and Et_2O for 0.5 h. The Et_2O layer was separated and the aqueous portion was extracted with Et_2O. The combined Et_2O extracts were washed with H_2O and saturated NaCl solution and dried (Na_2SO_4). After concentration in vacuo, the residual oil was redissolved in anhydrous Et_2O and treated with a solution of 3.0 g of maleic acid in MeOH. The light brown solid was collected and recrystallized from EtOH–Et_2O to yield 6.8 g (62%): mp 161.5–163.5 °C. Anal. ($C_{27}H_{30}N_3O_6F_3$) C, H, N.

2-Benzylthio-6-methoxy-8-nitroquinoline (9a). A mixture of 12.4 g (0.1 mol) of benzyl mercaptan (**8**, α-toluenethiol), 24.0 g (0.1 mol) of **1**, 11.0 g (0.11 mol) of triethylamine, and 100 mL of DMF was heated at 100 °C for 48 h. The dark solution was poured into ice–H_2O. A brownish solid was collected and recrystallized from benzene as a yellow brown material: mp 132–134 °C (66% yield). Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N, S.

Compound **9b** was prepared similarly (Table I).

2-Benzylthio-6-methoxy-8-aminoquinoline (10a). A mixture of 16.3 g (0.05 mol) of **9a**, 150 mL of H_2O, 2.5 mL of HOAc, and 17.5 g of iron fillings was stirred for 24 h at 80–90 °C.[9] The mixture was filtered and washed with warm H_2O and Me_2CO. The aqueous Me_2CO solution was extracted with Et_2O, dried (Na_2SO_4), treated with charcoal, and concentrated in vacuo. The solid residue was covered with petroleum ether, collected and recrystallized from EtOH–H_2O. The product (14.5 g, 98%) melted at 92–94 °C. Anal. ($C_{17}H_{16}N_2OS$) C, H, N, S.

Compound **10b** was obtained by reduction of **9b** with alcoholic hydrazine hydrate and 10% Pd/C[8] (Table I).

2-Benzylthio-6-methoxy-8-(4-amino-1-methylbutylamino)quinoline Maleate (12a). Compound **12a** (and **12b**) was obtained by the alkylation[11] and hydrazinolysis[12] method employed for **7a–f** (Table I).

2-(*p*-Chlorobenzyloxy)-6-methoxy-8-(4-phthalimido-1-methylbutyl)aminoquinoline (6d). In only one case was the intermediate phthalimido derivative **6** isolated.

A mixture of 6.3 g (0.2 mol) of **4d**, 6.0 g (0.02 mol) of **5**, 8.2 g (0.1 mol) of NaOAc, and 150 mL of 66% EtOH–H_2O was refluxed for 96 h.[10] On the third day, 0.03 mol of **5** and 0.1 mol of NaOAc were added to the reaction mixture; on the fourth day 0.1 mol of NaOAc was added. At the end of the reflux period the mixture was saturated with K_2CO_3 and EtOH removed in vacuo. The mixture was diluted with H_2O and extracted with Et_2O. The Et_2O was washed with H_2O and saturated NaCl solution, and the solution was dried ($MgSO_4$) and concentrated in vacuo. The residual oil was dissolved in 200 mL of MeOH and treated with excess 48% HBr. Dilution of this mixture with anhydrous Et_2O (300 mL) precipitated 4.5 g (47%) of salt which proved to be **4d**. Further dilution with Et_2O to a total volume of 1 L, and cooling for 2.5 days, gave 3.4 g (25%) of a salt (**6d**·HBr). The free base **6d** was liberated by aqueous Na_2CO_3 and recrystallized from EtOH: mp 94.5–96 °C. Anal. ($C_{30}H_{28}N_3O_4Cl$) C, H, N, Cl.

Isolation of **6d** in the triethylamine method[11] resulted in a 69% yield of the free base as compared to the 20–25% yields obtained by the NaOAc buffer method.[10]

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Synthesis of 5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones as Potential Antitumor Agents

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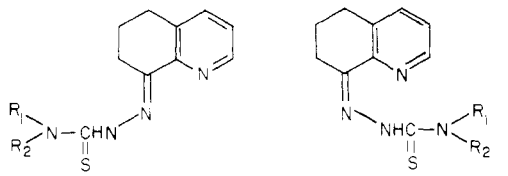
5,6-Dihydro-8(7*H*)-quinolinone was synthesized and converted into thiosemicarbazones which could be considered to be semirigid analogues of the 2-formylpyridine thiosemicarbazone class of antitumor agents. The *Z* and *E* isomers were separated and identified by ^1H NMR and UV. Although the compounds showed essentially no inhibitory activity against the enzyme alkaline phosphatase, several of these agents had demonstrable anticancer activity in mice bearing the P388 leukemia. The *E*-configuration analogues in general were slightly more active than their corresponding *Z* isomers.

Since the discovery by Brockman et al.[1] that 2-formylpyridine thiosemicarbazone (PT, **1**) had antileukemic activity in mice, a large number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones have been synthesized

Table I. 5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones

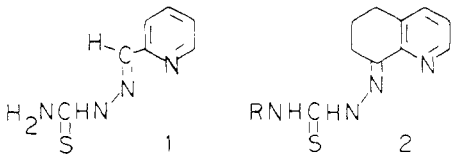


2*E* 2*Z*

Compd	R_1	R_2	Confign	^{1}H NMR[a] 2′-NH	^{1}H NMR[a] 4′-NH	Mp, °C	Yield, %	Formula[b]
2a	H	H	*Z*	14.32	8.1-8.5	187-190[c]	43	$C_{10}H_{12}N_4S$
2b	H	H	*E*	10.33	7.8-8.2	184-185[c]	20	$C_{10}H_{12}N_4S$
2c	CH_3	H	*Z*	14.55	6.6-7.6	140-141[d]	38	$C_{11}H_{14}N_4S$
2d	CH_3	H	*E*	8.95	8.8-9.3	134-136[c]	19	$C_{11}H_{14}N_4S$
2e	CH_2=$CHCH_2$	H	*Z*	14.68	7.8-8.0[e]	95-96[f]	39	$C_{13}H_{16}N_4S$
2f	CH_2=$CHCH_2$	H	*E*	8.89	8.0-8.3[e]	127-132[c]	16	$C_{13}H_{16}N_4S$

[a] All spectra were run in $CDCl_3$ with chemical shifts reported in parts per million downfield from an internal standard of Me_4Si. [b] All compounds were analyzed for C, H, N, and S and analytical results were within 0.4% of theory. [c] From C_6H_6. [d] From C_6H_6-c-C_6H_{12}. [e] Spectra were run in Me_2SO-d_6. [f] From c-C_6H_{12}.

and evaluated as antineoplastic agents.[2] It is generally accepted that the mechanism of the cytotoxic action of this class of agents involves the inhibition of ribonucleoside diphosphate reductase, an enzyme which catalyzes the conversion of ribonucleotides to deoxyribonucleotides. The thiosemicarbazones coordinate with ferrous ions through the conjugated N*–N*–S* tridentate ligand thus blocking the enzyme.[2] In addition, these compounds have been employed to inhibit the enzyme alkaline phosphatase through coordination of the essential cofactor zinc.[3]



1 2

In order to study the conformational aspects of the formylthiosemicarbazone moiety and the resulting biological activity, semirigid forms of PT were synthesized. The compounds employed were the thiosemicarbazones of 5,6-dihydro-8(7*H*)-quinolinone (**2**). Studies of the relationship between structure and activity with PT have indicated that the introduction of a Me group at the 3 position of PT enhances antitumor activity,[4] while substitution on the carbonyl carbon results in a loss of activity.[5] For this reason, alkyl substitution at the 3 position of compounds with structure **2** may be favorable for antitumor activity, while the presence of the ketone would be expected to decrease activity. However, it is possible that the decreased number of rotamers will offset the decreased activity.

Chemistry. 5,6,7,8-Tetrahydroquinoline (**3**) was prepared from 3-aminoacrolein and cyclohexanone[6] or from quinoline.[7] Although the direct oxidation of **3** to **6** with selenium dioxide[8] was not successful, the stepwise oxidation to **4** followed by rearrangement to **5** and oxidation to **6** resulted in the desired products (see Scheme I).[9,10] The formation of thiosemicarbazones of **6** resulted in recovery of two isomers, the major product (*Z*)-**2** and the minor one (*E*)-**2**. The assignment of the stereochemistry was based upon the x-ray single crystal structure of **2a**[11] and a characteristic chemical shift of the 2′-NH and its rate of exchange in the ^{1}H NMR spectrum. The 2′-NH is deshielded in the *Z* isomer and does not exchange readily, due to intramolecular bonding of this proton to the pair of electrons on the quinoline nitrogen. With the *E* isomer the 2′-NH is upfield and exchanges immediately (Table I). These results are in agreement with similar chemical shifts recently reported by Antonini and co-workers as concerns the *E* and *Z* configurations of PT and 1-formylisoquinoline thiosemicarbazone (IQ-1).[12] As with the 1-tetralone hydrazones,[13] the 7-H's were not well resolved and could not be used as an indication of configuration.

Scheme I

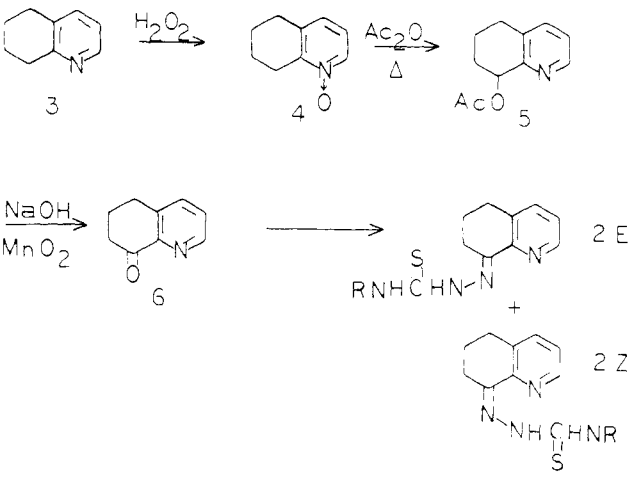


Table II. Ultraviolet Absorption Characteristics of 5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones

Compd	Confign	λ max, nm H_2O	λ max, nm C_6H_6	Δλ max
2a	*Z*	324	354	30
2b	*E*	321	327	6
2c	*Z*	324	353	29
2d	*E*	323	336	13
2e	*Z*	326	354	28
2f	*E*	325	336	11

The UV spectra of the various isomers also proved useful in structure assignment. With water as a solvent the λ max of (*E*)-**2** and (*Z*)-**2** were not significantly different, but with benzene as solvent the *Z* isomers showed large bathochromic shifts for λ max. This phenomenon can probably be accounted for by intramolecular H bonding in (*Z*)-**2** which would favor π-orbital overlap. No intramolecular H bonding is possible with (*E*)-**2** in either polar (H_2O) or nonpolar (C_6H_6) solvents and therefore no significant UV shift between solvents was observed (Table II).

Table III. Effect of 5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones on the Survival Time of Mice Bearing the P388 Leukemia

Compd	Daily dose, mg/kg[a]	Av change in body wt, %[b]	Av survival, days ± SE	% T/C[c]
Control		−2.4	11.2 ± 0.4	
2a	5	+6.7	13.0 ± 0.0	116
	10	−21.5	15.8 ± 0.4	141
	20	−30.8	9.2 ± 0.6	73
2b	2.5	−5.3	11.6 ± 0.5	104
	5	−3.8	11.8 ± 0.2	106
	10	−10.7	14.8 ± 0.2	132
2c	5	−7.7	12.0 ± 0.6	107
	10	−10.8	13.6 ± 0.8	121
	20		2.0 ± 0.0	18
2d	2.5	−9.3	13.4 ± 0.9	119
	5	−12.0	16.0 ± 0.0	143
	10		2.0 ± 0.0	18
2e	2.5	+4.6	10.2 ± 0.2	91
	5	0.0	9.8 ± 1.24	88
	10	−6.1	7.8 ± 0.8	70
2f	2.5	+0.7	11.2 ± 0.2	100
	5	−8.4	13.2 ± 0.2	118
	10	−9.1	14.0 ± 0.8	125

[a] Drugs were administered once daily for 6 consecutive days beginning 24 h after tumor implantation (1×10^6 cells/mouse). Each group contained 5–15 animals. [b] Average weight change from onset to termination of drug treatment. [c] T/C represents the ratio of the survival time of treated to control animals.

Biological Results. The effects of the 5,6-dihydro-8(7*H*)-quinolinone thiosemicarbazones on the survival time of mice bearing the P388 leukemia are shown in Table III. Compounds **2a,b,d,f** had demonstrable anticancer activity against P388 leukemia, although the therapeutic index was low as evidenced by the extensive loss in body weight of mice receiving these agents and the small dosage range over which the compounds were active. The data suggest, however, that the *E* configuration (**2b,d,f**) is slightly more active than the *Z* configuration with respect to anticancer activity. (*Z*)-PT and (*Z*)-IQ-1 are reported to be quite unstable and isomerize rapidly to their respective *E* isomers.[12] We have independently confirmed these results in polar solvents. Although **2a,c,e** are readily isomerized to **2b,d,f**, respectively, in ethanol with sodium ethoxide, the *Z* isomers appear to be stable under neutral conditions, but the possibility of some isomerization occurring in vivo cannot be ruled out.

Previous studies[3] had suggested that some substituted α-(N)-heterocyclic carboxaldehyde thiosemicarbazones were effective in inhibiting alkaline phosphatase activity and were considered as candidates to retard the degradation of the active nucleotide forms of the 6-thiopurines by this enzyme in drug-resistant cell lines. The test compounds **2a–f** were assayed against alkaline phosphatase prepared from *Escherichia coli* and were found to be inactive at concentrations up to 10^{-4} M.

Experimental Section

Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Satisfactory IR and ^{1}H NMR spectra were obtained for all compounds reported. IR spectra were recorded on a Perkin-Elmer Model 700 spectrophotometer, and ^{1}H NMR spectra were recorded on a Varian EM 360 spectrometer. UV data were obtained using a Beckman recording DB-GT spectrophotometer. Chromatography was performed with Brinkman silica gel. Enzyme inhibition studies were carried out using a Beckman DU-2 spectrophotometer.

Antitumor Activity. Experiments were performed on female CDF1 mice bearing the P388 leukemia. Animals were inoculated intraperitoneally with 1×10^6 cells 24 h prior to initiating therapy that was continued for 6 consecutive days at the indicated daily dosage levels. Mice were weighed daily and the percent weight change from onset to termination of therapy was determined and used as an indication of toxicity. The prolongation of the survival time of tumor-bearing animals was used as the measure of antineoplastic activity.

Enzyme Inhibition. The assay used to evaluate inhibitors of *E. coli* alkaline phosphatase was that used by Garen and Levinthal[14] with several modifications.[15] The reaction mixture contained 10^{-3} M *p*-nitrophenyl phosphate in 3.0 mL of 1 M Tris–HCl buffer (pH 9.0), 0.1 mL of inhibitor (in 50% dimethyl sulfoxide), and 0.1 mL of enzyme (0.021 unit) dissolved in 1 M Tris–HCl buffer (pH 9.0). One unit of alkaline phosphatase will hydrolyze 1 mol of *p*-nitrophenyl phosphate per minute at pH 9.0 and at 25 °C. The molar absorbancy of *p*-nitrophenol in 1 M Tris–HCl buffer, pH 9.0, is 1.57×10^4 (mol L^{-1})/cm. The reaction was initiated by adding substrate to a mixture of enzyme and test compound which had been allowed to incubate for 15 min at 37 °C. The reaction rate was determined spectrophotometrically by measuring the initial increase in absorption at 410 nm resulting from the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol. The 1-cm path length cell was maintained at room temperature. In all cases, controls consisted of incubating enzyme in the presence of 0.1 mL of 50% dimethyl sulfoxide for 15 min prior to adding substrate.

Chemistry. 5,6,7,8-Tetrahydroquinoline *N*-Oxide (4). A mixture of **3** (8.4 g, 0.063 mol), H_2O_2 (8.4 mL of 30%, 0.074 mol), and 25 mL of HOAc was heated at 70 °C for 3 h. An additional 8.4 mL of H_2O_2 was added and the mixture was heated at 70 °C for 24 h. The reaction mixture was concentrated in vacuo, made alkaline with 10% NaOH, and extracted with $CHCl_3$. The combined organic layer was dried ($MgSO_4$). Removal of the solvent, followed by recrystallization from Et_2O, gave 7.9 g (83%) of **4**, mp 77–79 °C.[9]

8-Acetoxy-5,6,7,8-tetrahydroquinoline (5). To **4** (7.0 g, 0.047 mol), maintained at 70 °C, was added acetic anhydride (10 g, 0.098 mol) with stirring. The temperature rose to 130 °C during the addition and was maintained at 100–110 °C for 10 h. The reaction mixture was concentrated, made alkaline with 10% NaOH, extracted with $CHCl_3$, and dried ($MgSO_4$). The residue (6.6 g) was subjected to chromatography on silica gel with C_6H_6–MeOH–Et_3N (93:4:3 v/v) as eluting solvent. The oily product was used without further purification in the next step.

8-Hydroxy-5,6,7,8-tetrahydroquinoline. A mixture of **5** (6.6 g, 0.034 mol) and 10% NaOH (60 mL) was heated at 70 °C for 2 h. The reaction mixture was extracted with Et_2O, dried ($MgSO_4$), and concentrated to give 4.0 g (77%) of **6**.[9]

5,6-Dihydro-8(7*H*)-quinolinone (6). A mixture of activated MnO_2[16] (19.9 g) and 8-hydroxy-5,6,7,8-tetrahydroquinoline (1.9 g, 0.013 mol) in $CHCl_3$ (200 mL) was heated under reflux for 7 h. The mixture was filtered and the MnO_2 was washed with Et_2O. The combined organic layer was decolorized with charcoal and dried ($MgSO_4$), and the residue was subjected to chromatography on silica gel using C_6H_6–MeOH–Et_3N (92:5:3 v/v) as eluting solvent. Unreacted starting material was the first product eluted from the column; this was followed by **6** (1.8 g) which was recrystallized from C_6H_6–Et_2O, mp 98–99.5 °C. Anal. (C_9H_9NO) C, H, N.

5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones (2a–f). The thiosemicarbazides (2 mol) were each dissolved in a minimum amount of 95% EtOH and added to a solution of **6** (1 mol) in 95% EtOH. The solution was heated under reflux for 2 h and concentrated, and the residue was subjected to chromatography on silica gel using MeOH–$CHCl_3$ (1:19, v/v) as eluting solvent. The first product from the column was the *Z* isomer, while the second product was considered to be the *E* isomer. Yields, recrystallizing solvents, and characterizing ^{1}H NMR absorption are reported in Table I.

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Synthesis and Determination of Antiviral Activity of the 2′(3′)-*O*-Methyl Derivatives of Ribavirin (1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide)

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Diazomethane treatment of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) in the presence of $SnCl_2$ as catalyst led to quantitative formation of the 2′-*O*-methyl and 3′-*O*-methyl derivatives of the parent compound. The products were successfully fractionated on a basic ion-exchange column and isolated in crystalline form. Identification was based on the elution sequence from the column and on ^1H NMR spectroscopy. Both derivatives were found to be inactive, relative to the parent compound, against several virus types in cell culture. Unlike ribavirin itself, the 2′(3′)-*O*-methyl derivatives did not suppress cellular DNA synthesis. NMR data showed that the loss of biologic activity upon 2′(3′)-*O*-methylation was not due to a change of conformation of the nucleoside sugar moiety.

Ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide,[1] originally referred to as "virazole", has been described as the first broad-spectrum antiviral agent which is not an interferon inducer.[2] Its spectrum encompasses nearly all major virus groups (adeno-, herpeto-, pox-, picorna-, toga-, orthomyxo-, paramyxo-, rhabdo-, and retroviridae). Particularly interesting are the inhibitory effects of ribavirin on influenza and parainfluenza virus replication,[2-7] both in cell cultures and in animals, including mice, hamsters, and ferrets.[7-10] The drug has been licensed for human use in several countries and is currently progressing through clinical trials.

It has been proposed that ribavirin inhibits virus multiplication via depletion of the GTP pool, although recent findings are at variance with this concept.[6] Ribavirin 5′-phosphate has, in fact, been found to competitively inhibit, in vitro, IMP dehydrogenase (IMP:NAD^+ oxidoreductase, E.C. 1.2.1.14),[11] presumably because of its structural resemblance to IMP and GMP.[12] If the mode of action of ribavirin in vivo does occur at the level of conversion of IMP to XMP, it must first be phosphorylated by the appropriate cellular kinase, probably deoxyadenosine kinase.[13]

With a view to further defining the molecular mechanism(s) involved in the antiviral activity of ribavirin, two new analogues have been prepared and their activities assayed in a number of virus cell systems, viz., 2′-*O*-methylribavirin (**1**) and 3′-*O*-methylribavirin (**2**) (Scheme I), both of which are theoretically capable of phosphorylation by cellular kinase(s).

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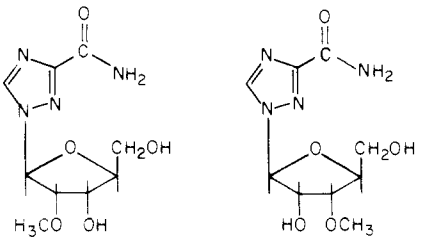
Scheme I



1 **2**

Chemistry. According to Robins et al.,[14] treatment of ribonucleosides with diazomethane in the presence of about 1 mM $SnCl_2 \cdot 2H_2O$ as catalyst leads to a quantitative conversion of the ribonucleosides to their 2′(3′)-*O*-methyl derivatives. The same treatment applied to ribavirin led to the formation of unidentified side products and incomplete etherification of the *cis*-hydroxyls. Subsequent trials demonstrated that, with a concentration of 5 mM catalyst in methanol and a molar ratio of ribavirin to catalyst of 4:1, formation of a mixture of the 2′- and 3′-*O*-methyl derivatives was quantitative. The course of the reaction was followed by TLC on silica gel.

The two monomethylated products **1** and **2** were then fractionated according to Dekker[15] on a Dowex (OH^-) column. Because of the strongly basic nature of this column, and the alkaline lability of the 3-carboxamide substituent, it was essential to achieve fractionation as rapidly as possible. The time required was brought down to 1 h with the use of a small column and a rapid elution rate; under these conditions, hydrolysis of the carboxamide substitutent to carboxyl was about 15%. Use of a larger