Potential Antitumor Agents. 13. 4-Methyl-5-amino-1-formylisoquinoline Thiosemicarbazone

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4-Methyl-5-amino-1-formylisoquinoline thiosemicarbazone has been synthesized in an attempt to obtain (a) high affinity for the target enzyme ribonucleotide reductase, (b) water solubility as an acid salt of the amine, (c) steric protection of the amino group from in vivo acetylation, and (d) insensitivity to O-glucuronidation, a major factor in inactivity in man of 5-hydroxy-2-formylpyridine thiosemicarbazone. The synthesis was achieved by nitration of 1,4-dimethylisoquinoline at the 5 position followed by selective oxidation with selenium dioxide to the corresponding 1-carboxaldehyde. The aldehyde group was protected by conversion to the cyclic ethylene acetal which was then catalytically reduced to produce the 5-amino derivative. Reaction with thiosemicarbazide in the presence of hydrochloric acid yielded the desired derivative. This agent was found to be an effective antineoplastic agent in mice bearing Sarcoma 180 ascites cells and at the maximum effective daily dose of 10 mg/kg increased the average survival of animals threefold over untreated tumor-bearing controls.

The 5-amino derivative of 1-formylisoquinoline thiosemicarbazone (IQ-1) was initially synthesized to explore the relationship between ring substitution of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones and antineoplastic activity.[1] The insertion of the electron-donating NH_2 group onto position 5 of the IQ-1 structure resulted in a derivative capable of prolonging the life-span of tumor-bearing animals to essentially the same extent as that produced by the parent compound.[1] In addition, this modification yielded a compound which could be solubilized in water as a salt of an appropriate acid, a property essential for the ultimate formulation of heterocyclic carboxaldehyde thiosemicarbazones for parenteral administration to patients with cancer. Sodium salts of hydroxylated derivatives have been synthesized to achieve solubilization,[2] and 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP) was selected as the first representative of this class of compounds for human trial as an antineoplastic agent.[3,4] 5-HP, however, proved to have little utility in man due in part to (a) rapid metabolism and excretion as an *O*-glucuronide[3] and (b) relatively low inhibitory potency for the enzyme ribonucleoside diphosphate reductase, blockade of which appears to be essential for antineoplastic activity.[5] We have recently reported[6] that the 5-amino derivative of IQ-1 was about 100 times more potent than 5-HP as an inhibitor of ribonucleoside diphosphate reductase. Since 5-amino-IQ-1 is not susceptible to O-glucuronidation, it appeared to possess the necessary requisite properties for consideration for clinical trial. However, we have found that the 5-acetylamino derivative of IQ-1 is completely devoid of carcinostatic activity.[1] Since N-acetylation is a relatively ubiquitous metabolic reaction in vivo, the objective of this study was to alter the molecule in a manner which served to protect the 5-NH_2 group from metabolic inactivation. This goal was achieved by creating steric hinderance in the vicinity of the NH_2 function by inserting a CH_3 group at the 4 position of the isoquinoline ring. Such a substitution resulted in a marked decrease in enzymatic acetylation, as demonstrated in vitro employing a rat liver homogenate with labeled acetyl coenzyme A as the substrate.[7] In this paper we report the synthesis and antineoplastic activity of 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone (8).

Chemistry. The synthesis of 1,4-dimethylisoquinoline (3) was carried out utilizing the Bischler–Napieralski reaction[8] which was modified by use of polyphosphoric acid instead of $POCl_3$ as the dehydrating reagent. The employment of this modification resulted in an increase in the yield of 3 from 31 to 70%. Spath et al.[9] have reported a yield of 81% utilizing P_2O_5 in boiling tetralin; however, we were unable to obtain this high yield. The required starting material, *N*-acetyl-2-phenylpropylamine (1), for this reaction was synthesized from commercially available 3-phenylbutyric acid which was converted to an acid chloride using thionyl chloride followed by reaction with NH_4OH to give 3-phenylbutyramide. This amide was subjected to a Hoffman reaction to yield 2-phenylpropylamine which on acetylation produced 1. 1,4-Dimethyl-3,4-dihydroisoquinoline (2) generated from the dehydration of 1 was then dehydrogenated with diphenyl disulfide as reported earlier.[10] Nitration of 3 produced only one compound, 1,4-dimethyl-5-nitroisoquinoline (4, Scheme I), analogous to the nitration of 1-methylisoquinoline.[1] Although direct oxidation of the 1-CH_3 group of compound 3 with SeO_2 to the carboxaldehyde resulted in poor yields,[10] compound 4 could selectively be oxidized with SeO_2 to yield the corresponding 1-carboxaldehyde in greater than 70% yield. In order to reduce the NO_2 group in compound 5 to an amino function, the aldehyde was first protected by conversion to the cyclic ethylene acetal 6, which was then reduced by catalytic hydrogenation using Pd/C to yield the corresponding amino acetal 7. Compound 7 was then allowed to react with thiosemicarbazide in the presence of concentrated HCl to form the desired thiosemicarbazone hydrochloride; the free base (8) was liberated by treatment with Na_2CO_3 solution.

Biological Results and Discussion. The tumor-inhibitory properties of compound 8, the parent compound IQ-1 and its 5-amino derivative, and the clinically tested agent of this class 5-HP were determined by measuring their effects on the survival time of mice bearing Sarcoma 180 ascites cells; the results are shown in Table I. The 5-nitro derivative 9 was found to be completely devoid of carcinostatic activity and is not included in Table I. The maximum increase in the average survival time of tumor-bearing mice produced by the optimum dosage of each compound is listed; however, a wide range of daily dose levels from 5 to 100 mg/kg was tested for each agent. The isoquinoline derivatives, IQ-1, its 5-amino analogue, and compound 8, were more effective than 5-HP in prolonging the life-span of mice bearing this neoplasm. Compound 8 has an advantage over IQ-1 in that it can be formulated for use in man by solubilization in water as an acid salt. In addition, we have evidence[7] that compound 8, in contrast to 5-amino-1-formylisoquinoline thiosemicarbazone, was only minimally susceptible to enzymatic acetylation of the 5-NH_2 group, a reaction which might convert 5-amino-1-formylisoquinoline thiosemicarbazone in vivo to the 5-acetylamino derivative, an agent which has

Scheme I

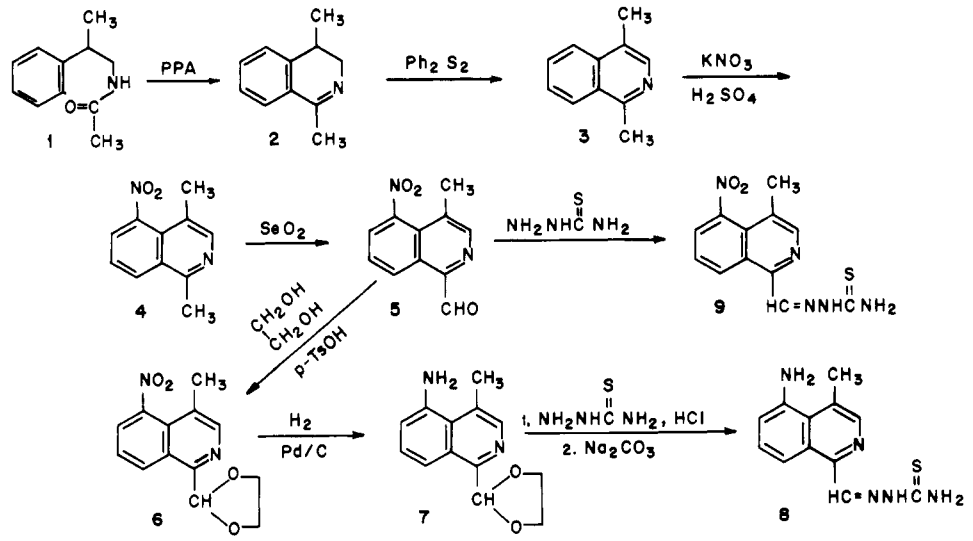

Table I. Comparative Effects of Thiosemicarbazones of 4-Methyl-5-amino-1-formylisoquinoline, 5-Amino-1-formylisoquinoline, 1-Formylisoquinoline, and 5-Hydroxy-2-formylpyridine on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells

Compd	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival time, days ± SE	50-Day survivors[c]	% T/C[d]
None		+18.2	12.6 ± 0.6	0/30	100
5-HP[e]	60	−3.3	30.4 ± 2.0	3/15	241
IQ-1[f]	40	−5.1	39.2 ± 3.0	3/10	311
5-NH_2-IQ-1[g]	40	−8.5	37.3 ± 3.2	3/10	296
8	10	−4.8	38.2 ± 2.8	3/10	303

[a] Administered once daily for six consecutive days, beginning 24 h after tumor implantation. Dose levels were administered in the range of 5–100 mg/kg per day for each compound; only the maximum effective daily dose is shown in the table. [b] Average weight change from onset to termination of drug treatment. [c] The number of tumor-bearing animals that survived at least 50 days; these mice were calculated as 50-day survivors in the determination of average survival time. [d] % T/C = (treated/control) × 100. [e] 5-Hydroxy-2-formylpyridine thiosemicarbazone. [f] 1-Formylisoquinoline thiosemicarbazone. [g] 5-Amino-1-formylisoquinoline thiosemicarbazone.

been shown by us to be completely devoid of antitumor activity.[1] The enzymatic data demonstrating steric protection of the 5-amino group from acetylation by the presence of a methyl group in the 4 position will be described elsewhere. Furthermore, compound 8 has been evaluated as an inhibitor of the target enzyme ribonucleoside diphosphate reductase and was found to be 60-fold more potent as an inhibitor of this enzyme from a rat hepatoma than was 5-HP[7] and twice as effective as 4-(*m*-amino)phenyl-2-formylpyridine thiosemicarbazone, an agent reported earlier from this laboratory as a candidate for clinical trial.[11] Compound 8 appears to be superior in its properties to any other agent in this series of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones reported to date and, therefore, possesses requisite properties for consideration as a second generation drug of this class for trial against cancer of man.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected. The ir absorption spectra were obtained with a Perkin-Elmer Model 257 spectrophotometer with thin films of liquids and KBr pellets of solids. NMR spectra were determined with a Varian T-60A spectrophotometer with Me_4Si as an internal standard. The spectral data were as expected and, therefore, only representative findings are included. Elemental analyses were performed by the Baron Consulting Co., Orange, Conn. Where analyses are indicated only by symbols of the element, the analytical results for those elements were within ±0.4% of the theoretical values.

Antitumor Activity. Experiments were performed on adult female CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Mass.). Transplantation of Sarcoma 180 ascites cells was carried out using a donor mouse bearing a 7-day tumor growth; the experimental details have been described earlier.[2] The percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Dose levels were administered in a range of 5–100 mg/kg/day for six consecutive days for each compound. Determination of the sensitivity of Sarcoma 180 ascites cells to these agents was based on the prolongation of survival time afforded by the drug treatments.

1,4-Dimethylisoquinoline (3). To a solution of 3-phenylbutyric acid (82 g, 0.5 mol) in 300 ml of benzene was added slowly 45 ml of thionyl chloride. The solution was refluxed for 2 h using a solution of NaOH as a gas trap. Excess thionyl chloride and benzene were removed under vacuum. The residual oil was added slowly through a dropping funnel to 400 ml of cold NH_4OH while stirring. The white precipitate which formed was filtered, washed with water, and dried to yield 70 g of crude amide. This material was added in small portions to a solution of sodium hypobromite obtained by dissolving 60 g of NaOH in 600 ml of water, cooling to 10°, and adding dropwise 20 ml of bromine. When the amide was completely dissolved the temperature was raised to 80° for 1 h and then an additional amount (120 ml) of 50% NaOH solution was added carefully. The resulting solution was again heated at 80° for 2 h. After cooling the oily layer of liberated amine was extracted with Et_2O (3 × 250 ml), dried ($MgSO_4$), filtered, and evaporated to leave an oil which was distilled, bp 68–73° (1 mm), to yield 40.6 g (70%).

To a solution of the above amine (40.6 g, 0.3 mol) in 150 ml of benzene was added acetic anhydride (31.5 ml, 0.3 mol) dropwise. The reaction mixture was refluxed for 2 h, benzene was removed under vacuum, and the remaining oil was distilled, bp 133–136° (0.1 mm), to yield 56.6 g (96%) of the acetamide derivative 1.

Compound 1 (35.4 g, 0.2 mol) was then heated with 275 g of polyphosphoric acid at 180° for 3 h. The reaction mixture was carefully added to ice water and filtered, and the filtrate was neutralized with a NaOH solution (20%). The resulting oil was extracted with Et_2O (3 × 300 ml), the ether layer was dried ($MgSO_4$), and the solvent was removed under vacuum. The residual oil 2 was distilled, bp 75–80° (0.1 mm), to yield 22.6 g (71%). The dehydrogenation was then carried out by heating

2 (15.9 g, 0.1 mol) in 80 ml of tetralin with 21.8 g (0.1 mol) of diphenyl disulfide at 200° with stirring. The thiophenol formed during the reaction was continuously allowed to distill over. After the reaction was complete (about 4 h), tetralin was removed by distillation using a water aspirator. The remaining oil, 3, was then distilled, bp 78–82° (0.05 mm), to yield 13.8 g (89%).

1,4-Dimethyl-5-nitroisoquinoline (4). A solution of 10.1 g (0.1 mol) of potassium nitrate in 50 ml of concentrated H_2SO_4 was added slowly from a dropping funnel to a solution of 15.7 g (0.1 mol) of 3 in 60 ml of concentrated H_2SO_4 kept at 0°. After the addition, the mixture was heated at 60° for 2 h and then poured slowly over crushed ice. The solution was made alkaline with NH_4OH; the resulting yellow precipitate was filtered, washed with water, dried, and crystallized from ethanol to yield 15.6 g (77%), mp 150–151°. Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

4-Methyl-5-nitroisoquinoline-1-carboxaldehyde (5). A solution of 10.1 g (0.05 mol) of 4 in 200 ml of dioxane was treated with 5.55 g (0.05 mol) of selenium dioxide (freshly resublimed) and the mixture was refluxed for 3 h. The precipitated selenium was removed by filtration, and the filtrate was flash evaporated. The residue was dissolved in dilute HCl and filtered, the filtrate was brought to pH 1.5 with solid $NaHCO_3$, and the mixture was again filtered through Celite. The clear solution was then alkalinized with a solution of Na_2CO_3. The precipitate was filtered, washed with water, dried, and crystallized from benzene and cyclohexane to yield 7.6 g (71%), mp 141–143°. Anal. ($C_{11}H_8N_2O_3$) C, H, N.

4-Methyl-5-nitro-1-formylisoquinoline Ethylene Acetal (6). To 10.8 g (0.05 mol) of 5 in 300 ml of benzene was added 0.5 g of *p*-toluenesulfonic acid and 10 ml of ethylene glycol. The mixture was refluxed with stirring for 24 h using a Dean-Stark trap to remove the water formed during condensation. The mixture was then washed with 25 ml of 10% $NaHCO_3$ solution followed by 25 ml of water. The benzene layer was dried ($MgSO_4$) and removed under vacuum, and the residue was recrystallized from ethanol to yield 10.3 g (87%), mp 114–115°. Anal. ($C_{13}H_{12}N_2O_4$) C, H, N.

4-Methyl-5-amino-1-formylisoquinoline Ethylene Acetal (7). Compound 6 (6.4 g, 0.025 mol) was dissolved in 250 ml of ethanol and 0.65 g of Pd/C (10%) was added. The mixture was hydrogenated at 50 psi for 1 h and then filtered to remove the catalyst. The ethanol was removed under vacuum and the residue was recrystallized from benzene to yield 4.6 g of 7 (80%), mp 130–131°. Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

4-Methyl-5-amino-1-formylisoquinoline Thiosemicarbazone (8). To 2.3 g (0.01 mol) of 7 in 50 ml of ethanol was added 5 ml of concentrated HCl and 0.91 g (0.01 mol) of thiosemicarbazide. The mixture was refluxed for 1.5 h; the precipitate of the hydrochloride salt of the desired compound was collected by filtration, washed with ethanol, and dried. The hydrochloride salt was then dissolved in 500 ml of boiling water and filtered into a flask containing 50 ml of 10% Na_2CO_3 solution. The yellow precipitate which formed was filtered, washed with water and ethanol, and dried to yield 2.2 g (81%), mp 216–218° dec. Anal. ($C_{12}H_{13}N_5S$) C, H, N.

4-Methyl-5-nitro-1-formylisoquinoline Thiosemicarbazone (9). To 1.08 g (0.05 mol) of compound 5 in 20 ml of ethanol was added a solution of 0.46 g (0.05 mol) of thiosemicarbazide dissolved in 10 ml of H_2O. The resulting solution was heated for 5 min and then cooled. The yellow precipitate was filtered, washed with water and ethanol, and then dried to yield 1.2 g (83%), mp 233–234° dec. Anal. ($C_{12}H_{11}N_5O_2S$) C, H, N.

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(Vinylaryloxy)acetic Acids. A New Class of Diuretic Agents. 4. Various [(2-Substituted and 2,2-disubstituted vinyl)aryloxy]acetic Acids

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A variety of [(2-substituted and 2,2-disubstituted vinyl)aryloxy]acetic acids was synthesized in which the substituents were primarily electron-withdrawing groups. These compounds were tested in dogs for their saluretic and diuretic properties. Many of the compounds exhibited significant activity; however, they were generally less potent than those reported in the three earlier papers in this series.

The three earlier papers in this series disclosed 2,2-diacylvinyl- (**1a**),[1] 2-acylvinyl- (**1b**),[2] and 2-nitrovinyl- (**1c**)[3] substituted aryloxyacetic acids, many of which possess a high order of saluretic and diuretic activity. These studies prompted the extension of the investigation to include other [(2-substituted and 2,2-disubstituted vinyl)aryloxy]acetic acids, in which the substituents are electron-withdrawing groups such as carboxy, carbethoxy, carbamoyl, cyano, sulfamoyl, and alkylsulfonyl.

Chemistry. The [(2,2-disubstituted vinyl)aryloxy]acetic acids presented in Table I were generally prepared by the process outlined below involving the ammonium acetate or piperidine acetate catalyzed Knoevenagel condensation of ethyl formylaryloxyacetates (**2a**) or their corresponding