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# Synthesis and Antitumor Activity of 3- and 5-Hydroxy-4-methylpyridine-2-carboxaldehyde Thiosemicarbazones

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To develop an α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazone with clinical utility as an anticancer agent, two analogues, 3-hydroxy-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (3-HMP) and 5-hydroxy-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (5-HMP), of 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP) have been designed and synthesized by two different methods. 3-HMP and 5-HMP both showed better antitumor activity than their respective parent compounds, 3-hydroxypyridine-2-carboxaldehyde thiosemicarbazone and 5-HP, in mice bearing the L1210 leukemia.

The α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones (HCTs) constitute, as a class, the most potent known inhibitors of ribonucleoside diphosphate reductase. The reductive conversion of ribonucleotides to their deoxyribonucleotide counterparts is a particularly critical step in the synthesis of DNA, since deoxyribonucleotides are present in extremely low levels in mammalian cells, and Corey and Chiba[1] have presented arguments that an inhibitor of ribonucleotide reductase could be more effective than an inhibitor of DNA polymerase in blocking DNA synthesis. Thus, it seems reasonable that a strong inhibitor of ribonucleotide reductase would be a useful weapon in the therapeutic armamentarium against cancer. 5-Hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP) is the only member of the HCT series that has been administered to man as part of a phase 1 study. The selection of 5-HP for clinical trial was due to (a) its activity against a spectrum of transplanted tumors and spontaneous dog lymphomas and (b) its ease of parenteral administration as the sodium salt. The results of two independent phase 1 studies[2,3] showed that transient decreases in blast counts occurred in 6 of 25 patients with leukemia, while no antitumor effects were observed in 18 patients with solid tumors. Administration of relatively large doses of drug was limited primarily by gastrointestinal toxicity. In addition, the most aggressive drug regimens also produced myelosuppression, hemolysis, anemia, hypertension, and hypotension. The exceedingly weak antileukemic activity of 5-HP that was observed in the phase 1 trial was attributed to the relatively short biological half-life of 5-HP in humans, which was due to the rapid formation and elimination of the *O*-glucuronide conjugate. Thus, the $t_{1/2}$ of 5-HP in the blood of mice was 15 min, while the drug had a $t_{1/2}$ in humans of 2.5 to 10.5 min, depending upon the patient. Twenty percent of a therapeutic dose of 5-HP was excreted in the urine of the mouse within 24 h; whereas, a therapeutic dose of 5-HP was excreted 2- to 3.5-times faster in man. Approximately 75% of the material found in the urine of patients was in the form of an *O*-glucuronide,[2] which had no activity against ribonucleotide reductase. In an attempt to circumvent this problem, our laboratory designed and synthesized 5-amino-4-methylisoquinoline-1-carboxaldehyde thiosemicarbazone, an isoquinoline derivative containing a 5-amino function to permit formulation as an acid salt and a 4-methyl group, which we have shown provides steric protection of the 5-$NH_2$ substituent from enzymatic acetylation, a reaction that eliminates anticancer activity.[4,5] Unfortunately, this promising agent was judged to not be sufficiently water soluble to permit adequate formulation for use in man. For this reason, we have synthesized hydroxy-substituted pyridine thiosemicarbazones, which are significantly more soluble as sodium salts.

## Chemistry

3-Hydroxy-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**14**, 3-HMP) and 5-hydroxy-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**17**, 5-HMP) were synthesized by well-documented methodology[6-8] as shown in Schemes I and II, respectively. Compounds **2**–**7** were synthesized by minor modifications to the procedures described by Furukawa.[9] 2,4-Lutidine (**1**) was nitrated to give the two isomers, 2,4-dimethyl-3- and 5-nitropyridine (**2** and **3**, respectively), in approximately equal amounts. Catalytic hydrogenation of compounds **2** and **3** over 5% Pd/C in absolute ethanol gave the corresponding amino derivatives **4** and **5**. Diazotization of compounds **4** and **5**

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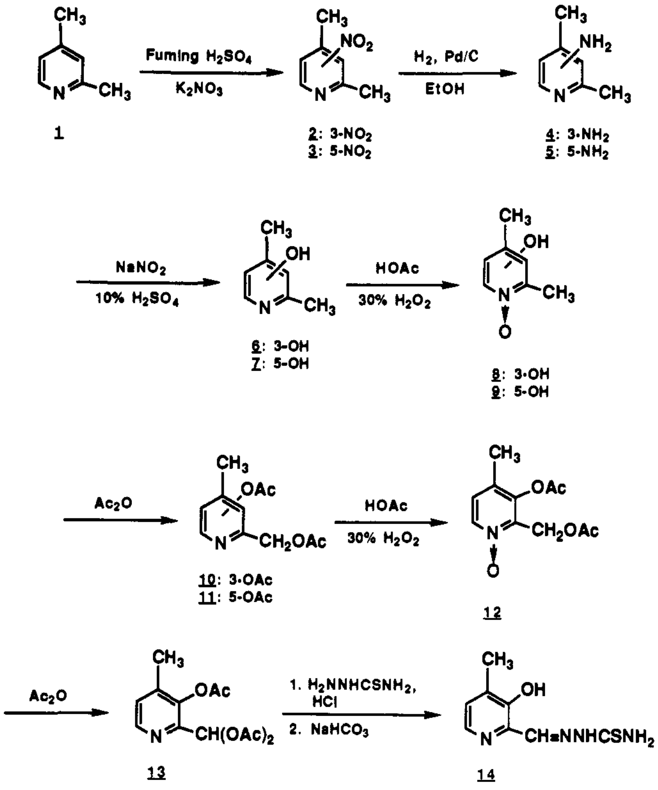
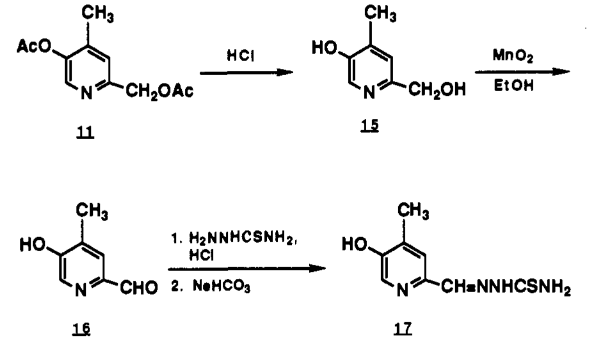
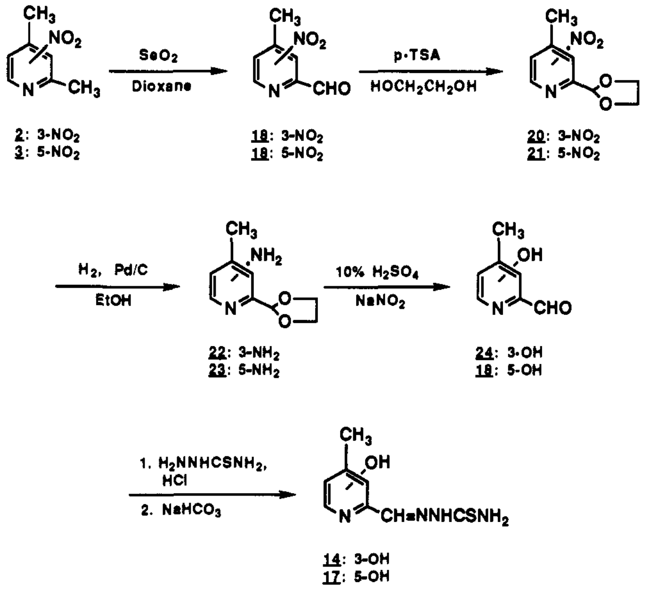
**Scheme I**



**Scheme II**



**Scheme III**



with sodium nitrite in 10% sulfuric acid, followed by hydrolysis of the resulting products, gave the respective hydroxy compounds **6** and **7**. Treatment of **6** and **7** with 30% hydrogen peroxide in glacial acetic acid produced the *N*-oxides **8** and **9**, which were then refluxed with acetic anhydride to give the acetates **10** and **11**. A repeat of the N-oxidation procedure with compound **10**, followed by rearrangement of the resulting *N*-oxide **12** in refluxing acetic anhydride, yielded the corresponding 2-pyridinealdehyde diacetate derivative **13**. Treatment of **13** with thiosemicarbazide in the presence of hydrochloric acid[6] produced 3-hydroxy-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (**14**, Scheme I). Hydrolysis of the acetate **11** with hydrochloric acid gave 5-hydroxy-2-(hydroxymethyl)-4-methylpyridine (**15**). Oxidation of **15** with manganese oxide in ethanol yielded the corresponding aldehyde **16**, which was then condensed with thiosemicarbazide to afford the desired compound **17**[7,8] (Scheme II). Conversion of 3-hydroxy-2-(hydroxymethyl)-4-methylpyridine, the isomer of 5-hydroxy-2-(hydroxymethyl)-4-methylpyridine (**15**), to the corresponding aldehyde by oxidation with manganese oxide, however, has not been successful, probably because 3-hydroxy-4-methylpyridine-2-carboxaldehyde is not stable under these oxidation conditions. The overall yields of the syntheses of 3-HMP (**14**) and 5-HMP (**17**) were 0.42% and 8.3%, respectively, based upon the corresponding 3- and 5-nitrolutidines. Because these low overall yields were unsatisfactory, especially for the synthesis of 3-HMP, another more efficient synthetic route has been devised (Scheme III). Selective oxidation[10] of 2,4-dimethyl-3- and 5-nitropyridines (**2** and **3**, respectively) with selenium dioxide in dioxane gave the corresponding aldehydes, **18** and **19**, which were then refluxed in toluene with ethylene glycol and *p*-toluenesulfonic acid to yield the corresponding 1,3-dioxolanes (**20** and **21**).[8] These protected intermediates were reduced by catalytic hydrogenation in the presence of 10% Pd/C to produce the respective amino derivatives, **22** and **23**, which were then converted to the 3- and 5-hydroxy-4-methylpyridine-2-carboxaldehydes, **24** and **16**, by treatment with sodium nitrite in 10% sulfuric acid. Condensation of **24** and **16** with thiosemicarbazide afforded the desired compounds **14** and **17** in overall yields of 4.8% and 21%, respectively. French and Blanz[11] also reported the synthesis of 3-HMP by a different methodology; however, no synthetic procedure nor any spectroscopic data to confirm the structure of this compound were given. Furthermore, in contrast to our test results, which showed that 3-HMP had antitumor activity against the L1210 leukemia, they reported that this agent was inactive against this tumor cell line.[11]

It is interesting that the 2-methyl groups in compounds **2** and **3** were considerably more sensitive to selenium dioxide oxidation than their 4-methyl counterparts. 4-Methyl-3-nitropyridine-2-carboxaldehyde (**18**) and 4-methyl-5-nitropyridine-2-carboxaldehyde (**19**) were isolated in 20% and 55% yields, respectively, by silica gel column chromatography after the oxidation. In addition to unreacted starting material, an amount of 4-methyl-3- and 5-nitro-2-pyridinecarboxylic acid was also isolated.

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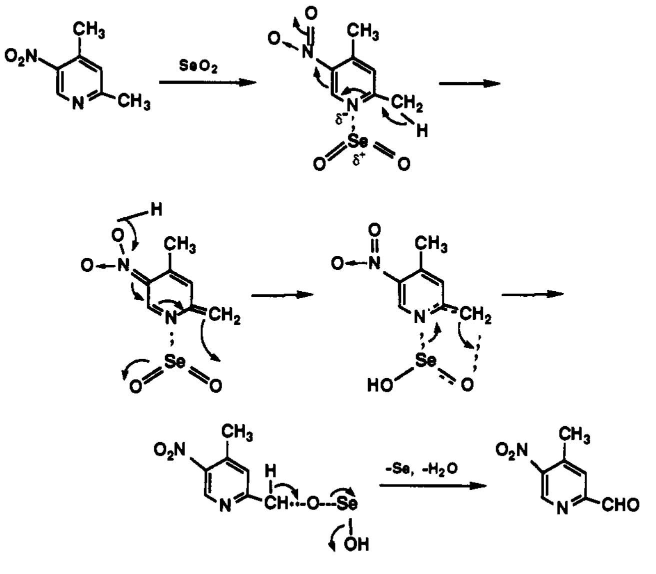
**Scheme IV**



When the reaction time was prolonged, the amount of the acid byproducts was increased; however, no detectable amounts of 2-methyl-3- and 5-nitropyridine-4-carboxaldehydes were found. A cyclic mechanism is proposed for the oxidation reaction of 5-nitro-2,4-lutidine (Scheme IV), which is analogous to the mechanism proposed by Corey and Schaefer[12] for the oxidation of 7-methylquinoline, except that a cyclic transition state is suggested. Such an intermediate may account for the selective oxidation of the 2-methyl group. A similar, but more hindered, cyclic transition state may be formed for the oxidation of 3-nitro-2,4-lutidine, which might explain why the 2-methyl group in the 3-nitro derivative is more difficult to oxidize than its 5-nitro counterpart.

## Biological Results and Discussion

The tumor-inhibitory properties of 3-HMP (14) and 5-HMP (17) were compared with those of 3-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (3-HP) and 5-HP by measuring their effects on the survival time of $CD_2$ $F_1$ female mice bearing the L1210 leukemia. Compounds were administered at daily dosage levels of from 10 to 60 mg/kg by intraperitoneal (ip) injection to groups of 5 tumor-bearing mice once a day for 6 consecutive days by methodology described previously.[13] The prolongation of life span produced by the maximum effective daily dose of each compound is shown in Table I. The 4-methyl-substituted derivatives, 3- and 5-HMP, were both equivalent to or more effective than their corresponding parent compounds, 3-HP and 5-HP, when administered following solubilization in DMSO or in suspension, respectively. The greater antitumor activity of the agents when administered in suspension presumably derives from their slow solubilization in the peritoneal cavity which provides a long-lasting effect. The greater activity of 3-HMP and 5-HMP than their non-methylated counterparts is consistent with the previous finding that the addition of methyl or other hydrophobic groups onto the 3, 4, or 5 carbon atoms of the pyridine ring increased activity as inhibitors of ribonucleotide reductase, probably due to a hydrophobic binding region in the target enzyme molecule.[14]

**Table I.** Comparative Effects of 3-HP, 5-HP, 3-HMP, and 5-HMP on Mice Bearing the L1210 Leukemia

| compd | injection form | optimum daily dosage[a] (mg/kg) | Av Δ wt[b] (%) | T/C[c] (%) |
|---|---|---|---|---|
| 3-HP | DMSO solution | 40 | +1.5 | 114 |
| 5-HP | DMSO solution | 40 | +1.8 | 132 |
| 3-HMP (14) | DMSO solution | 40 | +0.5 | 135 |
| 5-HMP (17) | DMSO solution | 40 | −7.4 | 138 |
| 5-HP | suspension | 60 | +4.6 | 146 |
| 3-HMP (14) | suspension | 50 | +0.9 | 168 |
| 5-HMP (17) | suspension | 40 | −3.4 | 186 |

[a] Administered once daily for six consecutive days, beginning 24 h after tumor implantation. [b] Average weight change of mice from onset to termination of drug treatment. [c] % T/C represents the ratio of the survival time of treated to control mice × 100.

## Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. $^1H$ NMR spectra were recorded at 90 MHz on a Varian EM-390 or at 500 MHz on a Bruker WM-500 spectrometer with $Me_4Si$ as the internal reference. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-SE mass spectrometer equipped with a VG 11-250 data system. The fast atom bombardment (FAB) spectrum was produced using the standard VG ION TECH LTD field gun with xenon gas at 8 kV anode potential. Accurate masses were calculated interactively with the data system using the peaks from poly(ethylene glycol) as reference masses. 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.

**2,4-Dimethyl-3- and 5-nitropyridine (2 and 3).**[9] Fuming sulfuric acid (1500 g, 15.3 mol) was added slowly to 2,4-lutidine (165 mL, 1.43 mol) and cooled in an ice bath with stirring. Potassium nitrate (262.5 g, 2.60 mol) was then added slowly. The reaction mixture was gradually heated to 100 °C and maintained at this temperature for 8 h. The reaction mixture was then heated at 120 °C for an additional 8 h. After cooling to room temperature, the reaction mixture was poured onto ice (2.5 kg). The solution was neutralized to pH 7 using potassium carbonate and extracted with chloroform (3 × 4 L). The organic layer was dried over anhydrous $Na_2SO_4$, and the solvent was evaporated; the remaining solution was distilled under reduced pressure. 2,4-Dimethyl-3-nitropyridine [(2; 41.7 g, 0.27 mol, 19%, 37 °C (0.24 mm Hg)], 2,4-dimethyl-5-nitropyridine [(3; 38.2 g, 0.25 mol, 18%, 44 °C (0.17 mm Hg)], and a mixture of 2,4-dimethyl-3- and 5-nitropyridine (13.74 g, 0.09 mol) were obtained. Compound 2: $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.33 (s, 3 H, 4-$CH_3$), 2.53 (s, 3 H, 2-$CH_3$), 7.02 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 8.35 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz). Compound 3: $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.70 (s, 6 H, 2- and 4-$CH_3$), 7.17 (s, 1 H, 3-H), 9.10 (s, 1 H, 6-H).

**3-Amino-2,4-dimethylpyridine (4).** To a solution of 2,4-dimethyl-3-nitropyridine (2; 31.4 g, 0.21 mol) in 200 mL of absolute ethanol was added 5% Pd/C (2 g). The mixture was hydrogenated under 60 psi of pressure for 2 h. The solution was filtered, and the solvent was evaporated in vacuo to give a solid (24.0 g, 98%): mp 48–50 °C (lit.[9] mp 51–53 °C). The product appeared homogeneous on TLC and by NMR analysis and was used without further purification: $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.17 (s, 3 H, 4-$CH_3$), 2.33 (s, 3 H, 2-$CH_3$), 3.60 (s, 2 H, 3-$NH_2$, $D_2O$ exchangeable), 6.85 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 7.85 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz).

**5-Amino-2,4-dimethylpyridine (5).** This compound was synthesized by methodology used for 4 except the starting

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material was **3**: yield 24.1 g (98%); mp 62–64 °C (lit.[9] mp 66–68 °C); $^1$H NMR (90 MHz, $CDCl_3$) δ 2.10 (s, 3 H, 4-$CH_3$), 2.37 (s, 3 H, 2-$CH_3$), 3.33 (s, 2 H, 3-$NH_2$, $D_2O$ exchangeable), 6.70 (s, 1 H, 3-H), 7.79 (s, 1 H, 6-H).

**2,4-Dimethyl-3-hydroxypyridine (6).** To a solution of 3-amino-2,4-dimethylpyridine (**4**; 25.0 g, 0.21 mol) in 10% sulfuric acid (400 mL) cooled to 0 °C by dry ice in acetone with stirring was added a solution of sodium nitrite (16.2 g, 0.23 mol) in 160 mL of water dropwise at 0–5 °C over a period of 7 min. The solution was maintained at 0 °C for an additional 15 min and then heated in a steam bath for 15 min. After cooling to room temperature, the solution was neutralized with $K_2CO_3$ to pH 7. The product was then extracted with chloroform (3 × 500 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent was removed in vacuo. The product was recrystallized from acetone and the mother liquid was purified by silica gel column chromatography (EtOAc) to afford an additional amount of the pure product. The total yield was 12.7 g (51%) as a colorless solid: mp 105–106 °C (lit.[9] mp 99–101 °C); $^1$H NMR (90 MHz, $CDCl_3$) δ 2.25 (s, 3 H, 4-$CH_3$), 2.50 (s, 3 H, 2-$CH_3$), 6.97 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 7.95 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz), 11.20 (s, 1 H, 3-OH, $D_2O$ exchangeable).

**2,4-Dimethyl-5-hydroxypyridine (7).** This compound was synthesized by methodology used for **6** except the starting material was **5**: yield 12.6 g (51%) as a colorless solid; mp 146–148 °C (lit.[9] mp 144–146 °C); $^1$H NMR (90 MHz, $CDCl_3$) δ 2.20 (s, 3 H, 4-$CH_3$), 2.47 (s, 3 H, 2-$CH_3$), 6.87 (s, 1 H, 3-H), 7.97 (s, 1 H, 6-H), 11.43 (s, 1 H, 5-OH, $D_2O$ exchangeable).

**2,4-Dimethyl-3-hydroxypyridine *N*-Oxide (8).** To a stirred solution of 2,4-dimethyl-3-hydroxypyridine (**6**; 23.7 g, 0.19 mol) in 130 mL of glacial acetic acid was added dropwise 36 mL of 30% hydrogen peroxide. The reaction mixture was heated to 80 °C and two additional portions of 30% hydrogen peroxide (36 mL) were added at 3-h intervals. The solution was maintained at 80 °C for a total of 9 h and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/MeOH, 7:3, v/v) to give 10.3 g (38%) of product: mp 134–136 °C; $^1$H NMR (90 MHz, $Me_2SO$-$d_6$) δ 2.17 (s, 3 H, 4-$CH_3$), 2.32 (s, 3 H, 2-$CH_3$), 6.94 (d, 1 H, 5-H, $J_{5,6}$ = 6 Hz), 7.72 (s, 1 H, 6-H, $J_{5,6}$ = 6 Hz); HRMS (FAB) *m/z* calcd for $C_7H_9NO_2$, 140.0711; found, 140.0707. Anal. ($C_7H_9NO_2$) C, H, N.

**2,4-Dimethyl-5-hydroxypyridine *N*-Oxide (9).** This compound was synthesized by methodology used for **8** except the starting material was **7**: yield 10.0 g (37%); mp 229 °C dec; $^1$H NMR (90 MHz, $Me_2SO$-$d_6$) δ 2.10 (s, 3 H, 4-$CH_3$), 2.22 (s, 3 H, 2-$CH_3$), 7.07 (s, 1 H, 3-H), 7.70 (s, 1 H, 6-H); HRMS (FAB) *m/z* calcd for $C_7H_9NO_2$, 140.0711; found, 140.0722.

**3-Acetoxy-2-(acetoxymethyl)-4-methylpyridine (10).** A mixture of 2,4-dimethyl-3-hydroxypyridine *N*-oxide (**8**; 11.3 g, 81 mmol) and acetic anhydride (200 mL) was heated at 110 °C with stirring for 2.5 h. After cooling, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:1, v/v) to yield 13.5 g (74%) of product as a slightly yellow oil: $^1$H NMR (90 MHz, $CDCl_3$) δ 2.20 (s, 3 H, 4-$CH_3$), 2.37 (s, 6 H, 2 $OCOCH_3$), 5.17 (s, 2 H, 2-$CH_2$), 7.15 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 8.35 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz); HRMS (FAB) *m/z* calcd for $C_{11}H_{13}NO_4$, 224.0923; found, 224.0935. Anal. ($C_{11}H_{13}NO_4$) C, H, N.

**5-Acetoxy-2-(acetoxymethyl)-4-methylpyridine (11).** This compound was synthesized by methodology used for **10** except the starting material was **9**: yield 9.85 g (54%) as a yellow oil; $^1$H NMR (90 MHz, $CDCl_3$) δ 2.15 and 2.25 (two s, 6 H, two $OCOCH_3$), 2.35 (s, 3 H, 4-$CH_3$), 5.13 (s, 2 H, 2-$CH_2$), 7.23 (s, 1 H, 3-H), 8.23 (s, 1 H, 6-H); HRMS (FAB) *m/z* calcd for $C_{11}H_{13}NO_4$, 224.0923; found, 224.0943. Anal. ($C_{11}H_{13}NO_4$) C, H, N.

**3-Acetoxy-2-(acetoxymethyl)-4-methylpyridine *N*-Oxide (12).** To a solution of 3-acetoxy-2-(acetoxymethyl)-4-methylpyridine (**10**; 13.5 g, 60 mmol) in 74 mL of glacial acetic acid was added dropwise with stirring 21 mL of 30% hydrogen peroxide. The mixture was heated to 80 °C and two additional portions of 30% hydrogen peroxide (21 mL) were added at 3-h intervals. The solution was maintained at 80 °C for a total of 9 h. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography (EtOAc/MeOH, 7:3, v/v) to give 2.62 g (18%) of product: mp >360 °C. The product was used immediately for the next step.

**3-Acetoxy-2-(diacetoxymethyl)-4-methylpyridine (13).** A mixture of 3-acetoxy-2-(acetoxymethyl)-4-methylpyridine *N*-oxide (**12**; 2.77 g, 11.6 mmol) and 54 mL of acetic anhydride was heated with stirring at 110 °C for 2.5 h. After cooling, the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:1, v/v) to yield 1.54 g (47%) of product as a yellow oil: $^1$H NMR (90 MHz, $CDCl_3$) δ 2.10–2.40 (m, 12 H, 4-$CH_3$, three $OCOCH_3$), 5.17 (s, 2 H, 2-$CH_2$), 7.20–7.38 (m, 1 H, 5-H), 8.37–8.52 (m, 1 H, 6-H); HRMS (FAB) *m/z* calcd for $C_{13}H_{15}NO_6$, 282.0978; found, 282.0990. Anal. ($C_{13}H_{15}NO_6$) H, N; C: calcd, 55.51; found, 56.01.

**3-Hydroxy-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (14). Method A.** To a slurry of thiosemicarbazide (0.26 g, 2.9 mmol) in 5 mL of concentrated HCl and 15 mL of ethanol was added a solution of **13** (0.8 g, 2.9 mmol) in 10 mL of ethanol. The reaction mixture was stirred at 50 °C for 2 h and the precipitate was filtered after cooling. The yellow solid was recrystallized from aqueous ethanol solution (1:1, v/v) containing 5% concentrated HCl to afford 0.25 g (35%) of product as the hydrochloride salt: mp 243 °C dec; $^1$H NMR (500 MHz, $Me_2SO$-$d_6$) δ 2.52 (s, 3 H, 4-$CH_3$), 3.80 (br s, 1 H, 3-OH, $D_2O$ exchangeable), 7.73 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 8.27 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz), 8.35 (s, 1 H, 2-CH), 8.66 and 8.88 (two s, 2 H, $NH_2$, $D_2O$ exchangeable), 12.07 (s, 1 H, NH, $D_2O$ exchangeable); HRMS (FAB) *m/z* calcd for $C_8H_{10}N_4OS$, 211.0654; found, 211.0651. Anal. ($C_8H_{10}N_4OS{\cdot}HCl{\cdot}H_2O$) C, H, N.

The hydrochloride was stirred in 10% sodium bicarbonate to yield the free base: mp 227–228 °C dec (lit.[11] mp 223–224 °C); $^1$H NMR (500 MHz, $Me_2SO$-$d_6$) δ 2.23 (s, 3 H, 4-$CH_3$), 4.80 (br s, 1 H, 3-OH, $D_2O$ exchangeable), 7.26 (d, 1 H, 5 H, $J_{5,6}$ = 5 Hz), 8.05 (d, 1 H, 6-H, $J_{5,6}$ = 5 Hz), 8.20 (s, 2 H, $NH_2$, $D_2O$ exchangeable), 8.35 (s, 1 H, 2-CH), 11.80 (s, 1 H, NH, $D_2O$ exchangeable).

**Method B.** To a solution of 3-amino-2-(1,3-dioxolan-2-yl)-4-methylpyridine (**22**; 0.6 g, 3.3 mmol) in 15 mL of 10% $H_2SO_4$ at 0 °C (ice bath) with stirring was added dropwise a solution of $NaNO_2$ (0.38 g, 5.5 mmol) in 3 mL of water. The mixture was stirred at 0 °C for 15 min and then heated in a steam bath for 30 min. The resulting solution was evaporated at room temperature under reduced pressure to yield 3-hydroxy-4-methylpyridine-2-carboxaldehyde (**24**) as a syrup, which was dissolved in 15 mL of water, decolorized with charcoal, and filtered. To the filtrate was added a solution of thiosemicarbazide (0.31 g, 3.3 mmol) in 5 mL of 5% concentrated HCl. The mixture was refluxed for 30 min and then cooled, and the yellow precipitate was filtered, washed with water, and recrystallized from aqueous ethanol solution (1:1, v/v) containing 5% concentrated HCl to afford 0.21 g (30%) of product: the melting point and all spectroscopic data were identical with those obtained by Method A.

**5-Hydroxy-2-(hydroxymethyl)-4-methylpyridine (15).** A mixture of 5-acetoxy-2-(acetoxymethyl)-4-methylpyridine (**11**; 6.2 g, 4.5 mmol) and 200 mL of concentrated HCl was refluxed for 1 h. After cooling, the reaction mixture was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/MeOH, 7:3, v/v) to give 3.8 g (97%) of product: mp 161–162 °C: $^1$H NMR (90 MHz, $Me_2SO$-$d_6$) δ 2.33 (s, 3 H, 4-$CH_3$), 4.70 (s, 2 H, 2-$CH_2$), 7.67 (s, 1 H, 3-H), 8.22 (s, 1 H, 6-H); HRMS (FAB) *m/z* calcd for $C_7H_9NO_2$, 140.0711; found, 140.0736.

**5-Hydroxy-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (17). Method A.** To a solution of **15** (3.9 g, 28 mmol) in 100 mL of ethanol was added $MnO_2$ (10.0 g, 0.12 mol) and the reaction mixture was heated to reflux for 2 h with stirring. The mixture was filtered and the filtrate was concentrated under reduced pressure to 80 mL. Because the aldehyde (**16**) is unstable, concentrated HCl (8 mL) was added immediately. Thiosemicarbazide (1.5 g, 17 mmol) was added to the aldehyde solution with stirring, and the reaction mixture was heated to reflux for 30 min. The precipitate was filtered upon cooling and recrystallized in aqueous ethanol solution (1:1, v/v) containing 5% concentrated HCl to afford 3.3 g (81%) of product: mp 229 °C; $^1$H NMR (500 MHz, $Me_2SO$-$d_6$) δ 2.33 (s, 3 H, 4-$CH_3$), 4.01 (br s, 1 H, 5-OH, $D_2O$ exchangeable), 8.02 (s, 1 H, 3-H), 8.20 (s, 1 H, 6-H), 8.22 (s, 1 H, 2-CH), 8.58 (s, 2 H, $NH_2$, $D_2O$ exchangeable), 12.0 (s, 1 H, NH, $D_2O$ exchangeable); HRMS (FAB) *m/z* calcd for $C_8H_{10}N_4OS$, 211.0654; found, 211.0671. Anal. ($C_8H_{10}N_4OS{\cdot}HCl{\cdot}H_2O$) C, H, N.

The hydrochloride was stirred in 10% sodium bicarbonate to yield the free base: mp 220–222 °C dec; $^1H$ NMR (500 MHz, $Me_2SO$-$d_6$) $\delta$ 2.15 (s, 3 H, 4-$CH_3$), 7.95 (s, 1 H, 3-H), 7.97 (s, 1 H, 6-H), 8.01 (s, 1 H, 2-CH), 8.04 and 8.18 (two s, 2 H, $NH_2$, $D_2O$ exchangeable), 10.1 (s, 1 H, 5-OH, $D_2O$ exchangeable), 12.0 (s, 1 H, NH, $D_2O$ exchangeable).

**Method B.** This compound was also prepared from the corresponding 5-amino derivative 5-amino-2-(1,3-dioxolan-2-yl)-4-methylpyridine (**23**) via the aldehyde **16** by the same procedure described for the synthesis of compound **14** (method B): yield 0.32 g (46%); the melting point and all spectroscopic data were identical with those obtained by method A.

**4-Methyl-3-nitropyridine-2-carboxaldehyde (18).** A mixture of 2,4-dimethyl-3-nitropyridine (**2**; 5.0 g, 33 mmol) and selenium dioxide (4.5 g, 42 mmol) in anhydrous 1,4-dioxane (100 mL) was refluxed under an atmosphere of nitrogen for 35 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 (120 g) column ($CH_2Cl_2$/EtOAc, 10:1, v/v; $R_f$ 0.65) to afford 1.1 g (20%) of white crystals: mp 101–102 °C; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.35 (s, 3 H, 4-$CH_3$), 7.47 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 8.72 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz), 9.95 (s, 1 H, 2-CHO). Anal. ($C_7H_6N_2O_3$) C, H, N.

**4-Methyl-5-nitropyridine-2-carboxaldehyde (19).** This compound was prepared from the nitro derivative **3** by the same procedure described for the synthesis of compound **18**, except the reaction time was 4 h: yield 6.0 g (55%); mp 82–83 °C (lit.[6] mp 81–82 °C); TLC, $R_f$ 0.86 ($CH_2Cl_2$/EtOAc, 3:2, v/v); $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.70 (s, 3 H, 4-$CH_3$), 7.90 (s, 1 H, 3-H), 9.20 (s, 1 H, 6-H), 10.10 (s, 1 H, 2-CHO).

**2-(1,3-Dioxolan-2-yl)-4-methyl-3-nitropyridine (20).** To 0.75 g (14 mmol) of compound **18** in 100 mL of toluene was added 40 mg of *p*-toluenesulfonic acid monohydrate and 2 mL of ethylene glycol. The reaction mixture was refluxed with stirring, and a Dean-Stark trap was used to remove the water formed during condensation until complete disappearance of the starting material was observed. The mixture was cooled and then washed with 25 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 (120 g) column ($CH_2Cl_2$/EtOAc, 10:1, v/v; $R_f$ 0.42) to afford 1.1 g (85%) of white crystals: mp 46–48 °C; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.40 (s, 3 H, 4-$CH_3$), 4.07 (s, 4 H, $CH_2CH_2$), 6.05 (s, 1 H, 2-CH), 7.30 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 8.60 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

**2-(1,3-Dioxolan-2-yl)-4-methyl-5-nitropyridine (21).** This compound was synthesized by methodology used for **20** except the starting material was **19**: yield 2.3 g (91%); mp 77–79 °C; (lit.[10] mp 77 °C) TLC, $R_f$ 0.74 ($CH_2Cl_2$/EtOAc, 3:2, v/v); $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.65 (s, 3 H, 4-$CH_3$), 4.10 (s, 4 H, $CH_2CH_2$), 5.85 (s, 1 H, 2-CH), 7.50 (s, 1 H, 3-H), 9.12 (s, 1 H, 6-H).

**3-Amino-2-(1,3-dioxolan-2-yl)-4-methylpyridine (22).** The nitro derivative **20** (1.1 g, 5.2 mmol) was dissolved in 200 mL of ethanol and hydrogenated in a Parr appratus under 50 psi of pressure in the presence of 10% Pd/C (200 mg) for 20 h. After filtration, the filtrate was evaporated under reduced pressure to give the product (0.9 g, 94%) as a syrup: ninhydrin positive; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.12 (s, 3 H, 4-$CH_3$), 4.05 (m, 4 H, $CH_2CH_2$), 4.10 (br s, 2 H, 3-$NH_2$, $D_2O$ exchangeable), 5.76 (s, 1 H, 2-CH), 6.92 (d, 1 H, 5-H, $J_{5,6}$ = 4.5 Hz), 7.86 (d, 1 H, 6-H, $J_{5,6}$ = 4.5 Hz). Anal. ($C_9H_{12}N_2O_2$) C, H, N.

**5-Amino-2-(1,3-dioxolan-2-yl)-4-methylpyridine (23).** This compound was synthesized by methodology used for **22** except the starting material was **21**: yield 1.2 g (92%); mp 79–80 °C; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 2.15 (s, 3 H, 4-$CH_3$), 3.70 (br s, 2 H, 5-$NH_2$, $D_2O$ exchangeable), 4.10 (m, 4 H, $CH_2CH_2$), 5.70 (s, 1 H, 2-CH), 7.15 (s, 1 H, 3-H), 8.00 (s, 1 H, 6-H). Anal. ($C_9H_{12}N_2O_2$) C, H, N.

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