Synthesis and Antitumor Activity of 4- and 5-Substituted Derivatives of Isoquinoline-1-carboxaldehyde Thiosemicarbazone

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Received June 21, 1995[⊗]

Various substituted isoquinoline-1-carboxaldehyde thiosemicarbazones (12 compounds) have been synthesized and evaluated for antineoplastic activity in mice bearing the L1210 leukemia. Condensation of 4-bromo-1-methylisoquinoline (**4**) with ammonium hydroxide, methylamine, ethylamine, and *N*-acetylethylenediamine gave the corresponding 4-amino, 4-methylamino, 4-ethylamino, and 4-*N*-(acetylethyl)amino derivatives, which were then converted to amides and subsequently oxidized to aldehydes followed by condensation with thiosemicarbazide to yield thiosemicarbazones **8a–c**, **9a–c**, and **16**. Nitration of **4**, followed by oxidation with selenium dioxide, produced aldehyde **18**, which was then converted to the cyclic ethylene acetal **19**. Condensation of **19** with morpholine followed by catalytic reduction of the nitro group and treatment with thiosemicarbazide afforded 5-amino-4-morpholinoisoquinoline-1-carboxaldehyde thiosemicarbazone (**22**). *N*-Oxidation of 1,5-dimethylisoquinoline, followed by rearrangement with acetic anhydride, gave, after acid hydrolysis, 1,5-dimethyl-4-hydroxyisoquinoline, which was converted to its acetate and then oxidized to yield 4-acetoxy-5-methylisoquinoline-1-carboxaldehyde (**32**). Sulfonation of 1,4-dimethylisoquinoline, followed by reaction with potassium hydroxide, acetylation, and oxidation, gave 5-acetoxy-4-methylisoquinoline-1-carboxaldehyde (**40**). Condensation of compounds **32** and **39** with thiosemicarbazide afforded the respective 4- and 5-acetoxy(5- and 4-methyl)thiosemicarbazones **33** and **40**, which were then converted to their respective 4- and 5-hydroxy derivatives **34** and **41** by acid hydrolysis. The most active compounds synthesized were 4-aminoisoquinoline-1-carboxaldehyde thiosemicarbazone (**9a**) and 4-(methylamino)isoquinoline-1-carboxaldehyde thiosemicarbazone (**9b**), which both produced optimum % T/C values of 177 against the L1210 leukemia in mice when used at a daily dosage of 40 mg/kg for 6 consecutive days. Furthermore, when **9a** was given twice daily at a dosage of 40 mg/kg for 6 consecutive days, a T/C value of 165 was obtained and 60% of the mice were 60-day long-term survivors.

α-(*N*)-Heterocyclic carboxaldehyde thiosemicarbazones have been shown to be potent inhibitors of the biosynthesis of DNA in mammalian cells.[1] Studies with transplanted murine neoplasms have demonstrated that the enzymatic site responsible for the blockage of DNA replication is at the level of ribonucleoside diphosphate reductase, an enzyme of critical importance for the generation of the deoxyribonucleoside triphosphate precursors of these macromolecules.[2] The pyridine and isoquinoline heterocyclic ring systems have been extensively investigated for structure–activity relationships with this class of antineoplastic agents. One of the most potent inhibitors of the target enzyme in this class of agents, isoquinoline-1-carboxaldehyde thiosemicarbazone (IQ-1), can be envisioned as a 3,4-benzo derivative of pyridine-2-carboxaldehyde thiosemicarbazone (2-PT); addition of a 3,4-benzo group significantly increased the antitumor activity of the parent compound (Figure 1). Recently, we have reported that 5-aminopyridine-2-carboxaldehyde thiosemicarbazone (5-AP) showed significant antitumor activity in mice bearing the L1210 leukemia.[3] Since it was of interest to determine whether the conversion of 5-AP to the corresponding isoquinoline derivative would increase antitumor activity, a series of 4-amino-substituted isoquinoline-1-carboxaldehyde thiosemicarbazones was designed and synthesized.

Several years ago, 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP) was evaluated clinically in a phase 1 study. The results of this investigation conducted independently in two separate institutions[4,5] showed that transient decreases in blast counts occurred in 25% of patients with leukemia, whereas no antitumor effects were observed in patients with solid tumors. Administration of relatively large doses of the 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 observed in the phase 1 clinical trial was attributed to a relatively short biological half-life in humans, which was due to the rapid formation and elimination of the *O*-glucuronide conjugate. To determine whether the enzymatic conjugation of the hydroxyl group by glucuronic acid might be minimized by the introduction of bulky adjacent substituents, two pairs of model compounds, 2,4-dimethyl-5-hydroxypyridine, 5-hydroxy-2-methylpyridine and 1,4-dimethyl-5-hydroxyisoquinoline, 5-hydroxy-1-methylisoquinoline, were synthesized. The results of these enzymatic tests showed that, in the pair of isoquinoline derivatives, the hindered methyl group decreased the rate of glucuronidation by greater than 12-fold, whereas in the pyridine model compounds, the methyl group adjacent to the hydroxyl group was not capable of reducing the rate of glucuronidation. On the basis of these findings and the obser-

[⊗] Abstract published in *Advance ACS Abstracts*, October 1, 1995.

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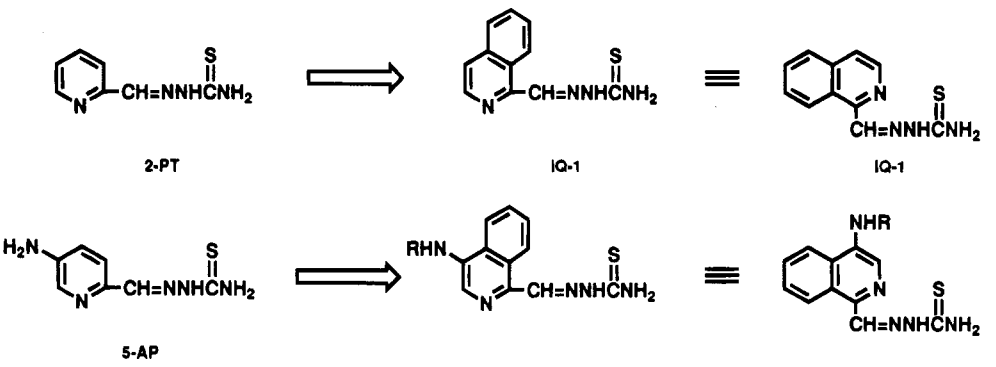


Figure 1.

vation that both 4-hydroxyisoquinoline-1-carboxaldehyde thiosemicarbazone and 5-hydroxyisoquinoline-1-carboxaldehyde thiosemicarbazone have significant antitumor activity in mice bearing transplanted tumors,[1] 4-hydroxy-5-methylisoquinoline-1-carboxaldehyde thiosemicarbazone (**34**) and 5-hydroxy-4-methylisoquinoline-1-carboxaldehyde thiosemicarbazone (**44**) were synthesized, as were a series of protected 4- and 5-amino-substituted isoquinoline-1-carboxaldehyde thiosemicarbazones, and these agents were evaluated for antitumor activity against the L1210 leukemia.

Chemistry

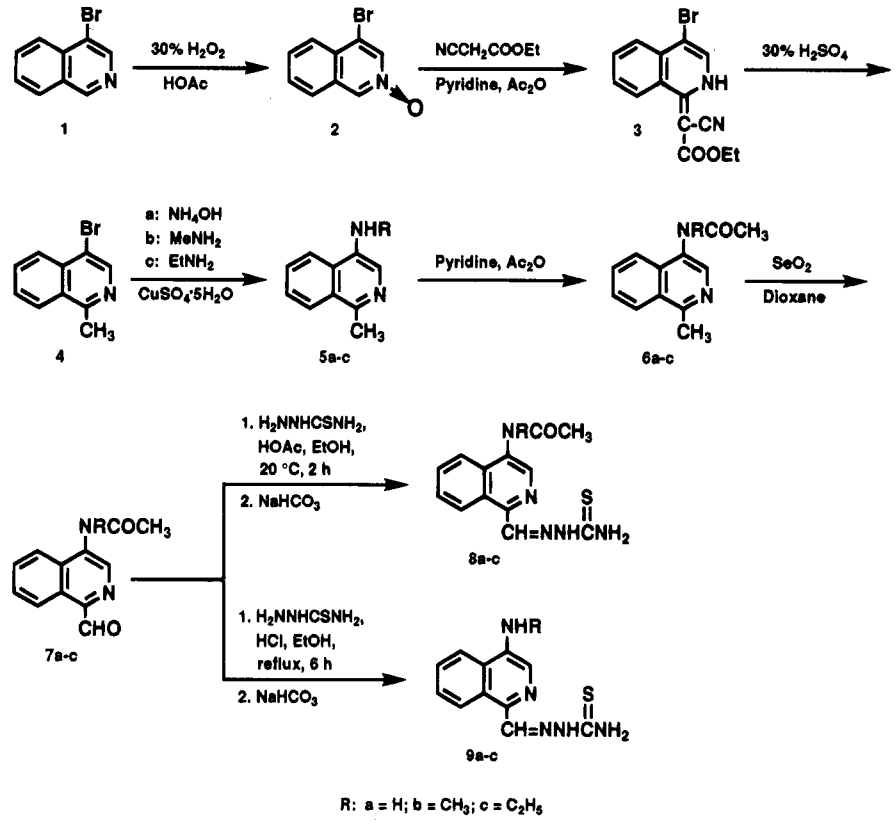
Various 4-amino-substituted isoquinoline-1-carboxaldehyde thiosemicarbazones were synthesized by methodology shown in Schemes 1–3. Oxidation of 4-bromoisoquinoline with 30% hydrogen peroxide in acetic acid by a modification of the procedure of Ochiai and Ikehara[6] gave 4-bromoisoquinoline *N*-oxide (**2**) in 93% yield. Reaction of compound **2** with ethyl cyanoacetate and acetic anhydride in the presence of pyridine,[7] followed by hydrolysis of the resulting ethyl 2-cyano-2,2-(4-bromo-1,2-dihydroisoquinolidene)acetate (**3**), produced 4-bromo-1-methylisoquinoline (**4**). The synthesis of compound **4** by this sequence is more simple and more efficient than the method reported by Sawanishi et al.[8] Condensation of compound **4** with ammonium hydroxide, methylamine, or ethylamine in the presence of $CuSO_4 \cdot 5H_2O$ in an autoclave yielded the 4-amino-substituted isoquinoline derivatives **5a–c**, respectively. To protect the amino function of **5a–c** for a later oxidation reaction, compounds **5a–c** were converted to the corresponding acetylamino derivatives **6a–c** by acetylation with acetic anhydride in pyridine. The reaction temperature for the acetylation varied from room temperature for the 4-amino and 4-methylamino derivatives to heating at 50–55 °C for the ethylamino derivative in order to complete the reaction. Oxidation of compounds **6a–c** with selenium dioxide in anhydrous dioxane afforded the aldehyde derivatives **7a–c**, which were then reacted with thiosemicarbazide in ethanol containing either 10% acetic acid at room temperature to form the 4-acetylamino-substituted thiosemicarbazone derivatives **8a–c** or 10% hydrochloric acid under reflux to produce the 4-amino-substituted thiosemicarbazone derivatives **9a–c** (Scheme 1).

Condensation of compound **4** with *N*-acetylethylenediamine in the presence of $CuSO_4 \cdot 5H_2O$ gave 4-[*N*-[2-(acetylamino)ethyl]amino]-1-methylisoquinoline (**10**) and a reductive side product, 1-methylisoquinoline (**11**). The 4-amino function of **10** could not be acetylated by reaction with acetic anhydride in pyridine even at 80 °C, presumably due to the steric effects of the bulky (acetylamino)ethyl group. However, the desired acetyl derivative **12** was obtained, together with the diacetylated derivative **13**, when the reaction was conducted at 100 °C for 4 h. Compounds **12** and **13** were both converted to the corresponding aldehydes **14** and **15** by oxidation with selenium dioxide in dioxane. Condensation of both **14** and **15** with thiosemicarbazide in refluxing ethanol containing 10% hydrochloric acid yielded the target compound **16** (Scheme 2).

Nitration of 4-bromo-1-methylisoquinoline (**4**) with potassium nitrate and concentrated sulfuric acid gave 4-bromo-1-methyl-5-nitroisoquinoline (**17**), which was then treated with selenium dioxide in dioxane to yield the aldehyde **18**. To protect the aldehyde group, compound **18** was refluxed in toluene with ethylene glycol and *p*-toluenesulfonic acid to form the corresponding 1,3-dioxolane **19**.[9] Reaction of compound **19** with refluxing morpholine afforded the 4-morpholino derivative **20**, which was then reduced by catalytic hydrogenation in the presence of 10% Pd/C to yield the respective amino derivative **21**. Condensation of **21** with thiosemicarbazide and concentrated hydrochloric acid in ethanol gave the desired thiosemicarbazone **22** (Scheme 3).

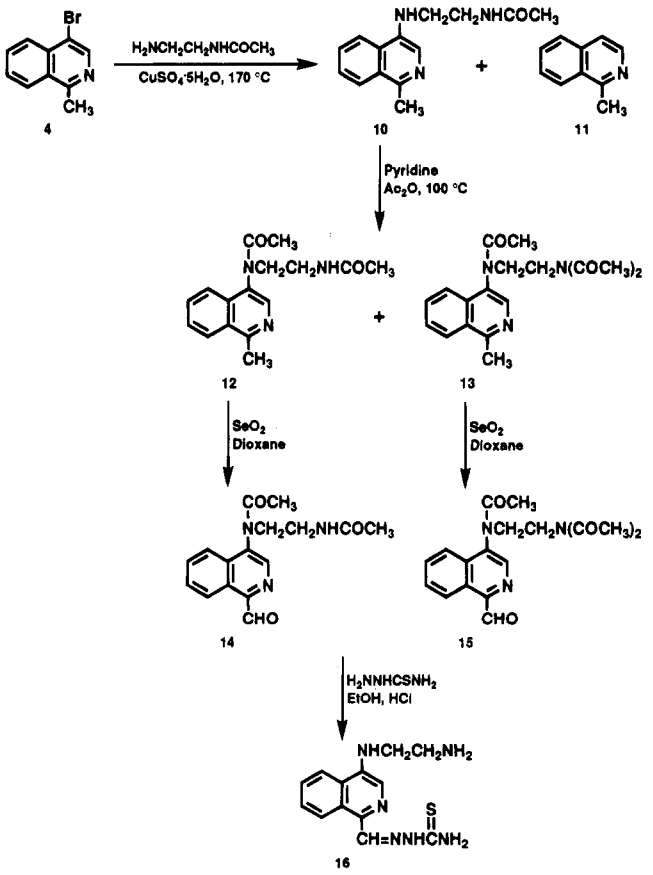
The synthesis of 4-hydroxy-5-methylisoquinoline-1-carboxaldehyde thiosemicarbazone (**34**) is shown in Scheme 4. The intermediate 1,5-dimethylisoquinoline (**27**) was prepared by a modification of the methodology of Spath et al.[10] Reduction of commercially available 2-methylbenzyl cyanide with lithium aluminum hydride and anhydrous aluminum chloride in anhydrous ether followed by acetylation with acetic anhydride in pyridine produced the amide derivative **25**. Dehydration of compound **25** with polyphosphoric acid afforded 1,5-dimethyl-3,4-dihydroisoquinoline (**26**), which was then dehydrogenated[9] with phenyl disulfide to give compound **27**. Oxidation of **27** with 30% hydrogen peroxide yielded the *N*-oxide derivative **28**, which was then subjected to the *N*-oxide rearrangement reaction in refluxing acetic anhydride to give two isomeric acetate derivatives, **29a,b**, which could not be separated by either silica gel column chromatography or fractional crystallization. Hydrolysis of the mixture of **29a,b** in hydrochloric acid afforded 1,5-dimethyl-4-hydroxyisoquinoline (**30**) and 5-methylisoquinoline-1-methanol (**31**). Compounds **30** and **31** were separated by converting **30** to the corresponding sodium salt, which was dissolved in water. The sodium salt was then converted back to compound **30** by acidification with 20% hydrochloric acid and precipitated from the aqueous solution. Compound

Scheme 1



R: a = H; b = CH_3; c = C_2H_5

Scheme 2



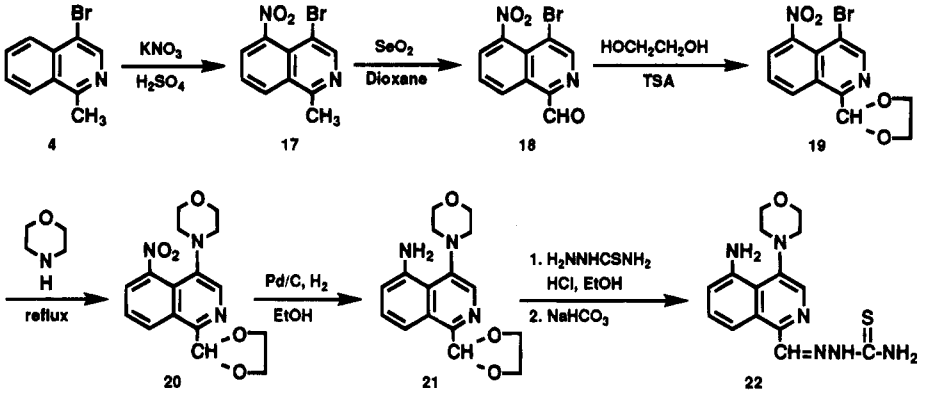
30 was converted back to the corresponding acetate **29a**, which was then oxidized with selenium dioxide in dioxane to give 4-acetoxy-5-methylisoquinoline-1-carboxaldehyde (**32**) (Scheme 4). Condensation of **32** with thiosemicarbazide followed by acidic hydrolysis of the resulting thiosemicarbazone **33** afforded the desired compound **34**.

The synthesis of 5-hydroxy-4-methylisoquinoline-1-carboxaldehyde thiosemicarbazone (**41**) is shown in Scheme 5. Treatment[11] of 1,4-dimethylisoquinoline (**35**) with fuming sulfuric acid followed by reaction of the resulting sulfonic acid derivative **36** with potassium hydroxide yielded 1,4-dimethyl-5-hydroxyisoquinoline (**37**). Acetylation of **37** with acetic anhydride yielded the acetylated derivative **38**, which was then selectively oxidized with selenium dioxide to give 5-acetoxy-4-methylisoquinoline-1-carboxaldehyde (**39**). Compound **39** was reacted with thiosemicarbazide to produce the thiosemicarbazone **40** which was then hydrolyzed to afford the final target compound **41**.

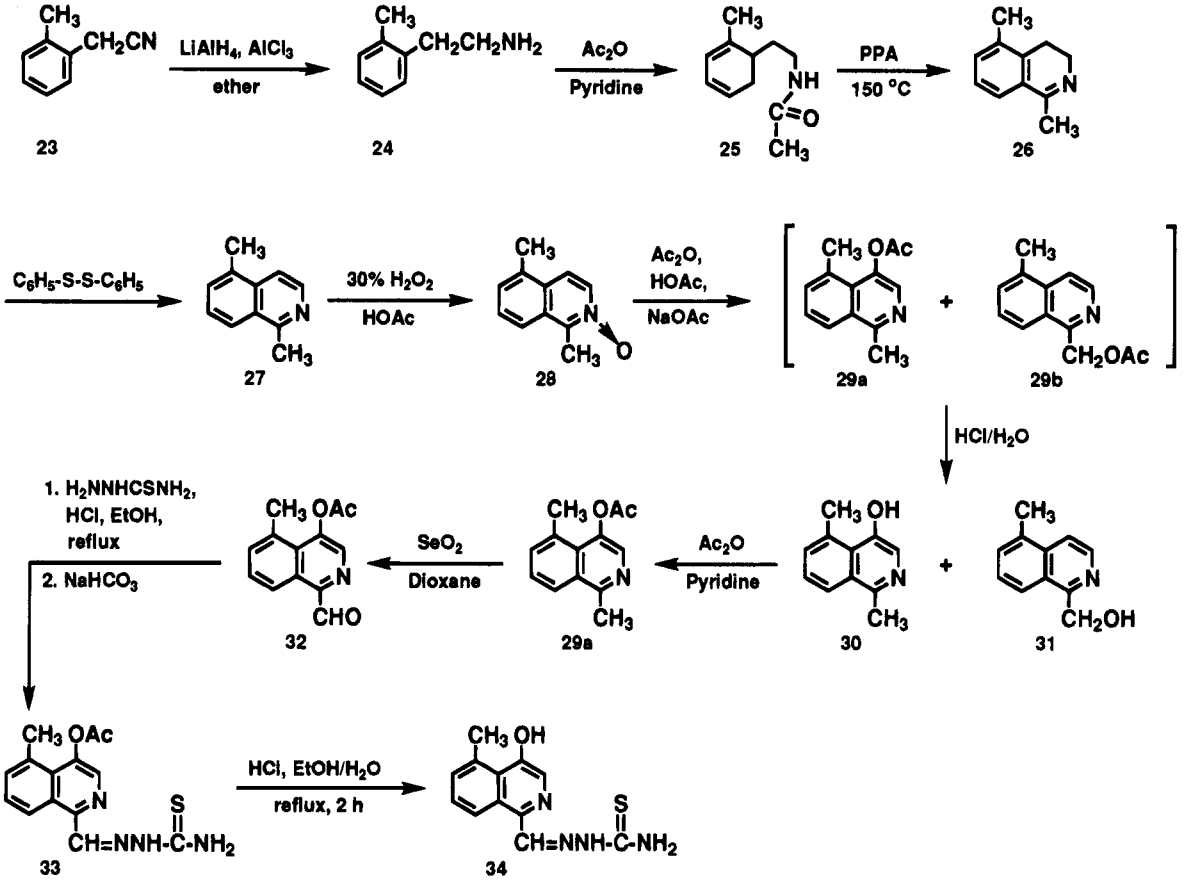
Biological Evaluation

Since *O*-glucuronidation represents a major inactivation pathway for 5-HP in humans, the effects of introducing a bulky methyl group adjacent to the hydroxyl moiety on the rate of glucuronidation were examined in two series of model compounds (Table 1). High lipid solubility is a prerequisite for a high rate of glucuronidation, and since the incorporation of a methyl group generally increases lipid solubility, two opposing actions result from the incorporation of a methyl functionality: (a) an increase in hydrophobicity, which acts to increase the rate of glucuronidation, and (b) steric hindrance to enzymic attack, which serves to decrease the rate of conjugation. With the hydroxyl-substituted pyridines examined, increasing hydrophobicity by substitution of methyl functions appeared to have the more dominant effect, and an increase in the rate of glucuronidation was observed, although the increase was

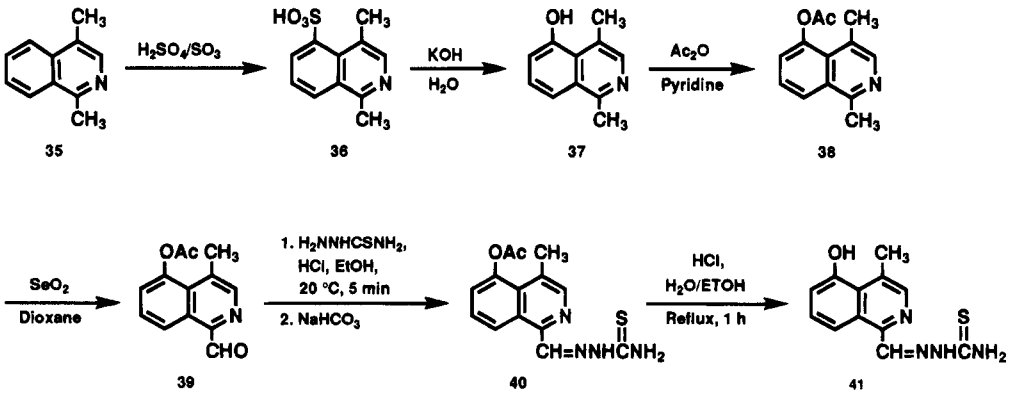
Scheme 3



Scheme 4

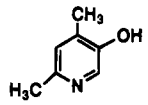
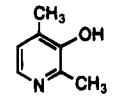
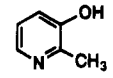
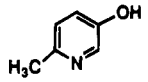
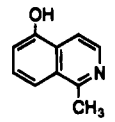
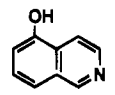
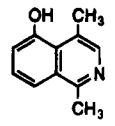


Scheme 5



lessened by the incorporation of two methyl groups adjacent to the hydroxyl function. In the hydroxyl-substituted isoquinoline series, the relatively rigid 4-methyl group in 2,4-dimethyl-5-hydroxyisoquinoline reduced the rate of glucuronidation more than 12-fold compared to 5-hydroxy-1-methylisoquinoline and ap-

Table 1. Relative Rates of Glucuronidation of Various Hydroxyl-Substituted Pyridine and Isoquinoline Derivatives

Compd	Relative rate
Hydroxyl-substituted Pyridine Derivatives	
	1.00
	0.81
	0.65
	0.59
Hydroxyl-substituted Isoquinoline Derivatives	
	1.00
	0.45
	0.08

peared to be highly effective in modifying this mode of drug elimination.

The tumor-inhibitory properties of the substituted isoquinoline-1-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 previously described methodology;[12] the results obtained with the active compounds are shown in Table 2. The two most active compounds synthesized were 4-aminoisoquinoline-1-carboxaldehyde thiosemicarbazone (**9a**) and 4-(methylamino)isoquinoline-1-carboxaldehyde thiosemicarbazone (**9b**) which produced the same % T/C value of 177 at a daily dose of 40 mg/kg for 6 consecutive days. 4-[*N*-(2-Aminoethyl)amino]isoquinoline-1-carboxaldehyde thiosemicarbazone (**16**) and 5-amino-4-morpholinoisoquinoline-1-carboxaldehyde thiosemicarbazone (**22**) produced % T/C values of 145 and 149 when 60 and 10 mg/kg, respectively, were administered daily for 6 consecutive days. These latter compounds produced antitumor activity comparable to that of 5-AP, which had a maximum % T/C value of 140 at a daily dosage of 20 mg/kg on the same delivery schedule. Other compounds either showed marginal activity or were inactive, and these test results are not included.

Table 2. Effects of 5-AP and 4-Amino-Substituted Isoquinoline-1-carboxaldehyde Thiosemicarbazone Derivatives on the Survival Time of Mice Bearing L1210 Leukemia

compd	substituted groups	daily dosage[a] (mg/kg)	av Δ wt[b] (%)	T/C[c] (%)
5-AP		40	−18.0	129
		20	−2.8	140
		10	0.0	138
9a	4-NH_2	40	3.2	177
		20	3.6	174
		10	3.0	162
9b	4-CH_3NH	40	1.0	177
		20	3.0	159
		10	8.0	179
16	4-$HNCH_2CH_2NH$	60	−4.6	145
		40	−5.0	120
		20	−4.6	116
22	5-NH_2, 4-morpholino	40	2.0	138
		20	7.3	141
		10	6.8	149

[a] Administered ip in suspension once daily for 6 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.

Table 3. Effects of 5-AP and 4-Aminoisoquinoline-1-carboxaldehyde Thiosemicarbazone (**9a**) Administered Twice Daily on the Survival Time of Mice Bearing the L1210 Leukemia

compd	optimum daily dosage[a] (mg/kg)	av Δ wt[b] (%)	av survival (days)[c]	T/C[d] (%)	long-term survivors[e]
5-AP	20 × 2	−7.6	19.2	234	1/5
9a	40 × 2	−7.8	13.5	165	3/5

[a] Drugs were administered in suspension by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily (ca. 12 h apart) for 6 consecutive days, with 5–10 mice/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 represents the ratio of the survival time of treated to control animals × 100. The average survival time of 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.

Compounds **9a** and 5-AP were further evaluated against the L1210 leukemia using the more frequent schedule of drug administration of twice a day for 6 consecutive days. The results of these tests are summarized in Table 3. Compound **9a** gave a % T/C value of 165 with 60% of the animals being 60-day long-term survivors (cures), whereas 5-AP, used as a positive control, produced a % T/C value of 234 with 20% of the mice being 60-day long-term survivors.

In summary, addition of a benzene ring onto the 3,4-positions of the pyrimidine ring of 5-AP to form the isoquinoline analogue 4-aminoisoquinoline-1-carboxaldehyde thiosemicarbazone (**9a**) led to an increase in the antitumor activity against the L1210 leukemia in mice. Introduction of a methyl group onto the 4-amino function did not affect the antitumor activity; however, replacement of the 4-amino group of **9a** with a larger substituent such as a (2-aminoethyl)amino group (**16**) or a morpholino moiety (**22**) resulted in a decrease in antitumor activity. Substitution of a 4- or 5-methyl group on the isoquinoline ring of 5- or 4-hydroxyisoquinoline-1-carboxaldehyde thiosemicarbazone, respectively, significantly decreased antitumor activity; this contrasted to the introduction of a 4-methyl function on

the isoquinoline ring of 5-aminoisoquinoline-1-carboxaldehyde thiosemicarbazone, a substitution that did not affect antitumor activity.[13,14] These findings suggest that the size, character, and number of substituents on the isoquinoline ring are important for antitumor activity.

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-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 are within ±0.4% of the theoretical value.

4-Bromoisoquinoline *N*-Oxide (2). To a stirred solution of 4-bromoisoquinoline (**1**; 25 g, 0.12 mol) in 500 mL of glacial acetic acid was added dropwise 30 mL of 30% hydrogen peroxide. The reaction mixture was heated to 65–70 °C, and two additional portions of mixtures of 150 mL of glacial acetic acid and 7.5 mL of 30% hydrogen peroxide were added at 10-h intervals. The solution was maintained at 65–70 °C for a total of about 30 h until TLC (CH_2Cl_2/AcOEt, 1:1, v/v) showed the starting material had disappeared. After the solvent was removed under reduced pressure, the residue was coevaporated with ethanol (100 mL), dissolved in 50% aqueous ethanol, and neutralized with ammonium hydroxide, and the solvent was evaporated again under reduced pressure. The residue was stirred with 150 mL of water for 1 h, filtered, and washed with cold water to give 25 g (93%) of product: mp 168–169 °C (lit.[6] mp 169 °C); ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 3H, 5-H, 7-H, 8-H), 8.12 (m, 1H, 6-H), 8.48 (s, 1H, 3-H), 8.75 (s, 1H, 1-H).

Ethyl 2-Cyano-2,2-(4-bromo-1,2-dihydroisoquinolidene)-acetate (3). To a stirred suspension of 11.2 g (50 mmol) of 4-bromoisoquinoline *N*-oxide (**2**), 6.9 g of ethyl cyanoacetate (6.9 g, 60 mmol), 4.7 g of pyridine (60 mmol), and 25 mL of methylene chloride was added dropwise 6.1 g of acetic anhydride (60 mmol) at 0–5 °C. The reaction mixture was stirred at 0–5 °C for 12 h and at room temperature for an additional 24 h and then evaporated to dryness under reduced pressure. The residue was partitioned between methylene chloride and water, and 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 column (CH_2Cl_2, R_f 0.83) to yield 9.6 g (60%) of product as yellow crystals: mp 201–202 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, CH_3), 4.30 (q, 2H, CH_2), 7.70 (m, 4H, ArH), 9.45 (d, 1H, 3-H), 14.52 (br s, 1H, 2-NH, D_2O exchangeable). Anal. ($C_{14}H_{11}BrN_2O_2$) C, H, N.

4-Bromo-1-methylisoquinoline (4). A suspension of compound **3** (4.3 g, 13.5 mmol) in 40 mL of 35% sulfuric acid was heated with stirring at 100–105 °C until a clear solution was formed and then refluxed for 2 days. After cooling to room temperature, the reaction mixture was poured onto ice (250 g). The solution was neutralized to pH 7 using 20% sodium hydroxide and extracted with methylene chloride. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated; the residue was purified by silica gel column chromatography (CH_2Cl_2/AcOEt, 1:1, v/v) to give 2.7 g (90%) of product: mp 50 °C (lit.[7] mp 49–50 °C); ^{1}H NMR ($CDCl_3$) δ 2.85 (s, 3H, 1-CH_3), 7.65 (m, 2H, 5-H, 8-H), 8.05 (m, 2H, 6-H, 7-H), 8.55 (s, 1H, 3-H).

4-(Acetylamino)-1-methylisoquinoline (6a). A mixture of **4** (2 g, 9 mmol), $CuSO_4 \cdot 5H_2O$ (0.4 g), and ammonium hydroxide (25 mL) was heated in an autoclave at 160–165 °C for 24 h. After cooling, the reaction mixture was extracted with methylene chloride (120 mL), washed with water, and dried over $MgSO_4$. The filtrate was evaporated in vacuo to yield crude **5a** (1.3 g) as an oil.

To a stirred solution of crude **5a** in 20 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), dissolved in methylene chloride, washed with 5% sodium bicarbonate, brine, and water, and dried over $MgSO_4$. After removing the solvent, the residue was recrystallized from ethanol to yield 1.2 g (67%) of product: mp 110–111 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H, $COCH_3$), 2.88 (s, 3H, 1-CH_3), 7.25 (br s, 1H, NH, D_2O exchangeable), 7.35–7.85 (m, 4H, ArH), 8.40 (s, 1H, 3-H). Anal. ($C_{12}H_{12}N_2O$) C, H, N.

4-(*N*-Acetyl-*N*-methylamino)-1-methylisoquinoline (6b). This compound was prepared from **4** (4.3 g, 19 mmol) by the procedure employed for the synthesis of **6a**: yield 2.5 g (60%); mp 95–96 °C; TLC R_f 0.65 (CH_2Cl_2/EtOH, 10:0.8, v/v); ^{1}H NMR ($CDCl_3$) δ 1.85 (s, 3H, $COCH_3$), 3.05 (s, 3H, 1-CH_3), 3.40 (s, 3H, N-CH_3), 7.60–8.40 (m, 5H, ArH). Anal. ($C_{13}H_{14}N_2O$) C, H, N.

4-(*N*-Acetyl-*N*-ethylamino)-1-methylisoquinoline (6c). This compound was prepared from **4** (2.0 g, 9 mmol) by the procedure employed for the synthesis of **6a**, except that the acetylation was carried out at 50–55 °C: yield 0.82 g (41%); mp 107–108 °C; TLC R_f 0.72 (CH_2Cl_2/EtOH, 10:1, v/v); ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, CCH_3), 1.75 (s, 3H, $COCH_3$), 3.05 (s, 3H, 1-CH_3), 3.40 (m, 1H, 4-NCH_A), 4.25 (m, 1-H, 4-NCH_B), 7.70–8.30 (m, 5H, ArH). Anal. ($C_{14}H_{15}N_2O$) C, H, N.

4-(*N*-Acetylamino)isoquinoline-1-carboxaldehyde (7a). A mixture of **6a** (0.7 g, 3.5 mmol) and selenium dioxide (0.6 g, 4.3 mmol) in 1,4-dioxane (40 mL) was refluxed under an atmosphere of nitrogen for 3 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, 1:1, v/v, R_f 0.35) to afford 0.45 g (61%) of white crystals: mp 203–204 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, $COCH_3$), 7.90 (m, 2H, 5-H, 8-H), 8.45 (m, 1H, 7-H), 9.30 (m, 1H, 6-H), 9.35 (s, 1H, 3-H), 10.25 (s, 1H, CHO), 10.30 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{12}H_{10}N_2O_2$) C, H, N.

4-(*N*-Acetyl-*N*-methylamino)isoquinoline-1-carboxaldehyde (7b). This compound was prepared from **6b** (2.1 g, 9.8 mmol) by the procedure employed for the synthesis of **7a**: yield 1.5 g (68%); mp 128–129 °C; TLC R_f 0.42 (AcOEt); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H, $COCH_3$), 3.40 (m, 1H, 4-NCH_3), 7.90 (m, 3H, 5-H, 7-H, 8-H), 8.70 (s, 1H, 3-H), 9.45 (m, 1H, 6-H), 10.40 (s, 1H, CHO). Anal. ($C_{13}H_{12}N_2O_2$) C, H, N.

4-(*N*-Acetyl-*N*-ethylamino)isoquinoline-1-carboxaldehyde (7c). This compound was prepared from **6c** (0.60 g, 2.6 mmol) by the procedure employed for the synthesis of **7a**: yield 0.56 g (88%); mp 110–112 °C; TLC R_f 0.50 (CH_2Cl_2/AcOEt, 1:1, v/v); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CCH_3), 1.95 (s, 3H, $COCH_3$), 3.50–4.25 (m, 2H, 4-NCH_2), 7.90–8.20 (m, 3H, 5-H, 7-H, 8-H), 8.65 (s, 1H, 3-H), 9.40 (m, 1H, 6-H), 10.45 (s, 1H, CHO). Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

4-(Acetylamino)isoquinoline-1-carboxaldehyde Thiosemicarbazone (8a). A mixture of **7a** (0.35 g, 1.8 mmol), thiosemicarbazide (0.25 g, 2.7 mol), 1 mL of glacial acetic acid, and 10 mL of ethanol was stirred at room temperature for 2 h, filtered, and washed with cooled water. The acetic acid salt was dissolved in hot water and filtered into 10 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 to give 0.42 g (82%) of product: mp 253–255 °C; MS *m/e* 288 ($M^+ + 1$); ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, $COCH_3$), 7.90 (m, 2H, 2-CH, 5-H, 8-H), 8.00 and 8.05 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.30 (m, 1H, 7-H), 8.70 (s, 1H, 1-CH), 8.90 (s, 1H, 3-H), 9.30 (m, 1H, 6-H), 10.20 (s, 1H, 4-NH, D_2O exchangeable), 11.40 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{13}H_{13}N_5OS$) C, H, N.

4-(*N*-Acetyl-*N*-methylamino)isoquinoline-1-carboxaldehyde Thiosemicarbazone (8b). This compound was prepared from **7b** (0.90 g, 3.9 mmol) by the procedure employed for the synthesis of **8a**: yield 0.42 g (36%); mp 215–216 °C; MS *m/e* 302 ($M^+ + 1$); ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H, $COCH_3$), 3.22 (s, 3H, N-CH_3), 7.90 (m, 2H, 5-H, 8-H), 8.00 and

8.05 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.50 (m, 1H, 7-H), 8.60 (s, 1H, 1-CH), 8.65 (s, 1H, 3-H), 9.30 (m, 1H, 6-H), 11.90 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{14}H_{15}N_5OS$) C, H, N.

4-(*N*-Acetyl-*N*-ethylamino)isoquinoline-1-carboxaldehyde Thiosemicarbazone (8c). This compound was prepared from **7c** (0.90 g, 3.9 mmol) by the procedure employed for the synthesis of **8a**: yield 0.42 g (36%); mp 215–216 °C; MS *m/e* 314 ($M^+ + 1$); ^1H NMR (DMSO-d_6) δ 1.15 (t, 3H, CH_3), 1.65 (s, 3H, $COCH_3$), 3.42 (m, 1H, N-CH_A), 4.10 (m, 1H, N-CH_B), 7.90 (m, 2H, 5-H, 8-H), 8.00 and 8.05 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.50 (m, 1H, 7-H), 8.55 (s, 1H, 1-CH), 8.65 (s, 1H, 3-H), 9.25 (m, 1H, 6-H), 11.80 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{15}H_{17}N_5OS \cdot HCl \cdot 0.25H_2O$) C, H, N.

4-Aminoisoquinoline-1-carboxaldehyde Thiosemicarbazone (9a). To a solution of **7a** (0.37 g, 1.7 mmol) in 10 mL of ethanol, 8 mL of water, and 4 mL of concentrated hydrochloric acid was added 0.25 g (2.7 mmol) of thiosemicarbazide. The mixture was refluxed overnight, cooled, and filtered. The yellow hydrochloride salt was dissolved in 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 0.35 g (83%); mp 189–190 °C dec; MS *m/e* 246 (M^+); ^1H NMR (DMSO-d_6) δ 6.58 (br s, 2H, 4-NH_2, D_2O exchangeable), 7.72 (m, 2H, 5-H, 8-H), 7.85 and 8.30 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.10 (s, 1H, 1-CH), 8.35 (m, 1H, 7-H), 8.60 (s, 1H, 3-H), 9.30 (m, 1H, 6-H), 11.55 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{11}H_{11}N_5S$) C, H, N.

4-(Methylamino)isoquinoline-1-carboxaldehyde Thiosemicarbazone (9b). This compound was prepared from **7b** (0.40 g, 1.8 mmol) by the procedure employed for the synthesis of **9a**: yield 0.33 g (73%); mp 160–162 °C; MS *m/e* 259 (M^+); ^1H NMR (DMSO-d_6) δ 2.95 (d, 3H, N-CH_3), 6.90 (m, 1H, 4-NH, D_2O exchangeable), 7.62 (m, 2H, 5-H, 8-H), 7.80 and 8.20 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 7.90 (s, 1H, 1-CH), 8.15 (m, 1H, 7-H), 8.45 (s, 1H, 3-H), 9.20 (m, 1H, 6-H), 11.35 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{12}H_{13}N_5S$) C, H, N.

4-(Ethylamino)isoquinoline-1-carboxaldehyde Thiosemicarbazone (9c). This compound was prepared from **7c** (0.40 g, 1.8 mmol) by the procedure employed for the synthesis of **9a**: yield 0.33 g (73%); mp 160–162 °C; MS *m/e* 271 (M^+); ^1H NMR (DMSO-d_6) δ 1.10 (t, 3H, CCH_3), 3.40–4.10 (m, 2H, CH_2), 7.80 (m, 2H, 5-H, 8-H), 8.00 and 8.05 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.50 (m, 1H, 7-H), 8.20 (s, 1H, 1-CH), 8.45 (s, 1H, 3-H), 9.25 (m, 1H, 6-H), 11.50 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{14}H_{15}N_5S$) C, H, N.

4-[[2-(Acetylamino)ethyl]amino]-1-methylisoquinoline (10). A mixture of **4** (2 g, 9 mmol), $CuSO_4 \cdot 5H_2O$ (0.4 g), water (10 mL), and *N*-acetylethylenediamine (12 mL) was heated at 130–135 °C for 14 h. The cooled reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and dried ($MgSO_4$). After removal of the solvent, the residue was chromatographed on a silica gel column, eluting first with CH_2Cl_2/AcOEt, 1:1, v/v, to give 0.3 g of 1-methylisoquinoline (identical with an authentic sample by TLC and ^1H NMR) and then with CH_2Cl_2/EtOH, 2:1, v/v, to yield 0.79 g (33%) of product: mp 163–165 °C; TLC R_f 0.30 (CH_2Cl_2/EtOH, 10:1, v/v); ^1H NMR ($CDCl_3$) δ 2.00 (s, 3H, $COCH_3$), 2.76 (s, 3H, 1-CH_3), 3.32–3.65 (m, 4H, CH_2CH_2), 5.15 (br s, 1H, ArNH, D_2O exchangeable), 6.27 (br s, 1H, CONH, D_2O exchangeable), 7.50–8.20 (m, 5H, ArH). Anal. ($C_{14}H_{17}N_3O$) C, H, N.

4-[*N*-Acetyl-*N*-[2-(acetylamino)ethyl]amino]-1-methylisoquinoline (12) and 4-[*N*-Acetyl-*N*-[2-(diacetylamino)ethyl]amino]-1-methylisoquinoline (13). A mixture of **10** (0.30 g, 1.2 mmol) and 2 mL of acetic anhydride in 20 mL of pyridine was heated at 100 °C for 4 h. The reaction mixture was evaporated in vacuo to dryness and coevaporated with ethanol (5 mL) twice. The residue was partitioned between methylene chloride and water; the organic layer was washed with 10% sodium bicarbonate solution, brine, and water and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH, 10:1, v/v) to give two products.

Compound **12**: yield 0.22 g (65%); mp 165–166 °C; TLC R_f 0.48 (CH_2Cl_2/EtOH, 10:1, v/v); ^1H NMR ($CDCl_3$) δ 1.80 and 2.00 (2 s, 6H, $2COCH_3$), 3.00 (s, 3H, 1-CH_3), 3.52–4.20 (m, 4H, CH_2CH_2), 6.65 (br s, 1H, CONH, D_2O exchangeable), 7.80–8.30 (m, 5H, ArH). Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

Compound **13**: yield 60 mg (15%); mp 169–171 °C; TLC R_f 0.72 (CH_2Cl_2/EtOH, 10:1, v/v); ^1H NMR (90 MHz, $CDCl_3$) δ 1.75 (s, 3H, $COCH_3$), 2.40 (s, 6H, $2COCH_3$), 3.05 (s, 3H, 1-CH_3), 3.42–4.10 (m, 4H, CH_2CH_2), 7.60–8.30 (m, 5H, ArH). Anal. ($C_{18}H_{21}N_3O_3$) C, H, N.

4-[*N*-Acetyl-*N*-[2-(acetylamino)ethyl]amino]isoquinoline-1-carboxaldehyde (14). A mixture of **12** (0.20 g, 0.7 mmol) and selenium dioxide (0.18 g, 1.6 mmol) in 1,4-dioxane (10 mL) was heated at 100 °C under an atmosphere of nitrogen for 3 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/EtOH, 10:1, v/v, R_f 0.75) to afford 0.14 g (67%) of white crystals: mp 106–108 °C; ^1H NMR ($CDCl_3$) δ 1.80 and 2.10 (2 s, 6H, $2COCH_3$), 3.55–4.25 (m, 4H, CH_2CH_2), 6.75 (br s, 1H, CONH, D_2O exchangeable), 7.90–8.30 (m, 3H, 5-H, 7-H, 8-H), 8.80 (s, 1H, 3-H), 9.50 (m, 1H, 6-H), 10.40 (s, 1H, 1-CHO). Anal. ($C_{16}H_{17}N_3O_3$) C, H, N.

4-[*N*-Acetyl-*N*-[2-(diacetylamino)ethyl]amino]isoquinoline-1-carboxaldehyde (15). This compound was prepared from **13** (0.12 g, 0.4 mmol) by the procedure employed for the synthesis of **14**: yield 0.10 g (80%); mp 112–114 °C; TLC R_f 0.55 (CH_2Cl_2/EtOH, 20:1, v/v); ^1H NMR ($CDCl_3$) δ 1.80 (s, 3H, $COCH_3$), 2.45 (s, 6H, $2COCH_3$), 3.80–4.20 (m, 4H, CH_2-CH_2), 7.90–8.20 (m, 3H, 5-H, 7-H, 8-H), 8.70 (s, 1H, 3-H), 9.40 (m, 1H, 6-H), 10.35 (s, 1H, 1-CHO). Anal. ($C_{18}H_{21}N_3O_3$) C, H, N.

4-[(2-Aminoethyl)amino]isoquinoline-1-carboxaldehyde Thiosemicarbazone (16). A mixture of **14** (0.24 g, 0.80 mmol), thiosemicarbazide (0.12 g, 1.3 mol), 2 mL of concentrated HCl, 2 mL of water, and 10 mL of ethanol was refluxed for 3 h. The cooled reaction solution was evaporated under reduced pressure to about one-third of the original volume until a yellow solid precipitated out. The solid was collected by filtration and recrystallized from ethanol to afford 0.20 g (77%) of product as yellow crystals: mp 220–222 °C dec; MS *m/e* 289 ($M^+ + 1$); ^1H NMR (DMSO-d_6) δ 3.30 (m, 2H, $ArNCH_2$), 3.75 (m, 2H, NCH_2), 4.20 (br s, 1H, ArNH, D_2O exchangeable), 7.65 (s, 1H, 2-CH), 8.00 (m, 2H, 5-H, 8-H), 8.15 and 8.30 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.45 (m, 1H, 7-H), 8.65 (m, 1H, 6-H), 8.80 (s, 1H, 3-H), 8.95 (br s, 2H, NH_2, D_2O exchangeable), 11.9 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{13}H_{16}N_6S \cdot 2HCl \cdot H_2O$) C, H, N.

4-Bromo-1-methyl-5-nitroisoquinoline (17). 4-Bromo-1-methylisoquinoline (1.0 g, 4.5 mmol) was added slowly to 6 mL of concentrated sulfuric acid and cooled in an ice bath with stirring. Potassium nitrate (0.51 g, 5 mmol) was then added slowly. The reaction mixture was gradually heated to 60 °C and maintained at this temperature for 2 h. After cooling to room temperature, the reaction mixture was poured into ice–water. The solution was neutralized to pH 7 using potassium carbonate, and the resulting yellow precipitate was collected by filtration; the solid was washed with water and recrystallized from ethanol to give 1.1 g (91%) of product: mp 165–166 °C; ^1H NMR ($CDCl_3$) δ 3.00 (s, 3H, 1-CH_3), 7.80 (m, 2H, 7-H, 8-H), 8.45 (d, 1H, 6-H), 8.75 (s, 1H, 3-H). Anal. ($C_{10}H_7BrN_2O_2$) C, H, N.

4-Bromo-5-nitroisoquinoline-1-carboxaldehyde (18). This compound was prepared from **17** (1.0 g, 3.7 mmol) by the procedure employed for the synthesis of **7a**: yield 0.60 g (55%); mp 140–141 °C; TLC R_f 0.82 (CH_2Cl_2/AcOEt, 10:1, v/v); ^1H NMR ($CDCl_3$) δ 7.80 (m, 2H, 7-H, 8-H), 9.10 (s, 1H, 3-H), 9.55 (m, 1H, 6-H), 10.4 (s, 1H, CHO). Anal. ($C_{10}H_5N_2O_3$) C, H, N.

4-Bromo-1-(1,3-dioxolan-2-yl)-5-nitroisoquinoline (19). To 0.56 g (2.0 mmol) of compound **18** in 30 mL of toluene were added 50 mg of *p*-toluenesulfonic acid monohydrate and 1 mL of ethylene glycol. The reaction mixture was refluxed with stirring, using a Dean–Stark trap to remove the water formed during condensation, until complete disappearance of the starting material was observed. 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/AcOEt, 4:1, v/v, R_f 0.62) to afford 0.46 g (71%) of product: mp 125–126 °C; ^1H NMR ($CDCl_3$) δ 4.21 (m, 4H, CH_2CH_2), 6.45 (s, 1H, 1-CH), 7.70–8.10 (m, 2H, 7-H, 8-H), 8.70 (d, 1H, 6-H), 8.90 (s, 1H, 3-H). Anal. ($C_{12}H_9BrN_2O_4$) C, H, N.

1-(1,3-Dioxolan-2-yl)-4-morpholino-5-nitroisoquinoline (20). A mixture of **19** (0.40 g, 1.2 mmol) and morpholine (12 mL) was refluxed for about 20 h until the starting material disappeared (monitored by TLC, CH_2Cl_2/EtOAc, 1:1, v/v, R_f 0.86, for the starting material, and R_f 0.50, for the product). The reaction mixture was evaporated in vacuo to dryness and coevaporated with toluene (2 × 10 mL). The residue was purified by silica gel chromatography to yield 0.26 g (63%) of yellow crystals: mp 228–229 °C; ^1H NMR ($CDCl_3$) δ 3.10 [m, 4H, $N(CH_2)_2$], 3.80 [m, 4H, $O(CH_2)_2$], 4.25 (m, 4H, CH_2CH_2), 6.45 (s, 1H, 1-CH), 7.75 (m, 2H, 7-H, 8-H), 8.55 (s, 1H, 3-H), 8.60 (d, 1H, 6-H). Anal. ($C_{16}H_{17}N_3O_5$) C, H, N.

5-Amino-1-(1,3-dioxolan-2-yl)-4-morpholinoisoquinoline (21). The nitro derivative **20** (0.26 g, 0.8 mmol) was dissolved in 150 mL of ethanol and hydrogenated in a Parr apparatus under 50 psi of pressure in the presence of 10% Pd/C (70 mg) for 1 h. After filtration, the filtrate was evaporated under reduced pressure to give the product (0.22 g, 91%) as a syrup, which was used for the next reaction without further purification: ninhydrin positive; ^1H NMR ($CDCl_3$) δ 3.05 [m, 4H, $N(CH_2)_2$], 3.75 [m, 4H, $O(CH_2)_2$], 4.15 (m, 4H, CH_2CH_2), 5.70 (br s, 2H, 5-NH_2, D_2O exchangeable), 6.35 (s, 1H, 1-CH), 6.85–7.52 (m, 3H, 6-H, 7-H, 8-H), 8.15 (s, 1H, 3-H).

5-Amino-4-morpholinoisoquinoline-1-carboxaldehyde Thiosemicarbazone (22). This compound was prepared from **21** (0.20 g, 0.66 mmol) by the procedure employed for the synthesis of **9a**: yield 0.18 g (82%); mp 210–212 °C; MS *m/e* 331 ($M^+ + 1$); ^1H NMR (DMSO-d_6) δ 3.15 [m, 4H, $N(CH_2)_2$], 3.85 [m, 4H, $O(CH_2)_2$], 7.20–7.42 (m, 3H, 6-H, 7-H, 8-H), 7.15 and 7.55 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 7.80 (s, 1H, 1-CH), 8.70 (s, 1H, 3-H), 9.10 (br s, 2H, NH_2, D_2O exchangeable), 11.9 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{15}H_{18}N_6OS$·HCl·0.5H_2O) C, H, N.

1,5-Dimethylisoquinoline (27). To a solution of lithium aluminum hydride (4.2 g, 0.11 mol) in 250 mL of anhydrous ether was added rapidly a solution of anhydrous aluminum chloride (13.3 g, 0.1 mol) in 150 mL of anhydrous ether with stirring followed by the addition of a solution of 2-methylbenzyl cyanide (13.1 g, 0.1 mol) in 150 mL of anhydrous ether at a rate sufficient to cause moderate refluxing. After the addition, the reaction mixture was refluxed further for 1 h, water (100 mL) was added dropwise to the cooled reaction mixture to decompose the excess hydride, and 140 mL of 6 N sulfuric acid was added. The aqueous layer was extracted with ether (2 × 100 mL). The combined ether solution was dried (anhydrous $MgSO_4$), filtered, and evaporated to give 12.6 g (93%) of compound **24** as an oil: ^1H NMR ($CDCl_3$) δ 1.70 (s, 2H, NH_2, D_2O exchangeable), 2.30 (s, 3H, CH_3), 2.70–3.05 (m, 4H, CH_2-CH_2), 7.15 (s, 4H, ArH).

Acetic anhydride (10 mL) was added dropwise to a stirred solution of **24** in 100 mL of anhydrous pyridine at 0–5 °C and stirred at room temperature overnight. The reaction mixture was evaporated to dryness in vacuo, and the residue was coevaporated with ethanol (10 mL) followed by toluene (10 mL) and partitioned between methylene chloride and water. The organic layer was washed with 10% sodium bicarbonate, brine, and water and then dried (anhydrous $MgSO_4$). The filtrate was evaporated in vacuo to yield 12.2 g (88%) of compound **25**: ^1H NMR ($CDCl_3$) δ 1.95 (s, 3H, $COCH_3$), 2.30 (s, 3H, $ArCH_3$), 2.85 (m, 2H, $ArCH_2$), 3.45 (m, 2H, NCH_2), 6.35 (br s, 1H, CONH, D_2O exchangeable), 7.10 (s, 4H, ArH).

A mixture of **25** (12.2 g, 69 mmol) and poly(phosphoric acid) (80 g) was heated at 160–165 °C for 4 h with stirring. The reaction mixture was cooled to 100–105 °C and poured into ice–water, alkalized with 20% sodium hydroxide solution, and extracted with toluene (3 × 250 mL). The combined toluene solution was dried (anhydrous $MgSO_4$), filtered, and evaporated to produce 10.9 g (99%) of compound **26**.

A mixture of **26** (5.2 g, 33 mmol) and phenyl disulfide (8.6 g, 39 mmol) in tetralin (40 mL) was stirred at 200–205 °C for 15 h, cooled, diluted with 80 mL of toluene, and extracted with 5% hydrochloric acid (2 × 40 mL). The combined aqueous solution was alkalinized with 30% potassium hydroxide solution, and the solid which precipitated was collected by filtration and washed with water to afford 4.8 g (94%) of product: mp 92–94 °C (lit.[10] mp 97–98 °C); ^1H NMR ($CDCl_3$) δ 2.65 (s, 3H, 5-CH_3), 2.90 (s, 3H, 1-CH_3), 7.40–7.70 (m, 3H, 4-H, 6-H, 8-H), 8.00 (m, 1H, 7-H), 8.46 (d, 1H, 3-H).

1,5-Dimethylisoquinoline *N*-Oxide (28). This compound was prepared from 1,5-dimethylisoquinoline (17.4 g, 0.11 mol) by the procedure employed for the synthesis of **2**: yield 17.0 g (86%); mp 137–138 °C; ^1H NMR ($CDCl_3$) δ 2.65 (s, 3H, 5-CH_3), 2.92 (s, 3H, 1-CH_3), 7.35–7.85 (m, 4H, 4-H, 6-H, 7-H, 8-H), 8.25 (s, 1H, 3-H). Anal. ($C_{11}H_{11}NO$) C, H, N.

1,5-Dimethyl-4-hydroxyisoquinoline (30). Method a: A mixture of **28** (3 g, 17.3 mmol), anhydrous sodium acetate (2.5 g), acetic acid (15 mL), and acetic anhydride (30 mL) was heated at 80–85 °C with stirring for 2 h. The cooled reaction mixture was evaporated under reduced pressure, and the residue was partitioned between methylene chloride and water. The organic layer was washed with 10% sodium carbonate and then water and evaporated to dryness. To the residue was added 50 mL of 10% hydrochloric acid, and the mixture was refluxed for 1 h, cooled, treated with Norit-A, and filtered. The filtrate was made strongly alkaline with 20% sodium hydroxide and extracted with ether (4 × 50 mL). After the aqueous layer was neutralized to pH 7 with 20% hydrochloric acid, the resulting off-white precipitate was collected by filtration, washed with cool water, and recrystallized from ethanol: yield 0.6 g (20%); mp 200–202 °C; ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H, 5-CH_3), 2.94 (s, 3H, 1-CH_3), 7.30 (m, 2H, 7-H, 8-H), 8.85 (m, 2H, 3-H, 6-H), 9.90 (br s, 1H, 4-OH, D_2O exchangeable). Anal. ($C_{11}H_{11}NO$) C, H, N.

The combined ether extract was washed with water, dried over $MgSO_4$, and filtered. After evaporation of the solvent, the residue was recrystallized from hexanes to give 1.1 g (37%) of compound **31** as off-white crystals: mp 75–77 °C; ^1H NMR ($CDCl_3$) δ 2.75 (s, 3H, 5-CH_3), 4.50 (br s, 1H, 1-COH, D_2O exchangeable), 5.50 (s, 2H, 1-CH_2), 7.40 (m, 2H, 6-H, 8-H), 7.70 (d, 1H, 4-H), 7.90 (m, 1H, 6-H), 8.40 (d, 1H, 3-H). Anal. ($C_{11}H_{11}NO$) C, H, N.

Method b: A mixture of **28** (3 g, 17.3 mmol) and 28 mL of acetic anhydride was refluxed for 2 h and processed as described in method a to yield 0.37 g (12%) of compound **30** and 0.90 g (30%) of compound **31**.

Addition of sodium acetate and acetic acid resulted in an increase in the yield of compound **30** from 12% to 20%.

4-Acetoxy-1,5-dimethylisoquinoline (29a). Acetic anhydride (2 mL) was added dropwise to a stirred solution of compound **30** (0.40 g, 2.3 mmol) in dry pyridine (20 mL) at 0–5 °C (ice–water bath). The reaction mixture was stirred at room temperature overnight and evaporated in vacuo to dryness. The residue was coevaporated with ethanol (10 mL), toluene (10 mL), and methylene chloride (10 mL) and then chromatographed on a silica gel column (CH_2Cl_2/AcOEt, 1:1, v/v, R_f 0.55) to afford 0.43 g (86%) of product: mp 92–94 °C; ^1H NMR ($CDCl_3$) δ 2.42 (s, 3H, $COCH_3$), 2.80 (s, 3H, 5-CH_3), 2.95 (s, 3H, 1-CH_3), 7.45 (m, 2H, 6-H, 8-H), 7.96 (d, 1H, 7-H), 8.15 (d, 1H, 3-H). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

4-Acetoxy-5-methylisoquinoline-1-carboxaldehyde (32). This compound was prepared from **29a** (0.40 g, 1.9 mmol) by the procedure employed for the synthesis of **7a**: yield 0.21 g (49%); mp 127–128 °C; TLC R_f 0.50 (CH_2Cl_2/AcOEt, 1:1, v/v); ^1H NMR ($CDCl_3$) δ 2.45 (s, 3H, $COCH_3$), 2.80 (s, 3H, 5-CH_3), 7.55 (m, 2H, 7-H, 8-H), 8.50 (s, 1H, 3-H), 9.35 (m, 1H, 6-H), 10.45 (s, 1H, CHO). Anal. ($C_{13}H_{11}NO_3$) C, H, N.

4-Acetoxy-5-methylisoquinoline-1-carboxaldehyde Thiosemicarbazone (33). This compound was prepared from **32** (0.29 g, 1.3 mmol) by the procedure employed for the synthesis of **9a**: yield 0.30 g (79%); mp 230–231 °C dec; MS *m/e* 303 ($M^+ + 1$); ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H, $COCH_3$), 2.85 (s, 3H, 5-CH_3), 7.70 (d, 1H, 8-H), 7.85 and 8.65 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.00 (m, 1H, 6-H), 8.45 (s,

1H, 1-CH), 8.55 (s, 1H, 3-H), 9.15 (m, 1H, 6-H), 11.95 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{14}H_{14}N_4O_2S$·HCl·H_2O) C, H, N.

4-Hydroxy-5-methylisoquinoline-1-carboxaldehyde Thiosemicarbazone (34). A mixture of **33** (0.30 g, 1 mmol), 2 mL of concentrated hydrochloric acid, and 8 mL of 50% ethanol was refluxed for 2 h, and the precipitate was filtered after cooling. The yellow solid was recrystallized from an aqueous ethanol solution (1:1, v/v) containing 5% concentrated HCl to afford 0.23 g (88%) of product: mp 275–277 °C. The hydrochloride was stirred in 10% sodium bicarbonate to yield the free base: mp 270–272 °C dec; ^{1}H NMR (500 MHz, Me_2SO-d_6) δ 2.91 (s, 3H, 5-CH_3), 7.85 (m, 2H, 7-H, 8-H), 8.11 (s, 1H, 3-H), 8.27 (d, 1H, 6-H, $J_{6,7}$ = 8.3 Hz), 8.79 (s, 1H, 1-CH), 8.85 and 8.90 (2 s, 2H, $CSNH_2$, D_2O exchangeable), 12.10 (s, 1H, NNH, D_2O exchangeable), 12.50 (s, 1H, 4-OH, D_2O exchangeable). Anal. ($C_{12}H_{12}N_4OS$·$2H_2O$) C, H, N.

1,4-Dimethylisoquinoline-5-sulfonic Acid (36). 1,4-Dimethylisoquinoline (**35**; 1.2 g, 7.6 mmol) was added dropwise to 12 mL of fuming (20%) sulfuric acid at 0–5 °C with stirring. The reaction mixture was stirred at 45–50 °C for 2 h, maintained at room temperature overnight, and then poured into 100 g of crushed ice. The resulting suspension was adjusted to pH 4 with 20% sodium hydroxide solution and cooled. The remaining precipitate of the sulfonic acid derivative was collected by filtration, washed with water and then ethanol, and dried to give 1.8 g (93%) of product: mp >300 °C; ^{1}H NMR (D_2O) δ 2.60 (s, 3H, 4-CH_3), 2.93 (s, 3H, 1-CH_3), 7.80–7.95 (m, 2H, 6-H, 7-H), 8.50–8.62 (m, 2H, 3-H, 8-H). Anal. ($C_{11}H_{11}NO_3S$·$1.4H_2O$) C, H, N.

1,4-Dimethyl-5-hydroxyisoquinoline (37). A mixture of **36** (4.3 g, 18 mmol), potassium hydroxide (17 g), and 10 mL of water was heated gradually to 290 °C and maintained at 290–300 °C for 15 min. The mixture was stirred vigorously; during the fusion, the color of the mixture became dark brown and frothing occurred. The cooled reaction mixture was dissolved in water (100 mL) and filtered. The filtrate was adjusted to pH 1–2 with 10% hydrochloric acid and filtered again. The resulting filtrate was alkalinized with sodium bicarbonate to precipitate the product, which was collected by filtration and washed with water. The crude product was recrystallized from ethanol to yield 1.3 g (42%) of white crystals: mp 280–282 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.58 (s, 3H, 4-CH_3), 2.85 (s, 3H, 1-CH_3), 7.32 (m, 2H, 6-H, 8-H), 7.90 (m, 2H, 3-H, 8-H), 10.10 (br s, 1H, 5-OH, D_2O exchangeable). Anal. ($C_{11}H_{11}NO$) C, H, N.

5-Acetoxy-1,4-dimethylisoquinoline (38). This compound was prepared from **37** (1.2 g, 6.9 mmol) by the procedure employed for the synthesis of **29a** from compound **30**: yield 0.80 g (54%); mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, $COCH_3$), 2.50 (s, 3H, 4-CH_3), 2.85 (s, 3H, 1-CH_3), 7.40 (d, 1-H, 8-H), 7.80 (m, 2H, 6-H, 7-H), 8.20 (s, 1H, 3-H). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

5-Acetoxy-4-methylisoquinoline-1-carboxaldehyde (39). This compound was prepared from **38** (0.80 g, 3.2 mmol) by the procedure employed for the synthesis of **7a**: yield 0.64 g (75%); mp 150–151 °C; TLC R_f 0.75 (CH_2Cl_2/AcOEt, 1:1, v/v); ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, $COCH_3$), 2.70 (s, 3H, 4-CH_3), 7.55 (d, 1H, 8-H), 8.00 (d, 1H, 7-H), 8.60 (s, 1H, 3-H), 9.10 (d, 1H, 6-H), 10.35 (s, 1H, CHO). Anal. ($C_{13}H_{11}NO_3$) C, H, N.

5-Acetoxy-4-methylisoquinoline-1-carboxaldehyde Thiosemicarbazone (40). This compound was prepared from **39** (0.23 g, 1.0 mmol) by the procedure employed for the synthesis of **9a**: yield 0.24 g (80%); mp 224–225 °C dec; MS *m/e* 303 (M^+ + 1); ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, $COCH_3$), 2.65 (s, 3H, 4-CH_3), 7.70 (d, 1H, 8-H), 7.75 and 8.60 (2 br s, 2H, $CSNH_2$, D_2O exchangeable), 8.20 (d, 1H, 6-H), 8.45 (s, 1H, 1-CH), 8.55 (s, 1H, 3-H), 9.15 (m, 1H, 7-H), 11.95 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N.

5-Hydroxy-4-methylisoquinoline-1-carboxaldehyde Thiosemicarbazone (41). This compound was prepared from **40** (0.23 g, 0.7 mmol) by the procedure employed for the synthesis of **34**: yield 0.16 g (90%); mp 258–259 °C dec; MS *m/e* 261 (M^+ + 1); ^{1}H NMR (500 MHz, Me_2SO-d_6) δ 2.56 (s, 3H, 5-CH_3), 7.36 (dd, 1H, 7-H, $J_{7,8}$ = 9.1 Hz, $J_{6,7}$ = 2.5 Hz), 7.46 and 8.48 (2 s, 2H, $CSNH_2$, D_2O exchangeable), 7.97 (d, 1H, 8-H, $J_{7,8}$ = 9.1 Hz), 8.24 (s, 1H, 3-H), 8.58 (d, 1H, 6-H, $J_{6,7}$ = 2.5 Hz), 8.60 (s, 1H, 1-CH), 10.37 (s, 1H, 5-OH, D_2O exchangeable), 11.7 (s, 1H, NNH, D_2O exchangeable). Anal. ($C_{12}H_{12}N_4OS$·$0.5H_2O$) C, H, N.

Measurement of the Rate of Glucuronidation. Measurement of the capacity of methyl groups substituted adjacent to the hydroxyl functions on the rate of glucuronidation of the hydroxyl group by bovine UDP-glucuronyltransferase was conducted by a modification of the method of Mulder and Van Doorn.[15] Because of a large interfering absorption by the α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, these compounds could not be tested directly by this method. However, model compounds lacking the thiosemicarbazone functionality were readily evaluable. The assay is based upon the enzymatic determination of UDP generated during the glucuronidation reaction (reaction 1):

$$\text{ROH} + \text{UDP-glucuronate} \xrightarrow{\text{UDP-glucuronyltransferase}} \text{R-O-glucuronide} + \text{UDP} \quad (1)$$

$$\text{UDP} + \text{phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{UTP} + \text{pyruvate} \quad (2)$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}^+ \quad (3)$$

The conversion of NADH into NAD^+ was followed spectrophotometrically at 340 nM using a Beckman model 25 spectrophotometer. An extinction coefficient of 6.22×10^6 cm^2 mol^{-1} for NADH was used to calculate the rate of glucuronidation. The procedure was as described by Mulder and Van Doorn[13] with the following modifications: The agents to be tested were dissolved in dimethyl sulfoxide instead of ethanol, and 10 μL of this solution was added per milliliter of assay mix to give final concentrations of 0.5 and 0.1 mM of hydroxyl-substituted pyridine and isoquinoline derivatives, respectively. The microsomal protein was replaced with 1 mg/mL of Sigma bovine liver UDP-glucuronyltransferase, and the assay was carried out at 30 °C instead of 29 °C.

Acknowledgment. This research was supported in part by United States Public Health Service Research Grant CA-53340 from the National Cancer Institute. We wish to thank Ms. Regina Loomis for her excellent technical assistance.

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JM950458P