Synthesis and Biological Activity of 3- and 5-Amino Derivatives of Pyridine-2-carboxaldehyde Thiosemicarbazone

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A series of 3- and 5-alkylamino derivatives, as well as other structurally modified analogues of pyridine-2-carboxaldehyde thiosemicarbazone, have been synthesized and evaluated as inhibitors of CDP reductase activity and for their cytotoxicity in vitro and antineoplastic activity in vivo against the L1210 leukemia. Alkylation of 3- and 5-amino-2-(1,3-dioxolan-2-yl)pyridines (**1**, **2**) resulted in corresponding 3-methylamino, 5-methylamino, 3-allylamino, 5-ethylamino, 5-allylamino, 5-propylamino, and 5-butylamino derivatives (**5**, **6**, and **11**−**15**), which were then condensed with thiosemicarbazide to yield the respective thiosemicarbazones (**7**, **8**, and **16**−**20**). Oxidation of 3,5-dinitro-2-methylpyridine (**21**) with selenium dioxide, followed by treatment with ethylene glycol and *p*-toluenesulfonic acid, produced the cyclic ethylene acetal, **23**. Oxidation of 2-(1,3-dioxolan-2-yl)-4-methyl-5-nitropyridine (**26**) with selenium dioxide, followed by sequential treatment with sodium borohydride, methanesulfonyl chloride, and morpholine afforded the morpholinomethyl derivative **30**. Catalytic hydrogenation of **23** and **30** with Pd/C yielded the corresponding amino derivatives **24** and **31**. Catalytic hydrogenation of 5-cyano-2-methylpyridine (**33**) with Raney nickel, followed by treatment with acetic anhydride, gave the amide derivative **35**. *N*-Oxidation of **35**, followed by rearrangement with acetic anhydride, produced the acetate derivative, 5-[(acetylamino)methyl]-2-(acetoxymethyl)pyridine (**37**). Repetition of the *N*-oxidation and rearrangement procedures with compound **37** yielded the diacetate derivative **39**. Condensation of compounds **24**, **31**, and **39** with thiosemicarbazide afforded the respective 3,5-diaminopyridine-, 4-(4-morpholinylmethyl)-5-aminopyridine-, and 5-(aminomethyl)pyridine-2-carboxaldehyde thiosemicarbazones (**25**, **32**, and **40**). The most biologically active compounds synthesized were the 5-(methylamino)-, 5-(ethylamino)-, and 5-(allylamino)pyridine-2-carboxaldehyde thiosemicarbazones (**8**, **17**, and **18**), which were potent inhibitors of ribonucleotide reductase activity with corresponding IC_{50} values of 1.3, 1.0, and 1.4 μM and which produced significant prolongation of the survival time of L1210 leukemia-bearing mice, with corresponding optimum % T/C values of 223, 204, and 215 being obtained when administered twice daily for six consecutive days at dosages of 60, 80, and 80 mg/kg, respectively.

Ribonucleoside diphosphate reductase is a critical enzyme in the synthesis of the deoxyribonucleotide precursors of DNA and, as such, is essential for cellular replication. Thus, its presence and activity is closely correlated with cellular growth rates.[1,2] Since deoxyribonucleoside triphosphates are present in extremely low levels in mammalian cells, a strong inhibitor of ribonucleoside diphosphate reductase would appear to be a particularly useful weapon in the therapeutic armamentarium against cancer, especially those neoplasms that are rapidly proliferating.[3] Several different classes of agents have been shown to be inhibitors of ribonucleoside diphosphate reductase. These include the α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones (HCTs),[4] hydroxyurea,[5] *N*-hydroxy-*N*′-aminoguanidine derivatives,[6−8] and polyhydroxybenzohydroxamates.[9,10] The HCTs, as a class, are among the most potent known inhibitors of ribonucleoside diphosphate reductase, depending upon the HCT, being 80−5000 times more potent than hydroxyurea, a clinically useful anticancer agent.[11,12] Members of the HCT class have shown anticancer activity against a wide spectrum of transplanted rodent neoplasms, as well as spontaneous lymphomas of dogs.[13,14] Such broad spectrum activity denotes clinical potential and suggests that a drug of this class may well have utility in cancer therapy.

A variety of nucleoside analogues are also active as inhibitors of ribonucleoside diphosphate reductase. These include 2,2′-difluoro-2′-deoxycytidine,[15−17] 2′-fluoroadenine arabinoside,[18] and 2-chloro-2′-deoxyadenosine.[19] However, the 5′-triphosphate of each of these compounds appears to be a more potent inhibitor of DNA polymerase than that of ribonucleoside diphosphate reductase, making DNA polymerase the more probable primary target of these nucleoside analogues.[18−20]

One of the members of the HCT series, 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP), has received a phase I trial in patients with cancer.[21,22] These studies have shown that the tumor-inhibitory activity of 5-HP in animal systems did not translate to patients with cancer. The inactivity of 5-HP as an antineoplastic agent in patients was attributed to a relatively short biological half-life in humans, which was due to the formation of an inactive *O*-glucuronide and the rapid elimination of this conjugate.

Recently, we have reported the synthesis and evaluation of several new HCTs that by virtue of their

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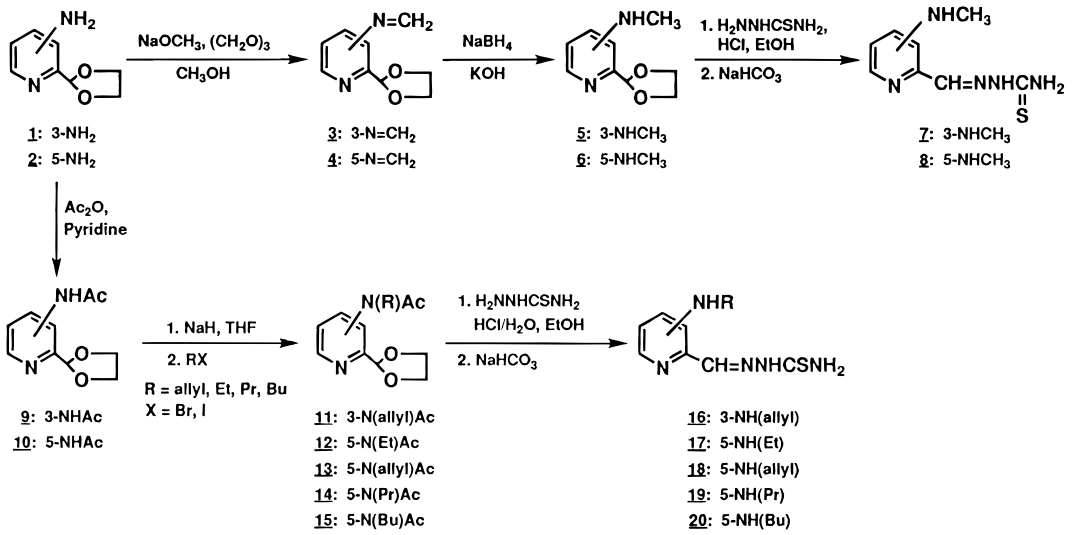
Scheme 1



structures are resistant to *O*-glucuronidation.[23−25] Among these compounds, the 3- and 5-aminopyridine-2-carboxaldehyde thiosemicarbazones (3-AP and 5-AP) showed significant antitumor activity in mice bearing the L1210 leukemia. 3-AP appeared to be the more promising, being 6 times more potent than 5-HP as an inhibitor of CDP reductase activity, and 3 and 7.5 times more potent than 5-HP as an inhibitor of the growth of wild-type and hydroxyurea-resistant L1210 cells, respectively.[26,27] In contrast, 5-AP was slightly more active than 5-HP as an inhibitor of CDP reductase activity and of the growth of parental L1210 cells but was 3 times more potent than 5-HP as an inhibitor of the growth of hydroxyurea-resistant cells. Since acetylamino derivatives of 3- and 5-AP were found to be devoid of carcinostatic activity and *N*-acetylation is a relatively ubiquitous metabolic reaction in vivo, we designed and synthesized a series of 3- and 5-alkylamino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone. Alkylation of the amino groups on the pyridine ring of 3- and 5-AP results in the conversion of these compounds from primary amines to the corresponding secondary amines and, therefore, would result in steric hindrance to the enzymatic acetylation of the amino function. Furthermore, introduction of an alkyl group would increase lipid solubility, which might improve the pharmacokinetic properties of the parent compounds. In addition, to further characterize the structural features required for optimal biological activity in the HCT series, several other amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone were also synthesized. The synthesized compounds were evaluated in vitro as inhibitors of CDP reductase activity and of the growth of L1210 cells and in vivo for antitumor activity against the L1210 leukemia.

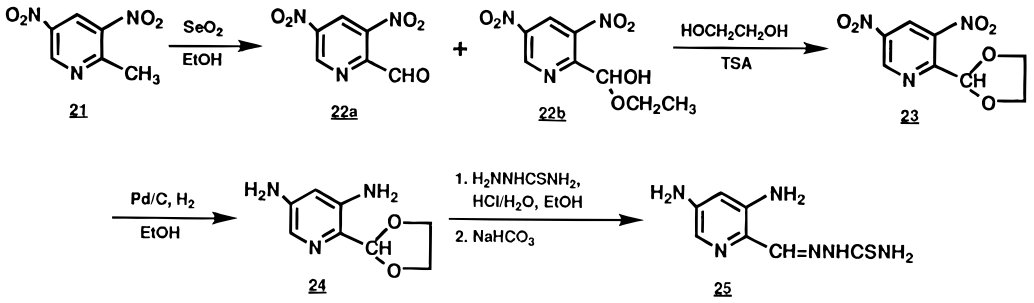
Chemistry

The synthesis of a variety of 3- and 5-mono(alkylamino) derivatives of pyridine-2-carboxaldehyde thiosemicarbazone is depicted in Scheme 1. Selective monomethylation of 3- and 5-amino-2-(1,3-dioxolan-2-yl)pyridines (**1** and **2**), which were prepared according to the procedure previously reported by our laboratory,[23] was achieved by the methodology of Barluenga et al.,[28] with minor modifications. Treatment of compounds **1** and **2** with sodium methoxide and paraformaldehyde in methanol, followed by reduction of the resulting methyleneamines (**3** and **4**) with $NaBH_4$ and 1 N KOH, gave the corresponding 3- and 5-(methylamino)-2-(1,3-dioxolan-2-yl)pyridines (**5** and **6**), which were then treated with thiosemicarbazide and concentrated hydrochloric acid in ethanol to afford the target products, 3- and 5-(methylamino)pyridine-2-carboxaldehyde thiosemicarbazones (**7** and **8**). Acetylation of compounds **1** and **2** with acetyl anhydride in anhydrous pyridine at room temperature gave the respective acetyl derivatives **9** and **10**. Treatment of compounds **9** and **10** with NaH in anhydrous THF, followed by an alkyl halide, afforded the corresponding 3- and 5-alkyl-substituted derivatives **11**−**15**. Direct condensation of compounds **11**−**15** with thiosemicarbazide in the presence of hydrochloric acid provided 3-(allylamino)-, 5-(ethylamino)-, 5-(allylamino)-, 5-(*n*-propylamino)-, and 5-(*n*-butylamino)pyridine-2-carboxaldehyde thiosemicarbazones (**16**−**20**), respectively.

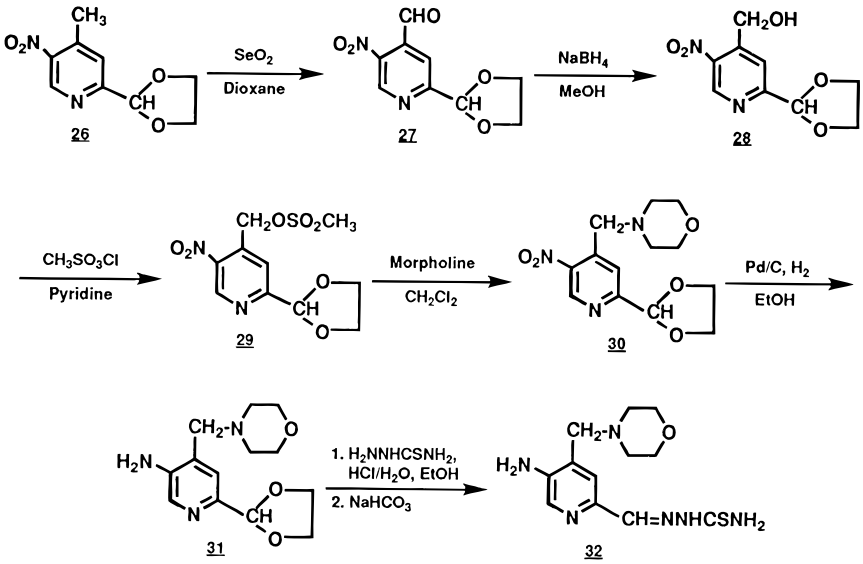
3,5-Diaminopyridine-2-carboxaldehyde thiosemicarbazone (**25**) was synthesized as illustrated in Scheme 2. The starting material, 3,5-dinitro-2-methylpyridine (**21**), was synthesized by methodology developed in our laboratory.[29] Oxidation of **21** with selenium dioxide by the minor modification of a method reported by Achremowicz[30] yielded a mixture of 3,5-dinitro-2-pyridine aldehyde (**22a**) and its ethyl hemiacetal (**22b**) in a ratio of 1:3 (estimated by NMR spectra), which, because of sensitivity to air, was treated directly with ethylene glycol and *p*-toluenesulfonic acid in toluene to form 3,5-dinitro-2-(1,3-dioxalan-2-yl)pyridine (**23**). Catalytic hydrogenation of compound **23** with 10% Pd/C in ethanol produced the corresponding diamino derivative **24**, which upon condensation with thiosemicarbazide under acidic conditions gave 3,5-diaminopyridine-2-carboxaldehyde thiosemicarbazone (**25**).

The synthesis of 5-amino-4-(morpholinomethyl)pyridine-2-carboxaldehyde thiosemicarbazone (**32**) is shown in Scheme 3. Oxidation of 2-(1,3-dioxolan-2-yl)-4-methyl-5-nitropyridine (**26**)[23] with selenium dioxide in dioxane gave the corresponding aldehyde **27**. Selective reduction of the aldehyde function of **27** with sodium borohydride in methanol yielded the 4-hydroxymethyl derivative **28**. Treatment of compound **28** with meth-

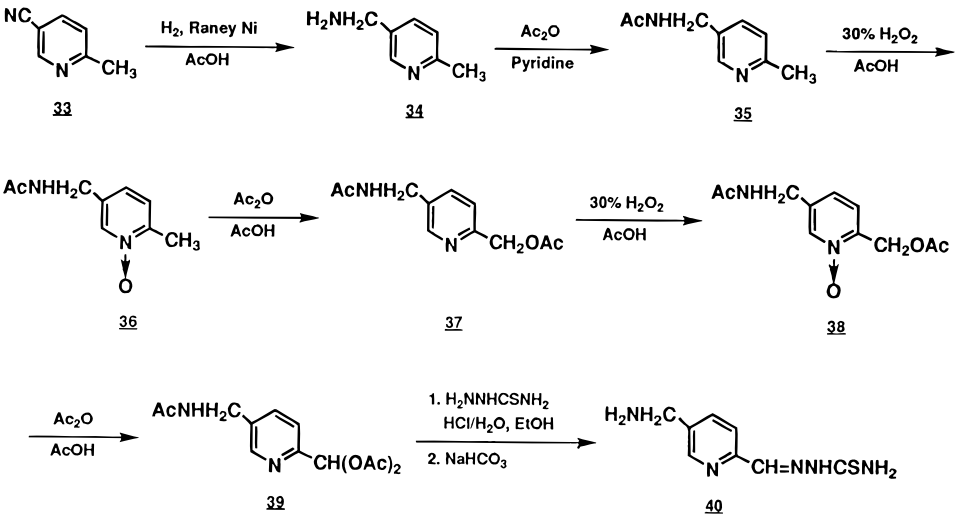
Scheme 2



Scheme 3



Scheme 4



anesulfonyl chloride in anhydrous pyridine followed by refluxing with morpholine produced the 4-morpholinomethyl derivative **30** via the intermediate **29**. Catalytic hydrogenation of compound **30** over 10% Pd/C in ethanol afforded the 5-amino derivative **31**, which was then converted to the target thiosemicarbazone **32** by condensation with thiosemicarbazide in the presence of hydrochloric acid. 5-(Aminomethyl)pyridine-2-carboxaldehyde thiosemicarbazone (**40**) was prepared as described in Scheme 4. Catalytic hydrogenation[31] of 5-cyano-2-methylpyridine (**33**) in the presence of Raney nickel in acetic acid gave the 5-aminomethyl derivative **34**. The pressure of the hydrogen and the reaction time are critical to the yield of the product. High hydrogen pressure or a relatively long reaction time led to mostly pyridine ring hydrogenated side products. Protection of the amino function of **34** by reaction with acetic anhydride in anhydrous pyridine yielded compound **35**. Treatment of **35** with 30% hydrogen peroxide in glacial acetic acid produced the *N*-oxide **36**, which was then refluxed with a mixture of acetic acid and acetic anhydride (1:2, v/v) to give the acetate **37**. Repeating the *N*-oxidation and rearrangement procedures with compound **37** and the resulting *N*-oxide **38**, respectively,

Table 1. Effects of Pyridine-2-carboxaldehyde Thiosemicarbazone Derivatives on CDP Reductase Activity and the Growth of L1210 Cells in Culture

	IC_{50} (μM)[a]	
compd	CDP reductase[b]	L1210 cell growth
3-AP	0.82	1.4
5-AP	2.7	2.5
7	1.0	1.5
8	1.3	2.4
16	1.7	≫10
17	1.0	3.4
18	1.4	4.4
19	1.4	5.1
20	2.5	7.8
25	9.0	1.1
32	11.0	≫10
40	2.5	2.1

[a] The concentration (μM) required to inhibit CDP reductase activity or L1210 cell growth by 50%. [b] CDP reductase IC_{50} values are the average of two separate determinations. The values varied by less than 10%.

produced the diacetate derivative **39**. Treatment of **39** with thiosemicarbazide in the presence of hydrochloric acid resulted in the desired compound **40**.

Biological Results and Discussion

The primary lesion created in cells by the HCTs is interference with the biosynthesis of DNA, and this action is primarily due to the potent inhibition of ribonucleoside diphosphate reductase activity. For this reason, the comparative effects of various 3- and 5-amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone on CDP reductase activity and on the growth of L1210 cells in culture were measured, and the results are shown in Table 1. Introduction of an alkyl substituent onto the amino group on the pyridine ring had different effects on the inhibitory activity of these agents, depending upon whether the amino group was in the 3- or the 5-position. Thus, alkylation of the amino group of 3-AP with a methyl or an allyl group, compounds **7** and **16**, respectively, produced agents that were slightly less active than 3-AP as inhibitors of CDP reductase activity, with IC_{50} values of 1.0 and 1.7 μM compared to an IC_{50} value of 0.82 μM for the parent compound 3-AP. However, while the 3-methylamino derivative, compound **7**, showed almost equivalent activity to 3-AP as an inhibitor of the growth of L1210 cells, with IC_{50} values of 1.5 and 1.4 μM, respectively, the 3-allylamino derivative **16** was inactive as an inhibitor of the growth of L1210 cells at up to 10 μM. In contrast to the results with the 3-substituted amino derivatives, alkylation of the amino group of 5-AP with methyl, ethyl, allyl, and propyl groups, compounds **8**, **17**, **18**, and **19**, respectively, resulted in agents that were more active as inhibitors of the reductase enzyme than 5-AP, with IC_{50} values of 1.3, 1.0, 1.4, and 1.4 μM, respectively, compared to an IC_{50} value of 2.7 μM for the parent compound 5-AP. Substitution of the amino group of 5-AP with the more bulky butyl group (compound **20**) showed activity similar to that of the parent compound 5-AP, having an IC_{50} value of 2.5 μM. However, while the 5-methylamino derivative, compound **8**, showed activity equivalent to that of 5-AP as an inhibitor of the growth of L1210 cells, with IC_{50} values of 2.4 and 2.5 μM, respectively, the inhibitory activity of compounds **17**, **18**, **19**, and **20** was gradually decreased as the size of the alkyl group was increased, with IC_{50} values of 3.4, 4.4, 5.1, and 7.8 μM, respectively. Thus, it appears that the amino function in the 5-position of the pyridine ring is more tolerant to the introduction of an alkyl group than the corresponding amino function in the 3-position for inhibitory activity on CDP reductase activity and growth inhibition of L1210 cells. The 3,5-diamino derivative, compound **25**, which combines the structural features of both 3-AP and 5-AP, was much less active as an inhibitor of CDP reductase activity than either monosubstituted agents. The relatively potent inhibitory activity of compound **25** against the growth of L1210 cells may well be the result of the production of a metabolic lesion other than inhibition of ribonucleoside diphosphate reductase. Introduction of a bulky morpholinomethyl group onto the 4-position of the pyridine ring of 5-AP resulted in an agent, compound **32**, that was much less active than the parent compound, 5-AP. Insertion of a methylene substituent between the 5-amino function and the pyridine ring of 5-AP resulted in the production of compound **40**, which showed activity comparable to that of the parent compound.

Table 2. Effects of Pyridine-2-carboxaldehyde Thiosemicarbazone Derivatives on the Survival Time of Mice Bearing the L1210 Leukemia

compd	optimum daily dosage[a] (mg/kg)	av Δ wt[b] (%)	av survival[c] (days)	T/C × 100[d]	long-term survivors[e]
3-AP	20	−2.4	18.0	225	0/5
5-AP	10	−4.3	15.5	194	0/5
7	40	−2.9	11.8	148	0/5
8	60	−4.4	17.8	223	0/5
16	80	−2.6	10.1	126	0/5
17	80	−3.4	16.3	204	0/5
18	60	−3.4	17.2	215	0/5
19	60	−5.2	11.7	146	0/5
20	40	−4.0	9.2	115	0/5
25	60	−17.1	10.8	135	2/5
32	40	−3.5	10.0	125	0/5
40	80	−2.4	13.4	168	1/5

[a] Drugs were administered in suspension by intraperitoneal injection, given as two daily divided doses, beginning 24 h after tumor implantation, for 6 consecutive days, with five mice per group. [b] Average change in body weight from onset to termination of therapy. [c] Average survival time includes only those mice that died prior to day 60. [d] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 8.0 days. [e] Long-term survivors are the number of mice that survived for >60 days relative to the total number of treated mice.

Since the various substituents would be expected to impact on the pharmacokinetic properties of the synthesized agents, the tumor-inhibitory properties of the 3- and 5-amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone were determined by measuring their ability to prolong the survival time of mice bearing the L1210 leukemia. Compounds were administered in suspension by intraperitoneal (ip) injection to groups of five tumor-bearing mice, twice daily for 6 consecutive days, by methodology previously described.[32] The results of these tests are shown in Table 2. A wide range of dosage levels of each compound of from 10 to 80 mg/kg per day × 6 were tested, and the prolongation of life produced by the optimum effective daily dose of each compound is listed. For comparison, the effects of the two parental compounds, 3-AP and 5-AP, are also included. As shown in Table 2, the 5-methylamino, 5-ethylamino, and 5-allylamino derivatives, compounds

8, **17**, and **18**, showed significant antitumor activity, producing corresponding % T/C values of 223, 204 and 215 at their optimum daily dosage levels. The antitumor activity of these compounds was comparable to that of 3-AP, which had a maximum % T/C value of 225, and slightly better than that of 5-AP, which had a maximum % T/C value of 194. The 3-methylamino and 5-propylamino derivatives, compounds **7** and **19**, exhibited moderate antitumor activity, and the 3-allylamino, 5-butylamino, and 5-amino-4-morpholinomethyl derivatives, compounds **16**, **20**, and **32**, showed little or no activity. In agreement with the effects of these agents on CDP reductase activity and the growth of L1210 cells, substitution of a bulky group such as a butyl group onto the 5-amino function of 5-AP (**20**) or a 4-morpholinomethyl group onto the pyridine ring of 5-AP (**32**) led to a decrease or loss of activity both in vitro and in vivo. The 3,5-diamino derivative, compound **25**, gave a % T/C value of 135 with 40% of the animals being 60-day long-term survivors. This effect was obtained, however, at the expense of a considerable loss in body weight (17.1%) post drug treatment. The 5-aminomethyl derivative, compound **40**, was comparable to 5-AP as an antineoplastic agent, with a % T/C value of 168 along with one of the five animals being a 60-day long-term survivor. In summary, the 5-methylamino, 5-ethylamino, and 5-allylamino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone (compounds **8**, **17**, and **18**) were good inhibitors of CDP reductase activity and showed significant antitumor activity in vivo with acceptable toxicity as indicated by loss in body weight, and therefore, further evaluation of these agents as potential anticancer drugs appears to be merited.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-390 90 MHz NMR spectrometer or a Bruker WM-250 250 MHz spectrometer with Me_4Si as the internal reference. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value.

3-(Methylamino)-2-(1,3-dioxolan-2-yl)pyridine (5). Sodium (0.6 g, 26 mmol) was added slowly to 15 mL of methanol. Once the evolution of hydrogen had ceased, 3-amino-2-(1,3-dioxolan-2-yl)pyridine[24] (1 g, 6.0 mmol) and paraformaldehyde (0.42 g, 14 mmol) were added. The mixture was stirred at 50 °C for 20 h, and then $NaBH_4$ (0.4 g, 10.6 mmol) was added. After the mixture was heated under reflux for 1 h, 10 mL of 1 N KOH was added and the resulting solution was refluxed for 2 h. The reaction mixture was cooled, and the solvent was removed under reduced pressure. The residue was partitioned between methylene chloride and water, and the aqueous layer was extracted with methylene chloride. The combined organic layer was washed with brine and then water and dried over $MgSO_4$. The filtrate was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel (120 g) column (CH_2Cl_2/EtOH, 20:1, v/v; R_f 0.33). The product was obtained as an oil (0.4 g, 37%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.80 (d, 3H, NCH_3), 4.10 (m, 4H, CH_2CH_2), 5.20 (br s, 1H, NH, D_2O exchangeable), 5.75 (s, 1H, 2-CH), 6.80−7.20 (m, 2H, 4-H and 5-H), 7.75 (dd, 1H, 6-H). Anal. ($C_9H_{12}NO_2$) C, H, N.

5-(Methylamino)-2-(1,3-dioxolan-2-yl)pyridine (6). This compound was prepared from 5-amino-2-(1,3-dioxolan-2-yl)-pyridine[24] (1 g, 6.0 mmol) by the procedure employed for the synthesis of 3-(methylamino)-2-(1,3-dioxolan-2-yl)pyridine: yield 0.7 g (63%); mp 85−87 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.85 (d, 3H, NCH_3), 4.05 (m, 4H, CH_2CH_2), 4.10 (br s, 1H, NH, D_2O exchangeable), 5.72 (s, 1H, 2-CH), 6.82 (m, 1H, 4-H), 7.30 (d, 1H, 3H), 7.90 (d, 1H, 6-H). Anal. ($C_9H_{12}NO_2$) C, H, N.

3-(Methylamino)pyridine-2-carboxaldehyde Thiosemicarbazone (7). To a solution of 3-(methylamino)-2-(1,3-dioxolan-2-yl)pyridine (**5**, 0.30 g, 1.6 mmol) in 5 mL of ethanol, 4 mL of water, and 1 mL of concentrated hydrochloric acid was added 0.16 g (1.7 mmol) of thiosemicarbazide. The mixture was stirred at room temperature overnight, refluxed for 1 h, cooled, and filtered. The crude yellow hydrochloride salt was dissolved in 20 mL of water and filtered. To the filtrate was added 10 mL of 5% sodium bicarbonate solution. The mixture was stirred at room temperature for 1 h, filtered, and washed with water and ethanol to yield 0.23 g (70%) of product as a yellow solid: mp 240−242 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.88 (d, 3H, NCH_3), 6.92 (br s, 1H, 3-NH, D_2O exchangeable), 7.08 (d, 1H, 4-H, $J_{4,5}$ = 6 Hz), 7.22 (dd, 1H, 5-H, $J_{4,5}$ = 6 Hz, $J_{5,6}$ = 4 Hz), 7.86 (d, 1H, 6-H, $J_{5,6}$ = 4 Hz), 8.24 and 8.36 (two s, 2H, $CSNH_2$, D_2O exchangeable), 8.32 (s, 1H, 2-CH), 11.05 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5S\cdot 0.2H_2O$) C, H, N.

5-(Methylamino)pyridine-2-carboxaldehyde Thiosemicarbazone (8). This compound was prepared from 5-(methylamino)-2-(1,3-dioxolan-2-yl)pyridine (**6**, 0.50 g, 2.7 mmol) by the procedure employed for the synthesis of 3-(methylamino)-pyridine-2-carboxaldehyde thiosemicarbazone (**7**): yield 0.45 g (80%); mp 217−218 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.75 (d, 3H, NCH_3), 6.25 (br s, 1H, 3-NH, D_2O exchangeable), 6.81 (dd, 1H, 4-H, $J_{3,4}$ = 7 Hz, $J_{4,6}$ = 2 Hz), 7.85 (d, 1H, 3-H, $J_{3,4}$ = 7 Hz), 7.90 (d, 1H, 6-H, $J_{4,6}$ = 2 Hz), 7.95 (s, 1H, 2-CH), 7.98 (s, 2H, $CSNH_2$, D_2O exchangeable), 8.32 (s, 1H, 2-CH), 11.05 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5S\cdot 0.25H_2O$) C, H, N.

3-(Acetylamino)-2-(1,3-dioxolan-2-yl)pyridine (9). To a stirred solution of **1** (2.0 g, 12 mmol) in 10 mL of anhydrous pyridine in an ice bath was added dropwise 2 mL of acetic anhydride at 0−5 °C. The reaction mixture was stirred overnight and evaporated in vacuo to dryness. The residue was dissolved in CH_2Cl_2 (30 mL), washed with 10% sodium bicarbonate, brine, and water, and then dried (anhydrous $MgSO_4$). The solvent was removed, and the residue was purified on a silica gel column (CH_2Cl_2/AcOEt, 1:4, v/v; R_f 0.27) to produce 2.0 g (80%) of product: mp 92−93 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.05 (s, 3H, CH_3), 4.12 (m, 4H, CH_2CH_2), 5.88 (s, 1H, 2-CH), 7.22 (dd, 1H, 5-H, $J_{4,5}$ = 4 Hz, $J_{5,6}$ = 8 Hz), 8.25 (d, 1H, 4-H, $J_{4,5}$ = 4 Hz), 8.60 (d, 1H, 6-H, $J_{5,6}$ = 8 Hz), 8.60 (br s, 1H, NH, D_2O exchangeable). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

3-(*N*-Acetyl-*N*-allylamino)-2-(1,3-dioxolan-2-yl)pyridine (11). To a solution of compound **9** (2.2 g, 11 mmol) in anhydrous THF (80 mL) was added carefully a suspension of NaH (0.64 g of 80% oil dispersion, 21.2 mmol) in 20 mL of anhydrous THF. After effervescence ceased, the reaction mixture was refluxed for 30 min and a solution of allyl bromide (2.6 g, 22 mmol) in 10 mL of anhydrous THF was added; the mixture was then refluxed for another 2 h. The cooled reaction mixture was evaporated in vacuo to dryness, and the oil residue was dissolved in CH_2Cl_2 (50 mL), washed with water, and dried ($MgSO_4$). The solvent was evaporated, and the residue was chromatographed on a silica gel column (CH_2Cl_2/EtOH, 20:1, v/v; R_f 0.40) to produce 2.3 g (87%) of product as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.85 (s, 3H, $COCH_3$), 3.60−3.90 (m, 2H, NCH_2), 4.05−4.32 (m, 4H, OCH_2CH_2O), 4.85−5.20 (m, 2H, $=CH_2$), 5.70−6.05 (m, 1H, =CH), 5.90 (s, 1H, 2-CH), 7.40 (m, 2H, 4-H and 5-H), 8.75 (m, 1H, 6-H). Anal. ($C_{13}H_{16}N_2O_3$) C, H, N.

Compounds **12**−**15** were synthesized from 5-(acetylamino)-2-(1,3-dioxolan-2-yl)pyridine (**10**)[24] and the appropriate allyl halide by methodology similar to that described for the preparation of compound **11**.

5-(*N*-Acetyl-*N*-ethylamino)-2-(1,3-dioxolan-2-yl)pyridine (12): isolated as an oil (0.34 g, 72%); TLC R_f 0.70 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.05−1.25 (t, 3H, CH_3), 1.90 (s, 3H, $COCH_3$), 3.65−3.90 (q, 2H, NCH_2),

4.10−4.20 (m, 4H, OCH_2CH_2O), 5.80 (s, 1H, 2-CH), 7.62 (m, 2H, 3-H and 4-H), 8.50 (s, 1H, 6-H). Anal. ($C_{12}H_{16}N_2O_3$) C, H, N.

5-(*N*-Acetyl-*N*-allylamino)-2-(1,3-dioxolan-2-yl)pyridine (13): isolated as an oil (1.1 g, 84%); TLC R_f 0.63 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.90 (s, 3H, $COCH_3$), 4.10−4.20 (m, 4H, OCH_2CH_2O), 4.25 (d, 2H, NCH_2), 5.00−5.20 (m, 2H, $=CH_2$), 5.75−5.90 (m, 1H, =CH), 5.88 (s, 1H, 2-CH), 7.55 (m, 2H, 3-H and 4-H), 8.75 (m, 1H, 6-H). Anal. ($C_{13}H_{16}N_2O_3$·0.25EtOH) C, H, N.

5-(*N*-Acetyl-*N*-*n*-propylamino)-2-(1,3-dioxolan-2-yl)pyridine (14): isolated as an oil (1.0 g, 76%); TLC R_f 0.65 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.90−1.05 (t, 3H, CH_3), 1.55−1.82 (m, 2H, CH_2), 1.92 (s, 3H, $COCH_3$), 3.62−3.85 (t, 2H, NCH_2), 4.10−4.20 (m, 4H, OCH_2CH_2O), 5.85 (s, 1H, 2-CH), 7.60 (m, 2H, 3-H and 4-H), 8.45 (d, 1H, 6-H). Anal. ($C_{14}H_{20}N_2O_3$) C, H, N.

5-(*N*-Acetyl-*N*-*n*-butylamino)-2-(1,3-dioxolan-2-yl)pyridine (15): isolated as an oil (1.1 g, 78%); TLC R_f 0.67 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.90 (t, 3H, CH_3), 1.20−1.50 (m, 4H, CH_2CH_2), 1.88 (s, 3H, $COCH_3$), 3.72 (t, 2H, NCH_2), 4.15 (m, 4H, OCH_2CH_2O), 5.85 (s, 1H, 2-CH), 7.55 (m, 2H, 3-H and 4-H), 8.35 (d, 1H, 6-H). Anal. ($C_{13}H_{18}N_2O_3$·0.25EtOH) C, H, N.

Compounds **16**−**20** were synthesized from the corresponding compounds **11**−**15** by methodology similar to that described for the preparation of compound **7**.

3-(Allylamino)pyridine-2-carboxaldehyde thiosemicarbazone (16): yield 1.9 g (77%); mp 215−217 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.00 (m, 2H, NCH_2), 5.14 (m, 2H, vinyl H), 5.90 (m, 1H, vinyl H), 6.72 (br s, 1H, 3-NH, D_2O exchangeable), 7.47 (d, 1H, 4-H, $J_{4,5}$ = 6 Hz), 7.53 (dd, 1H, 5-H, $J_{4,5}$ = 6 Hz, $J_{5,6}$ = 4 Hz), 7.98 (d, 1H, 6-H, $J_{5,6}$ = 4 Hz), 8.43 (s, 2H, $CSNH_2$, D_2O exchangeable), 8.52 (s, 1H, 2-CH), 10.08 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5S$·HCl) C, H, N.

5-(Ethylamino)pyridine-2-carboxaldehyde thiosemicarbazone (17): yield 0.34 g (72%); mp 197−198 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.15 (t, 3H, CH_3), 3.05 (m, 2H, NCH_2), 6.12 (br s, 1H, 3-NH, D_2O exchangeable), 6.82 (dd, 1H, 4-H, $J_{3,4}$ = 7 Hz, $J_{4,6}$ = 2 Hz), 7.76 (d, 1H, 3-H, $J_{3,4}$ = 7 Hz), 7.84 (d, 1H, 6-H, $J_{4,6}$ = 2 Hz), 7.86 (s, 2H, $CSNH_2$, D_2O exchangeable), 7.95 (s, 1H, 2-CH), 11.08 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_9H_{13}N_5S$) C, H, N.

5-(Allylamino)pyridine-2-carboxaldehyde thiosemicarbazone (18): yield 0.70 g (74%); mp 185−187 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.90 (m, 2H, NCH_2), 5.20 (m, 2H, vinyl H), 5.92 (m, 1H, vinyl H), 6.52 (br s, 1H, 3-NH, D_2O exchangeable), 7.47 (d, 1H, 4-H, $J_{4,5}$ = 6 Hz), 7.55 (dd, 1H, 5-H, $J_{4,5}$ = 6 Hz, $J_{5,6}$ = 4 Hz), 7.98 (d, 1H, 6-H, $J_{5,6}$ = 4 Hz), 8.22 (s, 1H, 2-CH), 8.42 and 8.75 (two s, 2H, $CSNH_2$, D_2O exchangeable), 10.05 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5S$) C, H, N.

5-(*n*-Propylamino)pyridine-2-carboxaldehyde thiosemicarbazone (19): yield 0.56 g (80%); mp 192−194 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.05 (t, 3H, CH_3), 1.50 (m, 2H, CH_2), 3.10 (m, 2H, NCH_2), 6.90 (br s, 1H, 3-NH, D_2O exchangeable), 7.10 (dd, 1H, 4-H, $J_{3,4}$ = 6 Hz, $J_{4,6}$ = 2 Hz), 7.86 (d, 1H, 3-H, $J_{3,4}$ = 6 Hz), 7.92 (d, 1H, 6-H, $J_{4,6}$ = 2 Hz), 7.95 (s, 1H, 2-CH), 8.12 and 8.25 (two s, 2H, $CSNH_2$, D_2O exchangeable), 11.08 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5S$·1.2H_2O) C, H, N.

5-(*n*-Butylamino)pyridine-2-carboxaldehyde thiosemicarbazone (20): yield 0.7 g (74%); mp 179−180 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.90 (t, 3H, CH_3), 1.32−1.52 (m, 4H, CH_2CH_2), 3.08 (m, 2H, NCH_2), 6.30 (br s, 1H, 3-NH, D_2O exchangeable), 6.90 (dd, 1H, 4-H, $J_{3,4}$ = 6 Hz, $J_{4,6}$ = 1.5 Hz), 7.86 (d, 1H, 3-H, $J_{3,4}$ = 6 Hz), 7.90 (d, 1H, 6-H, $J_{4,6}$ = 1.5 Hz), 7.93 (s, 1H, 2-CH), 8.00 and 8.10 (two s, 2H, $CSNH_2$, D_2O exchangeable), 10.64 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{11}H_{17}N_5S$) C, H, N.

3,5-Dinitro-2-(1,3-dioxolan-2-yl)pyridine (23). A mixture of 2,4-dinitro-2-methylpyridine (**21**, 2.9 g, 16 mmol) and selenium dioxide (2.9 g, 26 mmol) was refluxed in 20 mL of 95% ethanol under an atmosphere of nitrogen for 20 h. The reaction mixture was cooled and filtered to remove the precipitated black selenium. The filtrate was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel column (CH_2Cl_2/AcOEt, 20:1, v/v) to afford 3.8 g of a yellow syrup as a mixture of compounds **22a** and **22b**, which was further refluxed with ethylene glycol (3 mL) and 50 mg of *p*-toluenesulfonic acid monohydrate in toluene overnight. The mixture was cooled and then washed with 20 mL of 10% $NaHCO_3$ solution, followed by 25 mL of water. The toluene layer was dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column (CH_2Cl_2/EtOAc, 30:1, v/v; R_f 0.36) to afford 3.0 g (79%) of product as a white solid: mp 109−110 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.10 (m, 4H, CH_2CH_2), 6.65 (s, 1H, 2-CH), 9.12 (m, 1H, 4-H), 9.70 (d, 1H, 6-H). Anal. ($C_8H_7N_3O_3$) C, H, N.

3,5-Diamino-2-(1,3-dioxolan-2-yl)pyridine (24). The dinitro derivative **23** (0.50 g, 2.1 mmol) was dissolved in 120 mL of ethanol and hydrogenated in a Parr apparatus under 50 psi of pressure in the presence of 10% Pd/C (70 mg) for 3 h. After filtration, the filtrate was evaporated under reduced pressure to give the product (0.33 g, 89%) as an off-white solid, mp 60−62 °C, which was used for the next step in the reaction without further purification: ninhydrin positive; ^{1}H NMR (90 MHz, DMSO-d_6) δ 3.85 (m, 4H, CH_2CH_2), 4.70 and 4.85 (two br s, 4H, 3- and 5-NH_2, D_2O exchangeable), 5.40 (s, 1H, 2-CH), 6.15 (d, 1H, 4-H), 7.05 (d, 1H, 6-H).

3,5-Diaminopyridine-2-carboxaldehyde Thiosemicarbazone (25). This compound was prepared from **24** (0.22 g, 1.2 mmol) by the procedure employed for the synthesis of **7**: yield 0.22 g (80%); mp 239−241 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 5.50 and 6.05 (two br s, 4H, 3- and 5-NH_2, D_2O exchangeable), 6.15 (d, 1H, 4-H, $J_{4,6}$ = 2 Hz), 7.30 (d, 1H, 6-H, $J_{4,6}$ = 2 Hz), 7.60 and 7.70 (two s, 2H, $CSNH_2$, D_2O exchangeable), 8.10 (s, 1H, 2-CH), 10.90 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_7H_{10}N_6S$) C, H, N.

2-(1,3-Dioxolan-2-yl)-5-nitropyridine-4-carboxaldehyde (27). A mixture of 2-(1,3-dioxolan-2-yl)-4-methyl-5-nitropyridine (**26**, 6.5 g, 16 mmol)[23] and selenium dioxide (10 g, 90 mmol) was refluxed in anhydrous dioxane (100 mL) until TLC showed that the reaction was complete (about 40 h). The reaction mixture was evaporated in vacuo to dryness, and the residue was stirred with 200 mL of methylene chloride for 1 h and then filtered. The filtrate was concentrated to a small volume and chromatographed on a silica gel column (CH_2Cl_2/EtOH, 25:1, v/v; R_f 0.56) to afford 4.8 g (66%) of product as an off-white solid: mp 77−78 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.15 (m, 4H, CH_2CH_2), 5.90 (s, 1H, 2-CH), 7.95 (s, 1H, 3-H), 9.32 (s, 1H, 6-H), 10.50 (s, 1H, 4-CHO). Anal. ($C_9H_8N_2O_5$) C, H, N.

2-(1,3-Dioxolan-2-yl)-4-(hydroxymethyl)-5-nitropyridine (28). To a solution of compound **27** (4.6 g, 21 mmol) in 100 mL of methanol was added dropwise a solution of $NaBH_4$ (0.4 g) in 4 mL of water at 0−5 °C with stirring. After the addition of the $NaBH_4$, the reaction mixture was stirred for an additional 30 min, and then evaporated to dryness. The residue was stirred with 20 mL of water for 30 min and the white solid that formed was collected by filtration and washed with water to afford 3.4 g (77%) of product, which was used for the next step in the reaction. An analytical sample was obtained by purification of the crude product on a silica gel column (CH_2Cl_2/AcOEt, 4:1, v/v, R_f 0.45): mp 119−120 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.21 (m, 4H, CH_2CH_2), 5.00 (s, 2H, 4-CH_2), 5.05 (t, 1H, OH, D_2O exchangeable), 5.88 (s, 1H, 2-CH), 8.10 (s, 1H, 3-H), 9.21 (s, 1H, 6-H). Anal. ($C_9H_{10}N_2O_5$) C, H, N.

2-(1,3-Dioxolan-2-yl)-4-[[(methylsulfonyl)oxy]methyl]-5-nitropyridine (29). To a solution of compound **28** (0.50 g, 2.2 mmol) in 15 mL of methylene chloride was added dropwise 0.30 g (2.6 mmol) of methanesulfonyl chloride at 0−5 °C with stirring, followed by 0.3 mL of triethylamine. The reaction mixture was stirred for about 1 h, at which time TLC showed that the reaction was complete. The mixture was washed with water (2 × 5 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness, and the residue was purified by silica gel column chromatography (CH_2Cl_2/AcOEt, 4:1, v/v, R_f 0.75) to give 0.5 g (75%) of product as white crystals: mp 126−

127 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.15 (s, 3H, CH_3), 4.21 (m, 4H, CH_2CH_2), 5.82 (s, 2H, 4-CH_2), 6.00 (s, 1H, 2-CH), 8.05 (s, 1H, 3-H), 9.45 (s, 1H, 6-H). Anal. ($C_{10}H_{12}N_2O_7S$) C, H, N.

2-(1,3-Dioxolan-2-yl)-4-(4-morpholinylmethyl)-5-nitropyridine (30). A mixture of **29** (0.35 g, 1.2 mmol) and morpholine (0.62 g, 7.2 mmol) in 15 mL of methylene chloride was stirred until TLC showed that the reaction was complete (~2 d). The reaction mixture was evaporated to dryness, and the residue was purified by silica gel column chromatography (CH_2Cl_2/AcOEt, 4:1, v/v, R_f 0.44) to afford 0.27 g (76%) of product as an off-white solid: mp 98−99 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.40 [m, 4H, N(CH_2)$_2$], 3.62 [m, 4H, O(CH_2)$_2$], 3.75 (s, 2H, 4-CH_2), 4.10 [m, 4H, (OCH_2)$_2$], 5.75 (s, 1H, 2-CH), 7.72 (s, 1H, 3-H), 8.95 (s, 1H, 6-H). Anal. ($C_{13}H_{17}N_3O_5$) C, H, N.

2-(1,3-Dioxolan-2-yl)-4-(4-morpholinylmethyl)-5-aminopyridine (31). A solution of **30** (0.25 g, 0.85 mmol) in ethanol (60 mL) was hydrogenated in a Parr apparatus at 50 psi of hydrogen in the presence of 10% Pd/C (0.1 g) for 3 h. The reaction mixture was filtered through a Celite pad, and the catalyst was washed with ethanol. The combined filtrate and washings were evaporated in vacuo to dryness and coevaporated with benzene. The remaining solid was recrystallized from ethanol/ether to afford 0.21 g (95%) of product as white crystals: mp 102−104 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.40 [m, 4H, N(CH_2)$_2$], 3.45 (s, 2H, 4-CH_2), 3.62 [m, 4H, O(CH_2)$_2$], 4.05 [m, 4H, (OCH_2)$_2$], 4.60 (br s, 2H, 5-NH_2, D_2O exchangeable), 5.65 (s, 1H, 2-CH), 7.12 (s, 1H, 3-H), 8.00 (s, 1H, 6-H). Anal. ($C_{13}H_{19}N_3O_3$) C, H, N.

4-(4-Morpholinylmethyl)-5-aminopyridine-2-carboxaldehyde Thiosemicarbazone (32). This compound was prepared from **31** (0.20 g, 0.75 mmol) by the procedure employed for the synthesis of **7**: yield 0.21 g (95%); mp 248−249 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.32 [m, 4H, N(CH_2)$_2$], 3.85 [m, 4H, O(CH_2)$_2$], 4.50 (s, 2H, 4-CH_2), 4.60 (br s, 2H, 5-NH_2, D_2O exchangeable), 6.35 (s, 1H, 3-H), 7.15 (s, 1H, 6-H), 7.60 and 7.70 (two s, 2H, $CSNH_2$, D_2O exchangeable), 7.90 (s, 1H, 2-CH), 10.80 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{12}H_{18}N_6S$·HCl) C, H, N.

5-[(Acetylamino)methyl]-2-(acetoxymethyl)pyridine (37). A mixture of 5-cyano-2-picoline (**33**, 3.3 g, 28 mmol) and active Raney Ni (3.2 g) in acetic acid (100 mL) was hydrogenated in a Parr apparatus at 5 psi of hydrogen for 4 h. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH, 10:1, v/v, R_f 0.52) to afford **34** (1.5 g, 44%) as an oil; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.55 (s, 3H, 2-CH_3), 4.70 (s, 2H, 5-CH_2), 6.35 (s, 2H, NH_2, D_2O exchangeable), 7.25 (d, 1H, 3-H), 7.75 (d, 1H, 4-H), 8.45 (s, 1H, 6-H). The preceding product (**34**, 1.5 g) was refluxed with a mixture of 10 mL of acetic acid and 20 mL of acetic anhydride overnight and then evaporated in vacuo to dryness. The residue was dissolved in a mixture of 5 mL of 30% hydrogen peroxide and 25 mL of acetic acid and heated at 100 °C for 10 h. The reaction mixture was evaporated in vacuo to dryness and coevaporated with acetic acid. The residue was dissolved in a mixture of 10 mL of acetic acid and 20 mL of acetic anhydride and refluxed for 3 h. After removal of the solvent in vacuo, the residue was purified by silica gel column chromatography (CH_2Cl_2/AcOEt, 2:1, v/v, R_f 0.62) to yield 1.1 g (17% from **33**) of product as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.10 and 2.12 (two s, 6H, two CH_3), 5.15 and 5.23 (two s, 4H, 2- and 5-CH_2), 7.40 (d, 1H, 3-H), 7.75 (d, 1H, 4-H), 8.65 (s, 1H, 6-H). Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

5-[(Acetylamino)methyl]-2-(diacetoxymethyl)pyridine (39). To a solution of compound **37** (1.0 g, 4.5 mmol) in 60 mL of acetic acid was added dropwise with stirring 5 mL of 30% hydrogen peroxide. The mixture was heated at 70−75 °C overnight. An additional 3 mL of 30% hydrogen peroxide was added, and the reaction mixture was heated at 70−75 °C for another 12 h. The cooled reaction mixture was evaporated in vacuo, and the residue was coevaporated with acetic acid. The residue was dissolved in a mixture of 10 mL of acetic acid and 20 mL of acetic anhydride and heated at 70 °C for 20 h. The reaction mixture was cooled and evaporated in vacuo to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/AcOEt, 2:1, v/v, R_f 0.62) to yield 0.7 g (56%) of product as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.05, 2.08, and 2.10 (three s, 9H, three CH_3), 5.15 and 5.20 (s, 2H, 5-CH_2), 7.50 (d, 1H, 3-H), 7.65 (s, 1H, 2-CH), 7.77 (d, 1H, 4-H), 8.75 (s, 1H, 6-H). Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

5-(Aminomethyl)pyridine-2-carboxaldehyde Thiosemicarbazone (40). This compound was prepared from **39** (0.60 g, 2.1 mmol) by the procedure employed for the synthesis of **7**: yield 0.34 g (75%); mp 243−245 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.52 (s, 2H, CH_2), 5.20 (br s, 2H, NH_2, D_2O exchangeable), 7.95 (dd, 1H, 4-H, $J_{3,4}$ = 6 Hz, $J_{4,6}$ = 1.5 Hz), 8.04 (d, 1H, 6-H, $J_{4,6}$ = 1.5 Hz), 8.10 and 8.16 (two s, 2H, $CSNH_2$, D_2O exchangeable), 8.52 (s, 1H, 2-CH), 11.25 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5S$·0.25H_2O) C, H, N.

Assay of CDP Reductase Activity. Ribonucleoside diphosphate reductase was prepared as previously described[26] and assayed by the method of Steeper and Steuart.[33] [^{14}C]CDP (3.75 nmol, 0.03 μCi; New England Nuclear Corp., Boston, MA) was incubated with 150 mmol of ATP, 900 nmol of dithiothreitol, and aliquots of the non-heme iron subunit and the effector-binding subunit of ribonucleoside diphosphate reductase in a volume of 0.15 mL for 30 min at 37 °C. The reaction was terminated by heating in a boiling water bath for 4 min. After snake venom treatment to convert nucleotides to the nucleoside level, deoxycytidine was separated from cytidine on a Dowex-1-borate column.

Assay of the Inhibition of the Growth of L1210 Leukemia Cells. L1210 cell growth was assayed by the method of Cory et al.[34] Ninety-six well plates were seeded with 2000 cells/well, and test agents were added at various concentrations 24 h later. After 72 h, 5-(3-(carboxymethoxy)phenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl)tetrazolium, inner salt and phenazine methosulfate were added, and plates were incubated at 37 °C and read at 492 nm 2−3 h later as previously described.[34] IC_{50} values were determined using at least five different concentrations for each agent.

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References

(1) Elford, H. L.; Freese, M.; Passamani, E.; Morris, H. P. Ribonucleotide Reductase and Cell Production. *J. Biol. Chem.* **1970**, *245*, 5228−5233.
(2) Weber, G. Enzymology of Cancer Cells. *N. Engl. J. Med.* **1977**, *296*, 486−493.
(3) Cory, J. G.; Chiba, P. Combination Chemotherapy Directed at the Components of Nucleoside Diphosphate Reductase. In *Inhibitors of Ribonucleoside Diphosphate Reductase Activity*; Cory, J. G., Cory, A. M., Eds.; Pergamon Press: New York, 1989; pp 245−264.
(4) Sartorelli, A. C.; Agrawal, K. C. Development of α-(*N*)-Heterocyclic Carboxaldehyde Thiosemicarbazones with Clinical Potential as Antineoplastic Agents. *ACS Symp. Ser.* **1976**, No. 30, Cancer Chemotherapy, 1−14.
(5) Krakoff, I. H.; Brown, N. C.; Reichard, P. Inhibition of Ribonucleoside Diphosphate Reductase by Hydroxyurea. *Cancer Res.* **1968**, *28*, 1559−1565.
(6) Tai, A. W.; Lien, E. J.; Lai, M. M. C.; Khwaja, T. A. Novel *N*-Hydroxyguanidine Derivatives as Anticancer and Antiviral Agents. *J. Med. Chem.* **1984**, *27*, 236−238.
(7) Weckbecker, G.; Lien, E. J.; Cory, J. G. Properties of *N*-Hydroxy-*N*′-aminoguanidine Derivatives as Inhibitors of Ribonucleotide Reductase. *Biochem. Pharmacol.* **1988**, *37*, 529−534.
(8) Cory, J. G.; Cory, A. H.; Raber, N. K.; Narayanan, A.; Schneller, S. W. Structural Aspects of *N*-Hydroxy-*N*′-aminoguanidine as Inhibitors of L1210 Cell Growth and Ribonucleotide Reductase Activity. *Adv. Enzyme Reg.* **1993**, *33*, 129−140.
(9) Elford, H. L.; Wampler, G. L.; van't Riet, B. New Ribonucleotide Reductase Inhibitors with Antineoplastic Activity. *Cancer Res.* **1979**, *39*, 844−851.
(10) van't Riet, B.; Wampler, G. L.; Elford, H. L. Synthesis of Hydroxy- and Amino-Substituted Benzohydroxamic Acids: Inhibition of Ribonucleotide Reductase and Antitumor Activity. *J. Med. Chem.* **1979**, *22*, 589−593.

(11) Liu, M. C.; Lin, T. S.; Sartorelli, A. C. Chemical and Biological Activity of α-(*N*)-Heterocyclic Carboxaldehyde Thiosemicarbazones. In *Progress in Medicinal Chemistry, Vol. 32*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier: Amsterdam, 1995; pp 1−35.
(12) Sartorelli, A. C.; Agrawal, K. C.; Tsiftsoglou, A. S.; Moore, E. C. Characterization of the Biochemical Mechanism of Action of α-(*N*)-Heterocyclic Carboxaldehyde Thiosemicarbazones. *Adv. Enzyme Reg.* **1977**, *15*, 117−139.
(13) Creasey, W. A.; Agrawal, K. C.; Capizzi, R. L.; Stinson, K. K.; Sartorelli. A. C. Studies on the Antineoplastic Activity and Metabolism of α-(*N*)-Hetrocyclic Carboxaldehyde Thiosemicarbazones in Dogs and Mice. *Cancer Res.* **1972**, *32*, 565−572.
(14) French, F. A.; Blanz, E. J., Jr. α-(*N*)-Formylheteroaromatic Thiosemicarbazones. Inhibition of Tumor-Derived Ribonucleoside Diphosphate Reductase and Correlation with In Vivo Antitumor Activity. *J. Med. Chem.* **1974**, *17*, 172−181.
(15) Sunkara, P. S.; Lippert, B. J.; Snyder, R. D.; Jarvi, E. T.; Farr, R. A. Antitumor Activity of 2′-Deoxy-2′,2′-difluorocytidine, a Novel Inhibitor of Ribonucleotide Reductase. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 324.
(16) Heinemann, V.; Xu, Y. Z.; Chubb, S.; Sen, A.; Hertel, L. W.; Grindey, G. B.; Plunkett, W. Inhibition of Ribonucleotide Reduction in CCRF-CEM Cells by 2′,2′-Difluorodeoxycytidine. *Mol. Pharmacol.* **1990**, *38*, 567−572.
(17) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robins, M. J.; Lippert, B.; Jarvi, E.; Resvick, R. 2′-Deoxy-2′-methylenecytidine and 2′-Deoxy-2′,2′-difluorocytidine 5′-Diphosphates: Potent Mechanism-Based Inhibitors of Ribonucleotide Reductase. *J. Med. Chem.* **1991**, *34*, 1879−1884.
(18) Tseng, W. C.; Derse, D.; Cheng, Y. C.; Brockman, R. W.; Bennett, L. L., Jr. In Vitro Biological Activity of 9-β-D-Arabinofuranosyl-2-fluoroadenine and the Biochemical Actions of its Triphosphate on DNA Polymerases and Ribonucleotide Reductase from HeLa Cells. *Mol. Pharmacol.* **1982**, *21*, 474−477.
(19) Huang, M. C.; Ashmun, R. A.; Avery, T. L.; Kuehl, M.; Blakley, R. L. Effects of Cytotoxicity of 2-Chloro-2′-deoxyadenosine on Cell Growth, Clonogenicity, DNA Synthesis, and Cell Cycle Kinetics. *Cancer Res.* **1986**, *46*, 2362−2368.
(20) Huang, P.; Chubb, S.; Hertel, L. W.; Grindey, G. B.; Plunkett, W. Action of 2′,2′-Difluorodeoxycytidine on DNA Synthesis. *Cancer Res.* **1991**, *51*, 6110−6117.
(21) DeConti, R. C.; Toftness, B. R.; Agrawal, K. C.; Tomchick, R.; Mead, J. A. R.; Bertino, J. R.; Sartorelli, A. C.; Creasey, W. A. Clinical and Pharmacological Studies with 5-Hydroxy-2-formylpyridine Thiosemicarbazone. *Cancer Res.* **1972**, *32*, 1455−1462.
(22) Krakoff, I. H.; Etcubanas, E.; Tan, C.; Mayer, K.; Bethune, V.; Burchenal, J. H. Clinical Trial of 5-Hydroxypicolinaldehyde Thiosemicarbazone (5-HP; NSC-107392), With Special Reference to Its Iron-Chelating Properties. *Cancer Chemother. Rep.* **1974**, *58*, 207−212.
(23) Wang, Y.; Liu, M. C.; Lin, T. S.; Sartorelli, A. C. Synthesis and Antitumor Activity of 3- and 5-Hydroxy-4-methylpyridine-2-carboxaldehyde Thiosemicarbazones. *J. Med. Chem.* **1992**, *35*, 3667−3671.
(24) Liu, M. C.; Lin, T. S.; Sartorelli, A. C. Synthesis and Antitumor Activity of Amino Derivatives of Pyridine-2-carboxaldehyde Thiosemicarbazone. *J. Med. Chem.* **1992**, *35*, 3672−3677.
(25) Liu, M. C.; Lin, T. S.; Penketh, P.; Sartorelli, A. C. Synthesis and Antitumor Activity of 4- and 5-Substituted Derivatives of Isoquinoline-1-carboxaldehyde Thiosemicarbazone. *J. Med. Chem.* **1995**, *38*, 4234−4243.
(26) Cory, J. G.; Cory, A. H.; Rappa, G.; Lorico, A.; Liu, M. C.; Lin, T. S.; Sartorelli, A. C. Inhibitors of Ribonucleotide Reductase: Comparative Effects of Amino- and Hydroxy-Substituted Pyridine-2-carboxaldehyde Thiosemicarbazones. *Biochem. Pharmacol.* **1994**, *48*, 335−344.
(27) Cory, J. G.; Cory, A. H.; Rappa, G.; Lorico, A.; Liu, M. C.; Lin, T. S.; Sartorelli, A. C. Structure-Function Relationships for a New Series of Pyridine-2-carboxaldehyde Thiosemicarbazones on Ribonucleotide Reductase Activity and Tumor Cell Growth in Culture and in Vivo. *Adv. Enzyme Reg.* **1995**, *35*, 55−68.
(28) Barluenga, J.; Bayon, A. M.; Asensio, G. A New and Specific Method for the Monomethylation of Primary Amines. *J. Chem. Soc., Chem. Commun.* **1984**, 1334-1335.
(29) Liu, M. C.; Lin, T. S.; Sartorelli, A. C. A One-Pot Synthesis of 3-Nitro- and 3,5-Dinitro-2-picolines. *Synth. Commun.* **1990**, *20*, 2965−2970.
(30) Achremowicz, L. Synthesis of 3,5-Dinitropyridine Aldehydes. *Il Farmaco Ed. Sci.* **1978**, *35*, 161−166.
(31) Kingsbury, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Heicht, S. M.; Gallagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. Synthesis of Water-Soluble (Aminoalkyl)camptothecin Analogues: Inhibition of Topoisomerase I and Antitumor Activity. *J. Med. Chem.* **1991**, *34*, 98−107.
(32) Lin, T. S.; Xu, S. P.; Zhu, L. Y.; Cosby, L. A.; Sartorelli, A. C. Synthesis of 2,3-Diaziridinyl-1,4-naphthoquinone Sulfonate Derivatives as Potential Antineoplastic Agents. *J. Med. Chem.* **1989**, *32*, 1467−1471.
(33) Steeper, J. R.; Steuart, C. E. A Rapid Assay for CDP Reductase Activity in Mammalian Cell Extracts. *Anal. Biochem.* **1970**, *34*, 123−130.
(34) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. Use of an Aqueous Soluble Tetrazolium/Formazan Assay for Cell Growth Assays in Culture. *Cancer Commun.* **1991**, *3*, 207−212.

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