Synthesis and Antitumor Activity of Amino Derivatives of Pyridine-2-carboxaldehyde Thiosemicarbazone

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Various substituted pyridine-2-carboxaldehyde thiosemicarbazones (12 compounds) have been synthesized and evaluated for antineoplastic activity in mice bearing the L1210 leukemia. Oxidation of 3-nitro-2-picoline, 5-nitro-2-picoline, 3-nitro-2,4-lutidine, and 5-nitro-2,4-lutidine with selenium dioxide was employed to generate the corresponding pyridine-2-carboxaldehydes, which were then converted to cyclic ethylene acetals and subsequently reduced to amino and hydroxyamino derivatives by catalytic hydrogenation. Condensation of nitro aldehydes and acetals with thiosemicarbazide afforded the respective thiosemicarbazones. Acetylation of the amino acetals and alkylsulfonation of the 5-amino acetal, followed by condensation with thiosemicarbazide was employed to yield amide thiosemicarbazones. The most active compounds synthesized were 3-aminopyridine-2-carboxaldehyde thiosemicarbazone and 3-amino-4-methylpyridine-2-carboxaldehyde thiosemicarbazone which produced against the L1210 leukemia, % T/C values of 246 and 255, and 40% 60-day long-term survivors at two daily doses of 40 mg/kg and 10 mg/kg, respectively, for six consecutive days.

The reductive conversion of ribonucleotides to deoxyribonucleotides appears to be a rate-controlling step in the biosynthesis of DNA. Thus, the level of the enzyme activity that catalyzes this reaction, ribonucleoside diphosphate reductase, is closely correlated with the rate of cellular replication.[1] Furthermore, since deoxyribonucleoside triphosphates are present in extremely low levels in mammalian cells, Corey and Chiba[2] have hypothesized that DNA synthesis would be more affected by an inhibitor of ribonucleoside diphosphate reductase than by an inhibitor of DNA polymerase. α-(*N*)-Heterocyclic carboxaldehyde thiosemicarbazones (HCTs) represent some of the most potent known inhibitors of ribonucleoside diphosphate reductase, being 80–5000 times more effective, depending upon the HCT, than hydroxyurea, a clinically useful agent that acts, at least in part, by interfering with the enzymatic reduction of ribonucleotides. 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.[3,4] These studies have shown that the broad tumor inhibitory activity of 5-HP in animal systems did not occur in patients with cancer, although it is important to stress that this agent did not undergo phase II evaluation. The inactivity of 5-HP as an antineoplastic agent in humans appeared to be the result of (a) its relatively low inhibitory potency for the target enzyme ribonucleoside diphosphate reductase,[5] (b) its short biological half-life in humans, which resulted from the rapid formation and elimination of an inactive glucuronide conjugate,[3] and (c) its relatively low therapeutic index.[3] 5-Aminoisoquinoline-1-carboxaldehyde thiosemicarbazone (AIQ-1), which was about 100 times more potent than 5-HP as an inhibitor of ribonucleoside diphosphate reductase, was considered as a second generation drug of this class for trial against human cancer. However, since the 5-acetyl derivative of AIQ-1 was completely devoid of carcinostatic activity,[6] the 4-methyl derivative of AIQ-1 (MAIQ-1) was designed and synthesized to minimize the formation of the inactive 5-acetyl derivative. The presence of a 4-methyl group in MAIQ-1 created steric hindrance, minimizing enzymatic acetylation of the 5-amino group.[6,7] Unfortunately, MAIQ-1 did not appear to have sufficient water solubility to permit adequate formulation for use in humans.

French and Blanz[8] reported the synthesis and antitumor activity of 5-(acetylamino)pyridine-2-carboxaldehyde thiosemicarbazone (5-AAP, **26**), which showed both strong inhibitory activity against ribonucleoside diphosphate reductase in vitro and significant antitumor activity in vivo. By analogy to the relationship between AIQ-1 and its 5-acetyl derivative, we hypothesized that 5-aminopyridine-2-carboxaldehyde thiosemicarbazone (5-AP, **18**), might well be more effective than 5-AAP. Furthermore, since 5-AP was a relatively small molecule capable of

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(2) 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: Oxford, 1989; pp 245–264.

(3) 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.

(4) 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.

(5) Moore, E. C.; Booth, B. A.; Sartorelli, A. C. Inhibition of Deoxyribonucleotide Synthesis by Pyridine Carboxaldehyde Thiosemicarbazones. *Cancer Res.* **1971**, *31*, 235–238.

(6) Agrawal, K. C.; Mooney, P. D.; Sartorelli, A. C. Potential Antitumor Agents. 13. 4-Methyl-5-amino-1-formylisoquinoline Thiosemicarbazone. *J. Med. Chem.* **1976**, *19*, 970–972.

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(8) 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.

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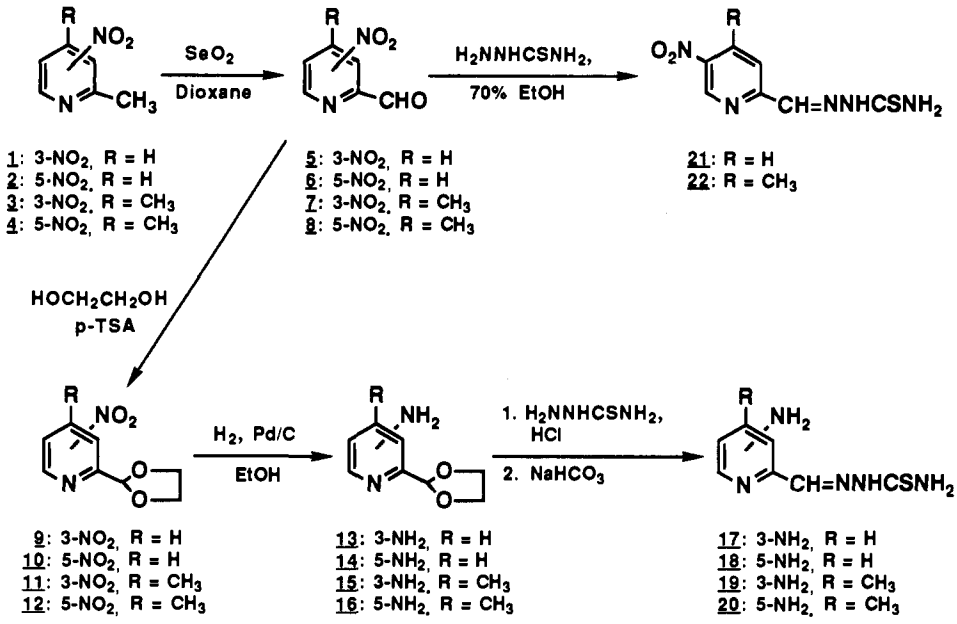
Scheme I



1: 3-NO_2, R = H
2: 5-NO_2, R = H
3: 3-NO_2, R = CH_3
4: 5-NO_2, R = CH_3

5: 3-NO_2, R = H
6: 5-NO_2, R = H
7: 3-NO_2, R = CH_3
8: 5-NO_2, R = CH_3

21: R = H
22: R = CH_3

9: 3-NO_2, R = H
10: 5-NO_2, R = H
11: 3-NO_2, R = CH_3
12: 5-NO_2, R = CH_3

13: 3-NH_2, R = H
14: 5-NH_2, R = H
15: 3-NH_2, R = CH_3
16: 5-NH_2, R = CH_3

17: 3-NH_2, R = H
18: 5-NH_2, R = H
19: 3-NH_2, R = CH_3
20: 5-NH_2, R = CH_3

forming an acidic salt of the amino group, we speculated further that it would be sufficiently water soluble to permit formulation for clinical usage. In this paper, we report the syntheses of 5-AP, the related agent 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, **17**), and their 4-methyl derivatives (3-AMP and 5-AMP, **19** and **20**, respectively), in which a methyl group has been placed at the 4-position of the pyridine ring in an effort to minimize enzymatic acetylation. For comparison, we have also synthesized 5-AAP (**26**) by a different method from that of French and Blanz[8] and two of its analogues, the 5-(acetylamino)-4-methyl derivative (**28**) and 3-(acetylamino)-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**27**). In addition, two nitro (**21** and **22**) and two alkylsulfonamide (**31** and **32**) derivatives were synthesized to evaluate further the structural features of this class of compounds that are required for antineoplastic activity.

Chemistry

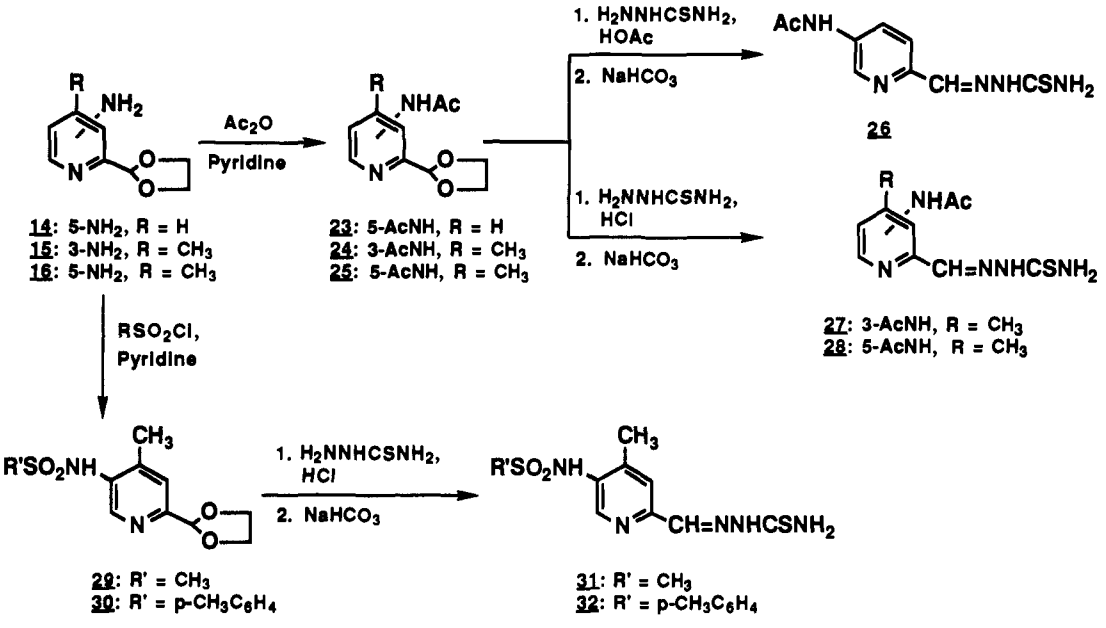
Various 3-amino, 5-amino-, and 5-nitro-substituted pyridine-2-carboxaldehyde thiosemicarbazones (**17–22**) were synthesized by methodology shown in Scheme I. Oxidation[9,10] of 3-nitro-, 5-nitro-, 4-methyl-3-nitro-, and 4-methyl-5-nitro-2-picolines (**1–4**) with selenium dioxide in refluxing dioxane yielded the corresponding pyridine-2-carboxaldehydes (**5–8**). To reduce the nitro groups in compounds **5–8** to amino functions, the aldehydes were protected by conversion to the cyclic ethylene acetals (**9–12**), which were then reduced by catalytic hydrogenation using Pd/C as a catalyst to give the corresponding amino acetals (**13–16**).[6] Compounds **13–16** were then reacted with thiosemicarbazide in ethanol containing 10% concentrated hydrochloric acid to form the desired thiosemicarbazone hydrochlorides; the free bases (**17–20**) were liberated by treatment with aqueous sodium bicarbonate solution. Condensation of 5-nitropyridine-2-carboxaldehyde and 4-methyl-5-nitropyridine-2-carboxaldehyde (**6** and **8**, respectively), with thiosemicarbazide in aqueous ethanol, yielded the corresponding 5-nitro-substituted thiosemicarbazones, **21** and **22**.

The acetamide and alkylsulfonamide derivatives of 3-amino- and 5-aminopyridine-2-carboxaldehyde thiosemicarbazone (**26–28** and **31**, **32**, respectively) were prepared as described in Scheme II. Acetylation of compounds **14–16** with acetic anhydride in anhydrous pyridine gave the acetamide derivatives **23–25**, which were then condensed with thiosemicarbazide to produce 5-(acetylamino)pyridine-2-carboxaldehyde thiosemicarbazone (**26**) and 3- and 5-(acetylamino)-4-methylpyridine-2-carboxaldehyde thiosemicarbazones (**27** and **28**, respectively). During the process of acidic hydrolysis of the ethylene acetal groups, some hydrolysis of the acetamide functions occurred even though reaction conditions were carefully controlled. The pure desired compounds were obtained, however, by recrystallization from ethanol or by silica gel chromatography. Treatment of **16** with methanesulfonyl chloride or *p*-toluenesulfonyl chloride in anhydrous pyridine afforded the corresponding 5-(methylsulfonyl)amino and 5-(*p*-tolylsulfonyl)amino derivatives, **29** and **30**, respectively, which were then treated with thiosemicarbazide in the presence of concentrated hydrochloric acid to afford the corresponding 5-(methylsulfonyl)amino and 5-(*p*-tolylsulfonyl)amino derivatives of 4-methylpyridine-2-carboxaldehyde thiosemicarbazones, **31** and **32**.

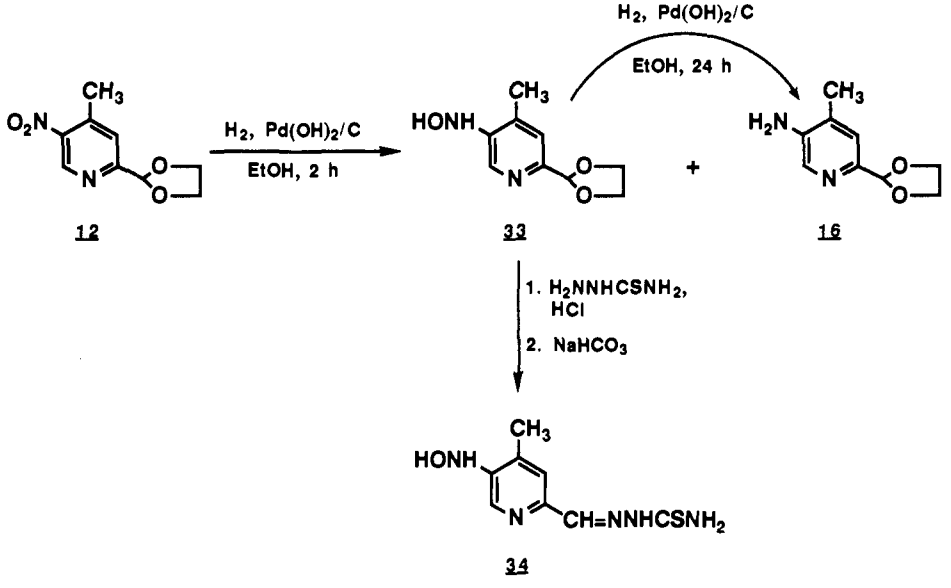
5-(Hydroxyamino)-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**34**) was synthesized by the procedure described in Scheme III. Hydrogenation of compound **12** in ethanol using $Pd(OH)_2/C$ as a catalyst under 50 psi of hydrogen yielded the 5-hydroxyamino derivative **33** contaminated with about 10–15% of the corresponding 5-amino derivative **16**. Compound **33** was easily purified by recrystallization from ethanol. The structure of **33** was assigned by NMR, mass spectroscopy, and elemental analysis. During the reduction process, the rate of absorption of hydrogen decreased considerably after the formation of the 5-hydroxyamino derivative **33** and the reaction was terminated at this stage. However, when the reaction was allowed to proceed until the absorption

(9) Achremowicz, L.; Syper, L. Reaction of 3-Nitropicoline *N*-Oxides with Acetic Anhydride. *Roczniki Chem.* **1972**, *46*, 409–419.
(10) 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.

Scheme II



Scheme III



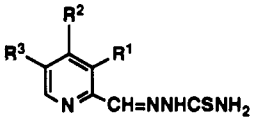
of hydrogen was complete (about 24 h), the 5-amino derivative **16** was obtained in nearly quantitative yield. Condensation of **33** with thiosemicarbazide in the presence of concentrated hydrochloric acid, followed by treatment with sodium bicarbonate, afforded the desired 5-(hydroxyamino)-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**34**).

Biological Evaluation

The tumor-inhibitory properties of the substituted pyridine-2-carboxaldehyde thiosemicarbazones were determined by measuring their effects on the survival time of CD_2F_1 mice bearing the L1210 leukemia. Compounds were administered in suspension by intraperitoneal (ip) injection to groups of 5–10 tumor-bearing mice by the methodology previously described;[11] the results of these tests are shown in Table I. The 5-nitro derivatives, **21** and **22**, the acetyl derivatives, **26–28**, and the alkylsulfonamide derivatives, **31** and **32**, were inactive and the results of these tests are not included. A wide range of dosage levels of each compound of from 10 to 60 mg/kg per day × 6 were tested; however, only the prolonged life span produced by the maximum effective daily dose of each compound is listed. For comparison, the effects of 5-HP, the agent of this series that has undergone clinical trial, was also included as a positive control. The 3-amino derivatives, **17** (3-AP) and **19** (3-AMP), were comparable in their antitumor efficacy against the L1210 leukemia and were the most effective of all the agents synthesized in prolonging the survival time of mice bearing this neoplasm. The 5-amino derivatives, **18** (5-AP) and **20** (5-AMP) and the 5-hydroxyamino derivative, **34**, were comparable to 5-HP as antineoplastic agents. The % T/C value of 5-HP against the L1210 leukemia obtained in these experiments was similar to our previous results with 5-HP;[7] however, a significant difference existed between our value for this agent (% T/C = 133) and that (% T/C

(11) 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.

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



compd	R^1	R^2	R^3	optimum daily dosage,[a] mg/kg	av Δ wt,[b] %	av survival, days	T/C × 100[c]
5-HP	H	H	OH	40	+2.0	10.4	133
17	NH_2	H	H	40	-5.9	14.6	187
18	H	H	NH_2	20	-2.8	11.0	140
19	NH_2	Me	H	60	+2.0	14.9	190
20	H	Me	NH_2	20	-7.0	10.8	138
34	H	Me	HONH	10	-2.7	10.6	136

[a] Drugs were administered in suspension by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days, with 5–10 mice per group. [b] Average change in body weight from onset to termination of therapy. [c] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of untreated L1210 tumor-bearing control animals was 7.8 days.

= 268) reported by French and Blanz.[8] This may be due to differences between the L1210 leukemia cell lines employed and/or to differences in the schedule of drug administration. Although 5-HP was administered daily by intraperitoneal injection starting 24 h after tumor inoculation in both studies, our experiments employed six daily treatments, while Blanz and French[12] used daily treatments that were continued until 50% of the animals had died.

The four most active compounds, i.e., the 3-amino derivatives, 3-AP (17) and 3-AMP (19) and the 5-amino derivatives, 5-AP (18) and 5-AMP (20), were further screened against L1210 leukemia bearing CD_2F_1 female mice with a schedule of drug administration of twice a day for six consecutive days. The results of this investigation are summarized in Table II. 3-AMP showed the least toxicity in this series and the group that received two daily doses of 40 mg/kg of 3-AMP gave a % T/C value of 255 and 40% 60-day long-term survivors. Conversely, the groups receiving two daily doses of 30 mg/kg of 3-AP and two daily doses of 30 mg/kg of 5-AP died after an average of 7.3 days (% T/C = 96) and 6.9 days (% T/C = 91), respectively. The dramatic loss in body weight post drug injection (an average decrease of 12.8% and 16.0% from the original body weight, respectively) suggests that their deaths were attributable to drug toxicity rather than to the leukemia. However, 3-AP and 5-AP showed much better antitumor activity at lower dosages and the groups that received two daily doses of 10 mg/kg of 3-AP and two daily doses of 10 mg/kg of 5-AP gave % T/C values of 246 and 232, and produced 40% 60-day long-term survivors, and 10% 60-day long-term survivors, respectively. 5-AMP was less toxic than 3-AP and 5-AP and its antitumor activity was at least equal to that of 5-AP. Comparison of the results in Table I and Table II shows that the activity of compounds 3- and 5-AP and 3- and 5-AMP were schedule dependent with much better therapeutic effects obtained by twice daily than by once daily treatment. 3-AP and 3-AMP appear to be superior in their activities against the L1210 leukemia to any other agent in this series of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones reported to date[8,13] and, therefore, further evaluation of these two promising agents as potential anticancer drugs is merited.

(12) Blanz, E. J., Jr.; French, F. A. The Carcinostatic Activity of 5-Hydroxy-2-formylpyridine Thiosemicarbazone. *Cancer Res.* 1968, *28*, 2419–2422.

Table II. Effects of 3-AP, 5-AP, 3-AMP, and 5-AMP Administered Twice Daily on the Survival Time of Mice Bearing the L1210 Leukemia

compd	daily dosage,[a] mg/kg	av Δ wt,[b] %	av survival, days[c]	T/C × 100[d]	long-term survivors[e]
3-AP (17)	10 × 2	-6.4	18.7	246	4/10
	15 × 2	-3.3	19.8	262	0/5
	20 × 2	-12.4	14.6	192	1/10
	30 × 2	-12.8	7.3	96	0/5
5-AP (18)	10 × 2	-3.8	17.6	232	1/10
	15 × 2	-7.7	16.8	221	0/5
	20 × 2	-12.3	14.0	185	1/10
	30 × 2	-16.0	6.9	91	0/5
3-AMP (19)	10 × 2	+1.4	14.8	195	2/10
	20 × 2	-3.8	16.9	222	0/10
	30 × 2	-2.4	17.6	232	1/10
	40 × 2	-8.1	19.4	255	2/5
5-AMP (20)	10 × 2	+1.4	18.6	245	1/10
	15 × 2	-7.1	21.0	276	0/10
	20 × 2	-12.1	17.9	236	0/10
	30 × 2	-12.4	14.4	190	0/5

[a] Drugs were administered in suspension by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily for 6 consecutive days, with 5–10 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 7.6 days. [e] Long-term survivors are the number of mice that survived for >60 days relative to the total number of treated mice.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 90 MHz NMR spectrometer or a Bruker WM-500 500 MHz spectrometer with Me_4Si as the internal reference. The mass spectra (at 70 eV) were provided by the Yale University Chemical Instrumentation Center. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the 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-Nitropyridine-2-carboxaldehyde (5). This compound was prepared by a reported procedure[9] with minor modification. A mixture of 3-nitropyridine[10] (1; 1.3 g, 9.4 mmol) and selenium dioxide (1.1 g, 9.4 mmol) in 1,4-dioxane (30 mL), containing 0.8 mL of water, was refluxed 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, R_f 0.82) to afford 0.32 g (23%) of white crystals: mp 61–62 °C (lit.[9] mp 63 °C); ^1H NMR (90 MHz, $CDCl_3$) δ 7.65 (dd, 1 H, 5-H, $J_{4,5}$ = 6.0 Hz, $J_{5,6}$ = 4.5 Hz), 8.32 (d, 1 H, 4-H, $J_{4,5}$ = 6.0 Hz), 9.05 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz), 10.31 (s, 1 H, 2-CHO).

5-Nitropyridine-2-carboxaldehyde (6). This compound was prepared from the nitro derivative 2 by the procedure employed for the synthesis of 5, except that anhydrous dioxane was used as the solvent and the reaction time was 4 h: yield 2.0 g (42%);

(13) Agrawal, K. C.; Sartorelli, A. C. The Chemistry and Biological Activity of the α-(*N*)-Heterocyclic Carboxaldehyde Thiosemicarbazones. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier/North-Holland Biomedical Press: New York, 1978; Vol. 15, pp 321–356.

mp 66–67 °C (lit.[9] mp 66.5–67.5 °C); TLC R_f 0.85 (EtOAc); ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.00 (d, 1 H, 3-H, $J_{3,4}$ = 4.5 Hz), 8.30 (d, 1 H, 4-H, $J_{3,4}$ = 4.5 Hz), 9.25 (s, 1 H, 6-H), 10.45 (s, 1 H, 2-CHO).

2-(1,3-Dioxolan-2-yl)-3-nitropyridine (9). A mixture of aldehyde **5** (1.5 g, 9.7 mmol), ethylene glycol (7.0 g, 62 mmol), and *p*-toluenesulfonic acid monohydrate (60 mg, 0.24 mmol) in toluene (150 mL) was refluxed until the starting material was no longer observed by TLC (CH_2Cl_2/EtOAc, 4:1, v/v). The reaction mixture was cooled and washed with aqueous sodium bicarbonate solution, water, and brine. The organic layer was 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/EtOAc, 4:1, v/v; R_f 0.50). The product was obtained as almost colorless needles (1.65 g, 87%): mp 65–67 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.15 (s, 4 H, CH_2CH_2), 6.52 (s, 1 H, 2-CH), 7.52 (m, 1 H, 5-H), 8.15 (dd, 1 H, 4-H, $J_{4,5}$ = 7 Hz, $J_{4,6}$ = 1 Hz), 8.85 (dd, 1 H, 6-H, $J_{5,6}$ = 4 Hz, $J_{4,6}$ = 1 Hz). Anal. ($C_8H_8N_2O_4$) C, H, N.

2-(1,3-Dioxolan-2-yl)-5-nitropyridine (10). This compound was prepared from **6** by the procedure employed for the synthesis of **9**: yield 2.0 g (84%); mp 104–105 °C; TLC R_f 0.73 (CH_2Cl_2/EtOAc, 1:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.10 (s, 4 H, CH_2CH_2), 5.92 (s, 1 H, 2-CH), 7.72 (d, 1 H, 3-H, $J_{3,4}$ = 8 Hz), 8.50 (dd, 1 H, 4-H, $J_{3,4}$ = 8 Hz, $J_{4,6}$ = 2 Hz), 9.42 (d, 1 H, 6-H, $J_{4,6}$ = 2 Hz). Anal. ($C_8H_8N_2O_4$) C, H, N.

3-Amino-2-(1,3-dioxolan-2-yl)pyridine (13). A solution of **9** (1.0 g, 5.1 mmol) in ethanol (100 mL) was hydrogenated overnight in a Parr apparatus at 50 psi of hydrogen in the presence of 10% Pd/C (0.1 g). 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 resulting solid was recrystallized from benzene to afford 0.81 g (96%) of product as white crystals: mp 73–74 °C; TLC R_f 0.4 (EtOAc); ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.10 (m, 4 H, CH_2CH_2), 4.15 (br s, 2 H, 3-NH_2, D_2O exchangeable), 5.80 (s, 1 H, 2-CH), 6.90 (m, 2 H, 4-H and 6-H), 7.95 (dd, 1 H, 5-H). Anal. ($C_8H_{10}N_2O_2 \cdot 0.1H_2O$) C, H, N.

5-Amino-2-(1,3-dioxolan-2-yl)pyridine (14). This compound was prepared from **10** by the procedure employed for the synthesis of **13**: yield 2.6 g (93%); mp 81–82 °C; TLC R_f 0.18 (EtOAc); ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.85 (br s, 2 H, 5-NH_2, D_2O exchangeable), 4.05 (m, 4 H, CH_2CH_2), 5.72 (s, 1 H, 2-CH), 6.95 (dd, 1 H, 4-H, $J_{3,4}$ = 8 Hz, $J_{4,6}$ = 2 Hz), 7.30 (d, 1 H, 3-H, $J_{3,4}$ = 8 Hz), 8.08 (d, 1 H, 6-H, $J_{4,6}$ = 2 Hz). Anal. ($C_8H_{10}N_2O_2$) C, H, N.

3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (17). To a solution of **13** (0.80 g, 4.8 mmol) in 10 mL of ethanol, 8 mL of water, and 2 mL of concentrated hydrochloric acid was added 0.48 g (5.3 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 50 mL of hot water and filtered. To the hot 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, followed by ethanol: yield 0.72 g (77%); mp 240–241 °C dec; MS *m/e* 194 (M^+); ^{1}H NMR (90 MHz, DMSO-d_6) δ 6.48 (br s, 2 H, 3-NH_2, D_2O exchangeable), 7.12 (m, 2 H, 4-H and 6-H), 7.83 (dd, 1 H, 5-H), 8.10 (br s, 2 H, $CSNH_2$, D_2O exchangeable), 8.10 (s, 1 H, 2-CH), 10.95 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_7H_9N_5S$) C, H, N.

5-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (18). This compound was prepared from **14** by the procedure employed for the synthesis of **17**: yield 1.9 g (82%); mp 205–207 °C; MS *m/e* 194 (M^+); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.60 (br s, 2 H, 3-NH_2, D_2O exchangeable), 6.95 (dd, 1 H, 4-H, $J_{3,4}$ = 8 Hz, $J_{4,6}$ = 1.5 Hz), 7.65 (s, 1 H, 2-CH), 7.75 (d, 1 H, 6-H, $J_{4,6}$ = 1.5 Hz), 7.90 (d, 1 H, 3-H, $J_{3,4}$ = 8 Hz), 7.85 and 8.10 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 11.05 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_7H_9N_5S \cdot 0.4H_2O$) C, H, N.

3-Amino-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (19). This compound was prepared from 15[14] by the procedure employed for the synthesis of **17**: yield 0.5 g (76%); mp 227–228 °C; MS *m/e* 208 (M^+); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (s, 3 H, 4-CH_3), 6.18 (s, 2 H, 3-NH_2, D_2O exchangeable), 7.01 (d, 1 H, 5-H, $J_{5,6}$ = 6 Hz), 7.78 (d, 1 H, 6-H, $J_{5,6}$ = 6 Hz), 7.90 (s, 2 H, $CSNH_2$, D_2O exchangeable), 8.32 (s, 1 H, 2-CH), 11.31 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5S \cdot HCl \cdot H_2O$) C, H, N.

5-Amino-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (20). This compound was prepared from 16[14] by the procedure employed for the synthesis of **17**: yield 0.64 g (78%); mp 235–236 °C; MS *m/e* 208 (M^+); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.10 (s, 3 H, 4-CH_3), 5.48 (s, 2 H, 5-NH_2, D_2O exchangeable), 7.80 (s, 2 H, $CSNH_2$, D_2O exchangeable), 7.80 (s, 1 H, 3-H), 7.95 (s, 1 H, 6-H), 8.00 (s, 1 H, 2-CH), 11.50 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5S \cdot HCl \cdot H_2O$) C, H, N.

5-Nitropyridine-2-carboxaldehyde Thiosemicarbazone (21). A mixture of **6** (0.50 g, 3.3 mmol) and thiosemicarbazide (0.36 g, 4 mmol) in 20 mL of 70% aqueous ethanol solution was refluxed for 2 h, cooled, and filtered. The yellow precipitate that formed was washed with water and recrystallized from ethanol to give 0.54 g (73%) of product: mp 215–217 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 8.25 (d, 1 H, 3-H), 8.35 and 8.55 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 8.90 (dd, 1 H, 4-H), 9.75 (d, 1 H, 6-H), 10.15 (s, 1 H, 2-CH), 11.95 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_7H_7N_5O_2S \cdot HCl$) C, H, N.

4-Methyl-5-nitropyridine-2-carboxaldehyde Thiosemicarbazone (22). This compound was prepared from **8** by the procedure employed for the synthesis of **21**: yield 0.38 g (88%); mp 220–222 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.55 (s, 3 H, 4-CH_3), 8.12 (s, 1 H, 3-H), 8.30 and 8.50 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 9.15 (s, 1 H, 6-H), 9.45 (s, 1 H, 2-CH), 11.85 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_8H_9N_5O_2S \cdot HCl$) C, H, N.

5-(Acetylamino)-2-(1,3-dioxolan-2-yl)pyridine (23). To a stirred solution of **14** (2.0 g, 12 mmol) in 15 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 coevaporated with ethanol (10 mL) and recrystallized from ethanol to yield 2.1 g (82%) of product: mp 145–147 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.05 (s, 3 H, CH_3), 4.10 (m, 4 H, CH_2CH_2), 5.82 (s, 1 H, 2-CH), 7.10 (dd, 1 H, 4-H, $J_{3,4}$ = 8 Hz, $J_{4,6}$ = 2 Hz), 7.45 (d, 1 H, 3-H, $J_{3,4}$ = 8 Hz), 8.40 (br s, 1 H, NH, D_2O exchangeable), 8.68 (d, 1 H, 6-H, $J_{4,6}$ = 2 Hz). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

3-(Acetylamino)-2-(1,3-dioxolan-2-yl)-4-methylpyridine (24). A mixture of **15** (500 mg, 2.78 mmol), 5 mL of acetic anhydride and 15 mL of anhydrous pyridine was refluxed overnight and evaporated in vacuo to dryness. The residue was dissolved in CH_2CH_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/CH_3OH, 10:1, v/v; R_f 0.67) to produce 440 mg (72%) of product: mp 77–79 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.10 (s, 3 H, $COCH_3$), 2.17 (s, 3 H, 4-CH_3), 2.20 (br s, 1 H, NH, D_2O exchangeable), 3.95 (m, 4 H, CH_2CH_2), 5.70 (s, 1 H, 2-CH), 7.15 (d, 1 H, 5-H, $J_{5,6}$ = 6 Hz), 8.42 (d, 1 H, 6-H, $J_{5,6}$ = 6 Hz). Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

5-(Acetylamino)-2-(1,3-dioxolan-2-yl)-4-methylpyridine (25). This compound was prepared from **16** by the procedure employed for the synthesis of **24**: yield 0.5 g (82%); mp 98–99 °C; TLC R_f 0.65 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.08 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, 4-CH_3), 4.05 (m, 4 H, CH_2CH_2), 5.72 (s, 1 H, 2-CH), 7.30 (s, 1 H, 3-H), 8.05 (br s, 1 H, NH, D_2O exchangeable), 8.58 (s, 1 H, 6-H). Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

5-(Acetylamino)pyridine-2-carboxaldehyde Thiosemicarbazone (26). A mixture of **23** (0.6 g, 3.6 mmol), thiosemicarbazide (0.4 g, 4.4 mmol), 1 mL of glacial acetic acid, and 10 mL of ethanol was heated with stirring at 50 °C for 6 h, cooled, and filtered. The acetic acid salt was dissolved in hot water and filtered into 15 mL of 5% sodium bicarbonate solution, and the mixture was stirred at room temperature for 1 h. The yellow precipitate that formed was filtered, washed with water, and recrystallized from ethanol twice to give 0.46 g (54%) of product: mp 215–217 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.05 (s, 3 H, $COCH_3$), 8.00 (m, 3 H, 2-CH, 3-H and 4-H), 8.05 and 8.15 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 8.80 (d, 1 H, 6-H, $J_{4,6}$ =

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1.5 Hz), 10.30 (s, 1 H, 5-NH, D_2O exchangeable), 11.30 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_9H_{11}N_5OS$) C, H, N.

3-(Acetylamino)-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (27). A mixture of **24** (0.41 g, 1.9 mmol), thiosemicarbazone (0.2 g, 2.2 mmol), 1 mL of concentrated hydrochloric acid and 10 mL of ethanol was stirred at room temperature overnight. The yellow precipitate (hydrochloride salt) that formed was filtered and washed with water, followed by ethanol. The hydrochloride salt was dissolved in hot water and stirred with 10 mL of 5% sodium bicarbonate solution for 1 h, filtered, and washed with water. The crude product was chromatographed on a silica gel column (CH_2Cl_2/CH_3OH, 4:1, v/v; R_f 0.52) to give 0.21 g (45%) of product: mp 225–227 °C; ^1H NMR (90 MHz, DMSO-d_6) δ 2.05 (s, 3 H, $COCH_3$), 2.18 (s, 3 H, 4-CH_3), 7.30 (d, 1 H, 5-H, $J_{5,6}$ = 6 Hz), 8.14 (d, 1 H, 2-CH), 7.90 and 8.30 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 8.35 (d, 1 H, 6-H, $J_{5,6}$ = 6 Hz), 9.71 (s, 1 H, 3-NH, D_2O exchangeable), 11.53 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5OS$) C, H, N.

5-(Acetylamino)-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (28). This compound was prepared from **25** by the procedure employed for the synthesis of **27**: yield 0.42 g (76%); mp 229–231 °C; TLC, R_f 0.52 (CH_2Cl_2/CH_3OH, 4:1, v/v); ^1H NMR (90 MHz, DMSO-d_6) δ 2.10 (s, 3 H, $COCH_3$), 2.25 (s, 3 H, 4-CH_3), 7.90 (s, 1 H, 3-H), 8.05 (s, 1 H, 2-CH), 8.05 and 8.35 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 8.60 (s, 1 H, 6-H), 9.62 (s, 1 H, 5-NH, D_2O exchangeable), 11.60 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5OS$) C, H, N.

2-(1,3-Dioxolan-2-yl)-5-[(methylsulfonyl)amino]-4-methylpyridine (29). To a stirred solution of **16** (0.8 g, 4.4 mmol) in 10 mL of anhydrous pyridine in an ice bath was added dropwise 0.6 g (5.3 mmol) of methanesulfonyl chloride at 0–5 °C. The mixture was stirred at room temperature overnight and evaporated in vacuo to dryness. The residue was coevaporated with toluene (10 mL) and then partitioned between CH_2CH_2 (30 mL) and water (10 mL). The organic layer was washed with 10% sodium bicarbonate, brine, and water, dried with anhydrous $MgSO_4$, and filtered. The filtrate was concentrated to a small volume and purified on a silica gel column (EtOAc, R_f 0.40) to give 0.68 g of product: mp 128–130 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 2.40 (s, 3 H, 4-CH_3), 3.03 (s, 3 H, CH_3SO), 4.12 (m, 4 H, CH_2-CH_2), 5.65 (s, 1 H, 2-CH), 7.40 (s, 1 H, 3-H), 8.10 (br s, 1 H, NH, D_2O exchangeable), 8.52 (s, 1 H, 6-H). Anal. ($C_{10}H_{14}N_2O_4S$) C, H, N.

2-(1,3-Dioxolan-2-yl)-4-methyl-5-[(*p*-tolylsulfonyl)amino]-pyridine (30). This compound was prepared from **16** with *p*-toluenesulfonyl chloride by a procedure similar to that employed for the synthesis of **29**: yield 0.75 g (77%); mp 155–156 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 2.05 (s, 3 H, $ArCH_3$), 2.32 (s, 3 H, 4-CH_3), 4.10 (m, 4 H, CH_2CH_2), 5.70 (s, 1 H, 2-CH), 6.50 (br s, 1 H, NH, D_2O exchangeable), 7.20–7.40 (m, 5 H, ArH and 3-H), 8.20 (s, 1 H, 6-H). Anal. ($C_{16}H_{18}N_2O_4S$) C, H, N.

5-[(Methylsulfonyl)amino]-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (31). A mixture of **29** (0.93 g, 3.6 mmol), thiosemicarbazide (0.37 g, 4.0 mmol), and 10 mL of 5% concentrated hydrochloric acid solution was heated with stirring at 60 °C for 4 h and cooled. The yellow precipitate (hydrochloride salt) that formed was filtered and washed with a small amount of water. The hydrochloride salt was then stirred in 10 mL of 1 N NaOH solution for 30 min and filtered. The filtrate was neutralized with dilute acetic acid, filtered, and washed with water followed by ethanol to give 0.65 g (63%) of product: mp 210–212 °C; ^1H NMR (90 MHz, DMSO-d_6) δ 2.35 (s, 3 H, 4-CH_3), 3.05 (s, 3 H, 4-CH_3SO), 8.05 (s, 1 H, 3-H), 8.22 (s, 1 H, 6-H), 8.30 (s, 1 H, 2-CH), 8.15 and 8.35 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 9.45 (br s, 1 H, SO_2NH, D_2O exchangeable), 11.45 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_9H_{13}N_5O_2S_2 \cdot 0.75H_2O$) C, H, N.

4-Methyl-5-[(*p*-tolylsulfonyl)amino]pyridine-2-carboxaldehyde Thiosemicarbazone (32). This compound was prepared from **30** with *p*-toluenesulfonyl chloride by the procedure employed for the synthesis of **31**: yield 0.43 g (80%); mp 234–236 °C; ^1H NMR (90 MHz, DMSO-d_6) δ 2.12 (s, 3 H, $ArCH_3$), 2.40 (s, 3 H, 4-CH_3), 7.30–7.40 (m, 4 H, ArH), 8.05 (s, 1 H, 3-H), 8.20 (s, 1 H, 6-H), 8.30 (s, 1 H, 2-CH), 8.35 and 8.55 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 9.40 (br s, 1 H, SO_2NH, D_2O exchangeable), 11.55 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_{15}H_{17}N_5O_2S_2$) C, H, N.

2-(1,3-Dioxolan-2-yl)-5-(hydroxyamino)-4-methylpyridine (33). A solution of **12** (1.8 g, 8.6 mmol) in ethanol (100 mL) was hydrogenated for 2 h in a Parr apparatus at 50 psi of hydrogen in the presence of 20% $Pd(OH)_2/C$ (0.2 g). 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. The residue was recrystallized from ethanol twice to give 1.0 g (60%) of product: mp 180–181 °C as white crystals; MS *m/e* 167 ($M^+ + 1$); ^1H NMR (500 MHz, DMSO-d_6) δ 2.08 (s, 3 H, 4-CH_3), 3.90–4.05 (m, 4 H, CH_2CH_2), 5.54 (s, 1 H, 2-CH), 7.13 (s, 1 H, 3-H), 8.18 (s, 1 H, 6-H), 8.29 (s, 1 H, NH, D_2O exchangeable), 8.45 (s, 1 H, OH, D_2O exchangeable). Anal. ($C_9H_{12}N_2O_3$) C, H, N.

5-(Hydroxyamino)-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (34). This compound was prepared from **33** by the procedure employed for the synthesis of **17**: yield 0.45 g (77%); mp 197–198 °C; MS *m/e* 224 (M^+); ^1H NMR (90 MHz, DMSO-d_6) δ 2.35 (s, 3 H, 4-CH_3), 8.12 (m, 3 H, 3-H, 6-H, and 2-CH), 8.55 and 8.75 (two br s, 2 H, $CSNH_2$, D_2O exchangeable), 9.15 (br s, 2 H, HONH, D_2O exchangeable), 12.10 (s, 1 H, NNH, D_2O exchangeable). Anal. ($C_8H_{11}N_5OS$) C, H, N.

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