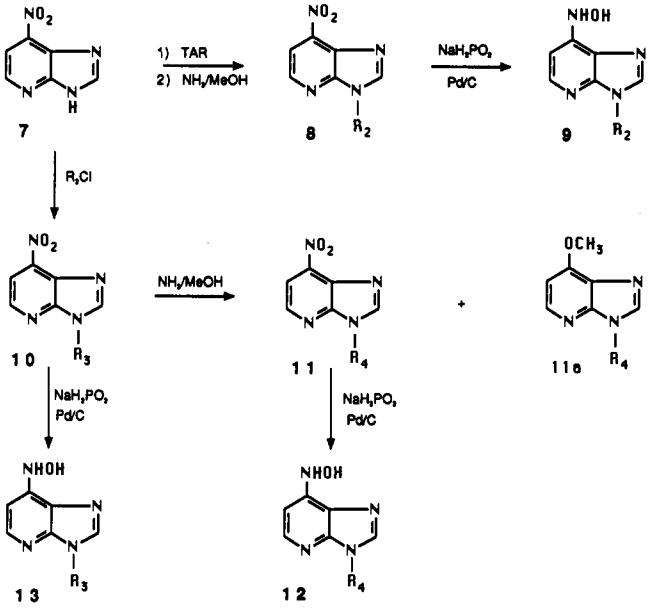
[a] Exponentially growing cells (5×10^5) were exposed to varying concentrations of drug for 72 h. The cells were then counted with Coulter Counter. [b] ID_{50} is the concentration of the compound in the culture media that produces 50% inhibition of the tumor cell growth as compared to the untreated controls.

N-7 isomer.[9] Treatment of **5** with refluxing ethanolic hydroxylamine gave the still blocked nucleoside **6**. Attempts to remove the toluoyl blocking groups of **6** under many classical conditions (methanolic ammonia, sodium methylate, triethylamine, or Amberlyst A_{26} OH^-) were unsuccessful, leading to 6-amino-2-chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine, which exhibited identical physical properties with that previously reported.[9]

The 7-(hydroxylamino)-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (1-deazaHAPR, **9**) was obtained from the nitro derivative **8**[6] by reduction with sodium hypophosphite and 5% Pd/C (Scheme II).[10] The synthesis of 7-(hydroxylamino)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**12**) was carried out according to Scheme II. Reaction of freshly prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose with the sodium salt of the 7-nitroimidazo[4,5-*b*]pyridine (**7**)[6] gave the blocked nucleoside **10**. Deblocking of **10** with methanolic ammonia yielded the nitro derivative **11** and also a small amount of 7-methoxy derivative **11a**. Reduction of **11** with sodium hypophosphite and 5% Pd/C in THF gave the hydroxylamino derivative **12**. The same reaction, starting from **10**, afforded the blocked hydroxylamino derivative **13**. The site of deoxyribosylation was confirmed by UV and ^1H NMR data. In fact the UV maxima of compound **12** were identical with those of the hydroxylamino **9**. The ^1H NMR spectrum of **12** displayed a singlet for the H-2 at δ 8.37 corresponding to a singlet at δ 8.33 for the same proton of compound **9**. All other new hydroxylamino derivatives of 1-deazapurine, reported in this paper, exhibited a singlet for the H-2 at δ 8.36 or 8.37.

The synthesis of 5-chloro-7-(hydroxylamino)-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**17**) was accomplished by the sequence shown in the Scheme III. Ribosylation of 5-chloro-7-nitro-3*H*-imidazo[4,5-*b*]pyridine (**14**)[11] was carried out in freshly distilled stannic chloride at room temperature for 12 h to give the blocked nucleoside **15**.[11] Deacetylation of **15** in methanolic ammonia at −20 °C for 4 h afforded a mixture of compound **16** and the corresponding 7-methoxy derivative **16a** in the ratio 3.5:1. When the same deacetylation was carried out at −0 °C, the isolate ratio of **16** and **16a** was 2:3. Reduction of **16** with sodium hypophosphite and 5% Pd/C in THF gave the desired compound **17**. Preparation of 5-chloro-7-(hydroxylamino)-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**19**) was accomplished by reaction of the usual freshly prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pento-

Scheme II



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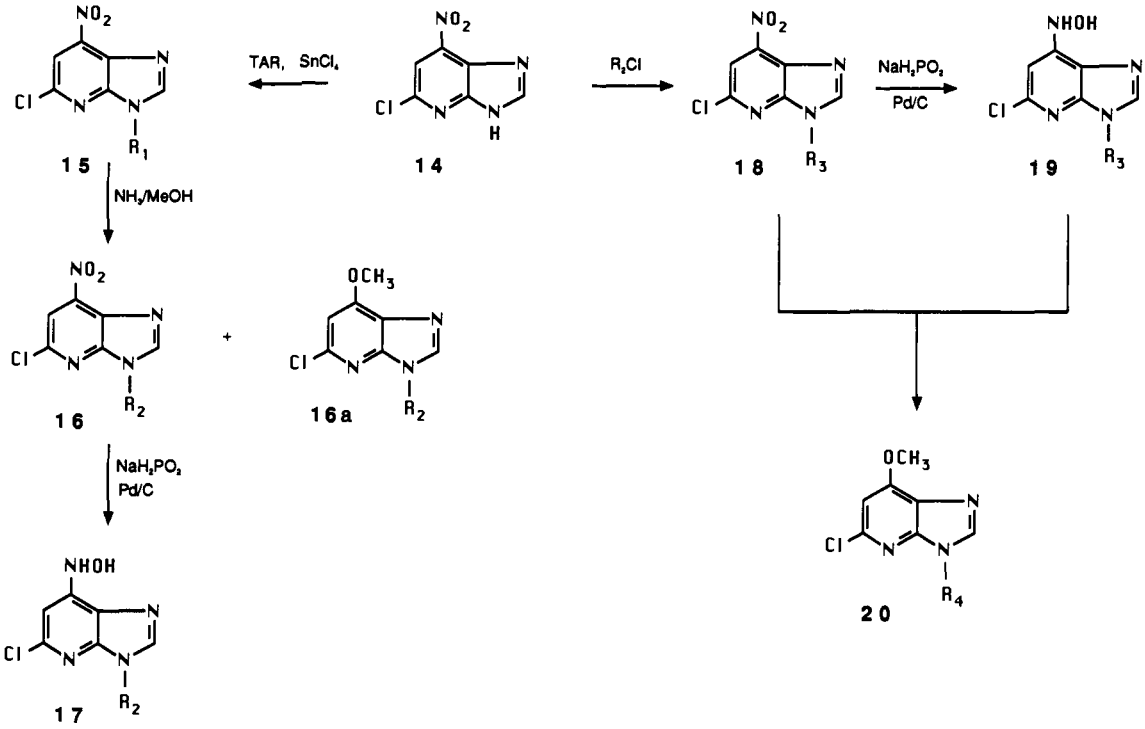
Scheme III



furanose with 5-chloro-7-nitro-3*H*-imidazo[4,5-*b*]pyridine (**14**) to give the blocked nucleoside **18** whose reduction with sodium hypophosphite and 5% Pd/C in THF afforded the hydroxylamino derivative **19**. As in the case of compound **6**, attempts to remove the toluoyl blocking groups, both from the nitro compound **18** and from the hydroxylamino derivative **19**, under several classical conditions were unsuccessful, leading to the methoxy derivative **20** as reported in Scheme III. In details, treatment of **18** with methanolic ammonia or sodium methylate or Amberlyst A26 (OH^-) in methanol gave 5-chloro-7-methoxy-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**20**). The same methoxy derivative **20** was obtained by treatment of **19** with Amberlyst A26 (OH^-) in methanol. Treatment of both **18** and **19** with Amberlyst A26 (OH^-) in 2-propanol or acetonitrile gave only starting material. Also no reaction was observed when **19** was treated with methanolic ammonia at room temperature. Treatment of both **18** and **19** with liquid ammonia at several temperatures gave only inseparable mixtures of products.

Biological Evaluation and Discussion

Antitumor Activity. The antitumor activity of the new hydroxylamino derivatives and of the nitro derivatives **11** and **16** was evaluated in vitro in murine leukemia cell lines (P388 and L1210). The results are reported in Table I. All the examined molecules displayed an activity comparable to that of the reference compounds HAPR and ara-A, their ID_{50} ranging from 0.9 μM (**16**) to >100 μM (**12**). Since compounds **6** and **19** were tested in the protected form, in order to clarify the influence of the toluoyl groups on the activity, the blocked form of the hydroxylamino derivative **12** was prepared and tested. The results showed that the presence of toluoyl groups increased the in vitro activity by about 4-fold (**13** ID_{50} = 22.5 μM versus **12** ID_{50} >100 μM). The nitro derivatives **11** and **16** were more potent than the corresponding hydroxylamino derivatives **12** and **17**. 2-Chloro-6-(hydroxylamino)-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**6**) and 5-chloro-7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]-pyridine (**16**), which appeared to be the most active compounds in the series of purines and 1-deazapurines, respectively, were tested also in vivo against leukemia L1210 in the mouse and found inactive.

Adenosine Deaminase Inhibitory Activity. Enzymatic tests on adenosine deaminase from calf intestine were also performed. The 6-hydroxylamino derivatives of deazapurines **9**, **12**, and **17** and also the blocked compound **13** are inhibitors of ADA whereas the purine derivatives **4** and **6** are resistant to the enzyme. Interestingly, compounds **12** and **13** are the first 1-deazapurine-2′-deoxynucleosides reported to have ADA inhibitory activity.

In conclusion, the introduction of a chlorine atom in position 2 of purine moiety of HAPR or the isosteric substitution of the nitrogen atom in position 1 led to compounds which are not affected by ADA or which are ADA inhibitors, while maintaining in vitro cytotoxic activity.

Potentiation of Antitumor Effect of Ara-A. On the basis of the knowledge that ara-A is rapidly deaminated to arabinosyl hypoxanthine by adenosine deaminase, and that EHNA, an inhibitor of the enzyme, greatly potentiates the toxicity of ara-A[12] and its antiherpes activity,[13] we tested the ability of 7-(hydroxylamino)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**12**), the less cytotoxic but the most active ADA inhibitor in the series ($K_i = 2.7 \times 10^{-7}$), to potentiate the antitumor activity of ara-A in vitro.

The results showed that ara-A and **12** have only a slight effect when administered separately at 5.8 μM. They brought about 70% of inhibition of leukemia L1210 cell growth when they are administered in combination at the same concentration. The contemporary supply of 23.4 μM ara-A and 5.8 μM **12** produced greater than 95% inhibition (Figure 1). In conclusion, 7-(hydroxylamino)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]-pyridine (**12**) greatly potentiates the antitumor activity of ara-A in vitro.

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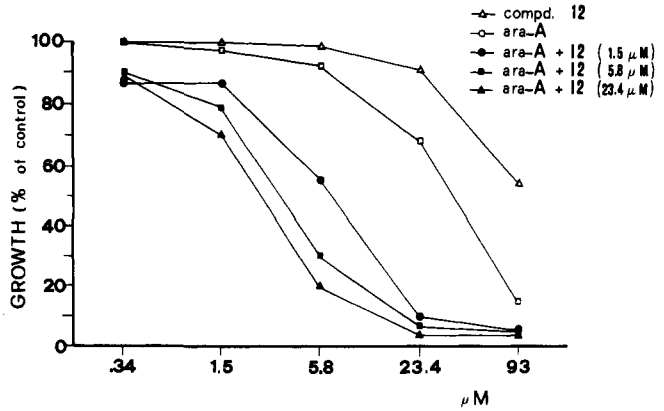

Figure 1. Potentiation of antitumor effect of ara-A by compound 12.

Experimental Section

Chemistry. Melting points were determined with a Buchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VXR 300 MHz spectrometer. UV spectra were recorded on a Cary 219 spectrophotometer. IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer. TLC were carried out on precoated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are indicated by atomic symbols and are within ±0.4% of theoretical values.

2-Chloro-6-(hydroxylamino)-9-β-D-ribofuranosylpurine (4). A solution of 0.50 g (1.12 mmol) of 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**3**)[7] in 5 mL of ethanol was added to 0.6 g (18 mmol) of NH_2OH in 15 mL of ethanol, and the reaction mixture was heated at reflux for 4 h and then concentrated in vacuo. The residue was dissolved in 50 mL of methanol saturated at 0 °C with ammonia and stirred under N_2 at room temperature for 12 h. The reaction mixture was evaporated, and the residue was flash chromatographed on a silica gel column with $CHCl_3$–MeOH (90:10) as eluent to give 0.11 g (31%) of **4** as a chromatographically pure solid: mp 196 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.63 (m, 2 H, CH_2-5′), 3.95 (m, 1 H, H-4′), 4.15 (m, 1 H, H-3′), 4.53 (m, 1 H, H-2′), 5.85 (d, 1 H, J = 5.5 Hz, H-1′), 8.42 (s, 1 H, H-8), 9.46 (br s, 1 H, NHOH), 10.97 (br s, 1 H, NHOH). Anal. ($C_{10}H_{12}ClN_5O_5$) C, H, N.

2-Chloro-6-(hydroxylamino)-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (6). A solution of 1.2 g (2.22 mmol) of 2,6-dichloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**5**)[9] in 20 mL of ethanol was added to 1.1 g (33 mmol) of NH_2OH in 40 mL of ethanol, and the reaction mixture was heated at reflux for 5 h and then concentrated in vacuo. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (95:5) as eluent to give 0.9 g (78%) of **6** as a chromatographically pure solid: mp 121–124 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.38 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3), 2.81 (m, 1 H, H-2′), 3.24 (m, 1 H, H-2″), 4.61 (m, 3 H, CH_2-5′ and H-4′), 5.81 (m, 1 H, H-3′), 6.52 (t, 1 H, J = 6.2 Hz, H-1′), 7.37 and 7.95 (m, 4 H each, HPh), 8.41 (s, 1 H, H-8), 9.46 (s, 1 H, NHOH), 11.01 (s, 1 H, NHOH). Anal. ($C_{26}H_{24}ClN_5O_6$) C, H, N.

7-(Hydroxylamino)-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (9). To a suspension of 2.2 g (7.4 mmol) of 7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**8**)[6] in 27 mL of THF under an atmosphere of N_2 was added 1.8 g (21 mmol) of NaH_2PO_2 in 27 mL of H_2O and 140 mg of 5% Pd/C. The reaction mixture was stirred under N_2 for 1.5 h, then the catalyst was removed, and the filtrate was concentrated to dryness. The residue was dissolved in methanol, and the phosphorus salts were precipitated with EtOAc and removed by filtration. The filtrate was concentrated in vacuo and the residue flash chromatographed on a silica gel column with $CHCl_3$–MeOH–NH_3 (83:15:2) as eluent to provide 1.4 g of the crude product. Recrystallization from H_2O gave 1.15 g (55%) of **9** as a white solid: mp 123–125 °C; UV λ_{max} (MeOH) 271 nm (ε 14200), 284 (sh) (ε 12700); ^{1}H NMR (Me_2SO-d_6) δ 3.63 (m, 2 H, CH_2-5′), 4.00 (m, 1 H, H-4′), 4.19 (m, 1 H, H-3′), 4.72 (m, 1 H, H-2′), 5.95 (d, 1 H, J = 6 Hz, H-1′), 6.76 (d, 1 H, $J_{6,5}$ = 5 Hz, H-6), 8.02 (d, 1 H, $J_{5,6}$ = 5 Hz, H-5), 8.33 (s, 1 H, H-2), 8.90 (s, 1 H, NHOH), 9.67 (s, 1 H, NHOH). Anal. ($C_{11}H_{14}N_4O_5$) C, H, N.

7-Nitro-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (10). To a suspension of 0.50 g (3.05 mmol) of 7-nitroimidazo[4,5-*b*]pyridine (**7**)[6] in 20 mL of dry acetonitrile under an atmosphere of N_2 was added NaH (80% in oil, 100 mg, 3.33 mmol), and the mixture was stirred at room temperature for 30 min. To the ice-cooled mixture was added 1.35 g (3.47 mmol) of freshly prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[8] and the suspension was stirred at 0 °C for 3 h. The reaction mixture was filtered to remove the insoluble material, and the filtrate was concentrated to a residue which was chromatographed on a silica gel column. Elution with $CHCl_3$–$(CH_3)_2CO$ (97:3) gave 0.94 g (60%) of **10** as a pure solid: mp 69–71 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.36 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.93 (m, 1 H, H-2′), 3.47 (m, 1 H, H-2″), 4.65 (m, 3 H, CH_2-5′ and H-4′), 5.90 (m, 1 H, H-3′), 6.80 (t, 1 H, J = 7.2 Hz, H-1′), 7.33 (m, 4 H, HPh), 7.90 (m, 4 H, HPh), 8.03 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-5), 8.68 (d, 1 H, $J_{6,5}$ = 5.0 Hz, H-6), 9.05 (s, 1 H, H-2). Anal. ($C_{27}H_{24}N_4O_7$) C, H, N.

7-Nitro-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (11) and 7-Methoxy-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (11a). A solution of 4.0 g (7.74 mmol) of **10** in 200 mL of methanol saturated at 0 °C with ammonia was set aside at room temperature for 40 h. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–NH_3 (88:10:2) as eluent to give 1.2 g (55%) of **11** and 0.3 g (15%) of **11a** as a chromatographically pure amber glass: (**11**) ^{1}H NMR (Me_2SO-d_6) δ 2.44 (m, 1 H, H-2′), 2.83 (m, 1 H, H-2″), 3.65 (m, 2 H, CH_2-5′), 3.96 (m, 1 H, H-4′), 4.52 (m, 1 H, H-3′), 6.64 (t, 1 H, J = 7.0 Hz, H-1′), 8.04 (d, 1 H, $J_{5,6}$ = 5.5 Hz, H-5), 8.75 (d, 1 H, $J_{6,5}$ = 5.5 Hz, H-6), 9.07 (s, 1 H, H-2). Anal. ($C_{11}H_{12}N_4O_5$) C, H, N. (**11a**) ^{1}H NMR (Me_2SO-d_6) δ 2.38 (m, 1 H, H-2′), 2.78 (m, 1 H, H-2″), 3.64 (m, 2 H, CH_2-5′), 3.93 (m, 1 H, H-4′), 4.09 (s, 3 H, OCH_3), 4.50 (m, 1 H, H-3′), 6.52 (t, 1 H, J = 7.0 Hz, H-1′), 6.92 (d, 1 H, $J_{6,5}$ = 5.5 Hz, H-6), 8.24 (d, 1 H, $J_{5,6}$ = 5.5 Hz, H-5), 8.52 (s, 1 H, H-2). Anal. ($C_{12}H_{15}N_3O_4$) C, H, N.

7-(Hydroxylamino)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (12). To a suspension of 0.96 g (3.42 mmol) of **11** in 30 mL of THF under an atmosphere of N_2 was added 0.88 g (10 mmol) of NaH_2PO_2 in 20 mL of H_2O and 70 mg of 5% Pd/C. The reaction mixture was stirred under N_2 for 10 h, then the catalyst was removed and washed with methanol, and the filtrate was concentrated to dryness. The residue was dissolved in methanol, and the phosphorus salts were precipitated with EtOAc and removed by filtration. The filtrate was concentrated in vacuo and the residue chromatographed on a silica gel column with $CHCl_3$–MeOH (85:15) as eluent to provide 0.51 g (55%) of **12** as a white solid: mp 66–69 °C; UV λ_{max} (MeOH) 269 nm (ε 20200), 284 (sh) (ε 18000); ^{1}H NMR (Me_2SO-d_6) δ 2.27 (m, 1 H, H-2′), 2.82 (m, 1 H, H-2″), 3.33 (m, 2 H, CH_2-5′), 3.96 (m, 1 H, H-4′), 4.46 (m, 1 H, H-3′), 6.47 (t, 1 H, J = 6.0 Hz, H-1′), 6.75 (d, 1 H, $J_{6,5}$ = 5.5 Hz, H-6), 8.04 (d, 1 H, $J_{5,6}$ = 5.5 Hz, H-5), 8.37 (s, 1 H, H-2), 8.89 (s, 1 H, NHOH), 9.65 (s, 1 H, NHOH). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

7-(Hydroxylamino)-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (13). To a suspension of 0.3 g (0.58 mmol) of **10** in 10 mL of THF under an atmosphere of N_2 was added 0.15 g (1.70 mmol) of NaH_2PO_2 in 5 mL of H_2O and 20 mg of 5% Pd/C. The reaction mixture was stirred under N_2 overnight and then the catalyst was removed and washed with methanol, and the filtrate was concentrated to dryness. The residue was dissolved in methanol, and the phosphorus salts were precipitated with EtOAc and removed by filtration. The filtrate was concentrated in vacuo and the residue chromatographed on a silica gel column with $CHCl_3$–cC_6H_{12}–MeOH (60:38:2) as eluent to provide 0.12 g (41%) of **13** as a chromatographically pure solid: ^{1}H NMR (Me_2SO-d_6) δ 2.37 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.86 (m, 1 H, H-2′), 3.21 (m, 1 H, H-2″), 4.56 (m, 3 H, CH_2-5′ and H-4′), 5.75 (m, 1 H, H-3′), 6.67 (t, 1 H, J = 7.2 Hz, H-1′), 6.78 (d, 1 H, $J_{6,5}$ = 7.4 Hz, H-6), 7.33 (m, 4 H, HPh), 7.90 (m, 4 H, HPh), 8.07 (d, 1 H, $J_{5,6}$ = 7.4 Hz, H-5), 8.36 (s, 1 H, H-2), 9.90 (s, 1 H, NHOH), 11.34 (s, 1 H, NHOH). Anal. ($C_{27}H_{28}N_4O_6$) C, H, N.

5-Chloro-7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (16) and 5-Chloro-7-methoxy-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (16a). A solution of 0.9 g (1.97 mmol) of 5-chloro-7-nitro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (15)[11] in 50 mL of methanol saturated at 0 °C with ammonia was set aside at −20 °C for 4 h. The reaction mixture was evaporated, and the residue was flash chromatographed on a silica gel column with $CHCl_3$–MeOH (97:3) as eluent to give 0.36 g (55%) of **16** and 0.1 g (16%) of **16a** as chromatographically pure amber glass: (**16**) ^{1}H NMR (Me_2SO-d_6) δ 3.69 (m, 2 H, CH_2-5′), 4.03 (m, 1 H, H-4′), 4.28 (m, 1 H, H-3′), 4.59 (m, 1 H, H-2′), 6.15 (d, 1 H, J = 5.5 Hz, H-1′), 8.16 (s, 1 H, H-6), 9.14 (s, 1 H, H-2). Anal. ($C_{11}H_{11}ClN_4O_6$) C, H, N. (**16a**) ^{1}H NMR (Me_2SO-d_6) δ 3.62 (m, 2 H, CH_2-5′), 4.06 (m, 2 H, H-4′ and H-3′), 4.10 (s, 3 H, OCH_3), 4.56 (m, 1 H, H-2′), 4.98 (d, 1 H, J = 5.5 Hz, H-1′), 7.01 (s, 1 H, H-6), 8.58 (s, 1 H, H-2). Anal. ($C_{12}H_{14}ClN_3O_5$) C, H, N.

5-Chloro-7-(hydroxylamino)-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (17). To a suspension of 0.30 g (0.91 mmol) of **16** in 10 mL of THF under an atmosphere of N_2 was added 230 mg (2.7 mmol) of NaH_2PO_2 in 5 mL of H_2O and 30 mg of 5% Pd/C. The reaction mixture was stirred under N_2 for 30 min, then the catalyst was removed, and the filtrate was concentrated to dryness. The residue was dissolved in methanol, and the phosphorus salts were precipitated with EtOAc and removed by filtration. The filtrate was concentrated in vacuo and the residue chromatographed on a silica gel column with AcOEt–MeOH (93:7) as eluent to provide 0.19 g (67%) of **17** as a white solid: mp 201–204 °C; UV λ_{max} (MeOH) 275 nm (ε 15 200); ^{1}H NMR (Me_2SO-d_6) δ 3.59 (m, 2 H, CH_2-5′), 3.95 (m, 1 H, H-4′), 4.12 (m, 1 H, H-3′), 4.55 (m, 1 H, H-2′), 5.87 (d, 1 H, J = 6.6 Hz, H-1′), 6.60 (s, 1 H, H-6), 8.37 (s, 1 H, H-2), 9.10 (s, 1 H, NHOH), 10.15 (s, 1 H, NHOH). Anal. ($C_{11}H_{13}ClN_4O_5$) C, H, N.

5-Chloro-7-nitro-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (18). To a suspension of 0.50 g (2.52 mmol) of 5-chloro-7-nitro-3*H*-imidazo[4,5-*b*]pyridine (14)[7] in 20 mL of dry acetonitrile under an atmosphere of dry N_2 was added 90 mg of 80% NaH, and the mixture was stirred at room temperature for 30 min. To the ice-cooled mixture was added 1.2 g (3.08 mmol) of freshly prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[8] and the suspension was stirred at 0 °C for 3 h. The reaction mixture was filtered to remove the insoluble material, and the filtrate was concentrate at 20 °C to a residue which was chromatographed on a silica gel column. Elution with C_6H_{12}–AcOEt (65:35) gave 0.68 g (50%) of **18** as a pure solid: mp 168–171 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.36 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 2.89 (m, 1 H, H-2′), 3.34 (m, 1 H, H-2″), 4.65 (m, 3 H, CH_2-5′ and H-4′), 5.82 (m, 1 H, H-3′), 6.69 (t, 1 H, J = 7.5 Hz, H-1′), 7.33 (m, 4 H, HPh), 7.88 (m, 4 H, HPh), 8.15 (s, 1 H, H-6), 9.06 (s, 1 H, H-2). Anal. ($C_{27}H_{23}ClN_4O_7$) C, H, N.

5-Chloro-7-(hydroxylamino)-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (19). To a suspension of 0.58 g (1.05 mmol) of **18** in 15 mL of THF under an atmosphere of N_2 was added 0.28 g (3.2 mmol) of NaH_2PO_2 in 10 mL of H_2O and 30 mg of 5% Pd/C. The reaction mixture stirred under N_2 for 14 h, then the catalyst was removed and washed with ethanol, and the filtrate was concentrated to dryness. Recrystallization from water of the white residue gave 0.31 g (55%) of **19** as white crystals: mp 184–186 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.37 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 2.77 (m, 1 H, H-2′), 3.23 (m, 1 H, H-2″), 4.59 (m, 3 H, CH_2-5′ and H-4′), 5.79 (m, 1 H, H-3′), 6.52 (t, 1 H, J = 7.0 Hz, H-1′), 6.63 (s, 1 H, H-6), 7.35 (m, 4 H, HPh), 7.91 (m, 4 H, HPh), 8.36 (s, 1 H, H-2), 9.14 (s, 1 H, NHOH), 10.17 (s, 1 H, NHOH). Anal. ($C_{27}H_{25}ClN_4O_6$) C, H, N.

5-Chloro-7-methoxy-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (20). To 0.15 mmol of **18** or **19** in 10 mL of methanol was added 1 g of Amberlyst A-26 (OH^- form), and the suspension was stirred at room temperature for 5 h. The reaction mixture was filtered to remove the insoluble material, and the filtrate was concentrate to a residue which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (94:6) gave 0.13 mmol (89%) of **20** as chromatographically pure amber glass: ^{1}H NMR (Me_2SO-d_6) δ 2.31 (m, 1 H, H-2′), 2.67 (m, 1 H, H-2″), 3.56 (m, 2 H, CH_2-5′), 3.87 (m, 1 H, H-4′), 4.08 (s, 3 H, OCH_3), 4.40 (m, 1 H, H-3′), 6.38 (t, 1 H, J = 7.0 Hz, H-1′), 6.99 (s, 1 H, H-6), 8.52 (s, 1 H, H-2). Anal. ($C_{12}H_{14}ClN_3O_4$) C, H, N.

Biological Studies. Enzyme Assay. The method used for the determination of activity against adenosine deaminase has been described in a preceding paper.[5]

Antitumor Activity in Vitro. Cell Lines. L1210 and P388 murine lymphhocytic leukemias, originally obtained from the N.C.I. Bethesda, MD, were obtained from in vivo and maintained in vitro in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 10% fetal calf serum (PAA, Gallneukirchen, Austria) and antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin). One and 2% of β-mercaptoethanol (1 mM) were added at L1210 and P388, respectively. The characteristic and the in vitro cultures of these cell lines have been previously described.[14-16]

Drugs. Compounds were dissolved at 1 mg/mL in 5% DMSO and then further diluted in water to the concentrations used in the test. The final DMSO concentration (0.5%) present at the highest drug dose is not toxic for both L1210 and P388.

Growth Inhibition Assay. Cells were plated at 3×10^4 mL^{-1} in 16-mm wells of a 24-well plate (Falcon) and immediately treated with a freshly prepared solution for 72 h. At the end of treatment the cells were counted by a Coulter Counter (Coulter Electronics ZM Model). Data were expressed as percentage of control. The 50% inhibitory concentration (IC_{50}) was calculated on concentration–response curves.

Antitumor Activity in Vivo. Animals and Tumors. First generation hybrid Balb/c × DBA/2 (CD_2F_1) adult female mice were used for the evaluation of antitumor activity. All animals were obtained from Charles River Italia (Calco, Como, Italy). The animals were 2–3 months old, weighed 25–30 g, and were kept under standard laboratory conditions.

The L1210 murine leukemia, originally obtained from the N.C.I., Bethesda, MD, was maintained by weekly ip passages of 10^5 cells in DBA/2 mice. For experimental purpose ip inocula of 1×10^5 cells/mouse into CDF_1 mice were used.

Drugs. Immediately before use, the compounds were dissolved in a 10% Tween 80 (Difco Laboratory) solution. Treatment was administered ip in a volume of 10 mL/kg of body weight at days 1 and 2.

Evaluation of Antitumor Activity and Toxicity. In experiments in leukemia models, drug activity was evaluated in terms of percent increase in median survival time in comparison to untreated controls (T/C). Toxicity was evaluated on the basis of the gross autopsy findings, as well as on the basis of reduction in survival time below that of untreated controls.

Acknowledgment. This work was supported by a grant from the Regione Marche. We thank M. Brandi, G. Rafaiani, G. Melegaro, and C. Tirabassi for technical assistence.

Registry No. **2**, 5451-40-1; **3**, 3056-18-6; **4**, 133832-61-8; **5**, 38925-80-3; **6**, 133832-62-9; **7**, 109151-82-8; **8**, 109151-84-0; **9**, 133832-63-0; **10**, 133832-64-1; **11**, 133832-65-2; **11a**, 133832-60-7; **12**, 133832-66-3; **13**, 133832-67-4; **14**, 101857-28-7; **15**, 101904-03-4; **16**, 101857-29-8; **16a**, 133832-72-1; **17**, 133832-68-5; **18**, 133832-69-6; **19**, 133832-70-9; **20**, 133832-71-0; adenine deaminase, 9027-68-3.

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