mp 233–234°. The salt (7.7 g) was suspended in MeOH–H_2O (1:1, 200 ml) and saturated aqueous $NaHCO_3$ solution was added at 0° until the mixture was alkaline. The resulting base **35** was collected, washed with H_2O, and dried to give 5.0 g (93.5%) of product: mp 138–140° (MeOH–H_2O). *Anal.* ($C_{12}H_{12}N_2O$) C, H.

5-Amino-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (36). To a suspension of **35** (0.2 g, 0.001 mol) in EtOH (2.0 ml) was added 25% NH_4OH (0.5 ml, 0.0014 mol) followed by 2.3 *N* HCl (0.5 ml, 0.01 mol). The mixture was refluxed for 0.5 hr, ether was added, and the resulting precipitate (200 mg, 98%) of the HCl salt was isolated. It had mp 233–235° (EtOH–EtOAc): nmr (DMSO) δ 5.03 (2, s, CH_2), 7.70 (4, m, aromatic H's), 10.0 (3, m, NH and NH_2). *Anal.* ($C_{10}H_{10}ClN_3$) H, Cl; C: calcd, 57.84; found, 57.43.

5-Hydrazino-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (38). A mixture of $H_2NNH_2 \cdot H_2O$ (3.0 g, 0.06 mol) and **35** (10.0 g, 0.05 mol) in MeOH (100 ml) was refluxed for 16 hr. Et_2O was added and the product (8.1 g, 87%) was obtained as a solid, mp 188–189°, after crystallization from an EtOH–$CHCl_3$–hexane mixture. The HCl salt had mp 310–312° (MeOH). *Anal.* ($C_{10}H_{11}ClN_4$) C, H, Cl.

5-(3′-Dimethylaminopropyl)-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (37). A mixture of **35** (5.0 g, 0.025 mol), 3-dimethylaminopropylamine (2.7 g, 0.026 mol), 2.3 *N* ethanolic HCl (12.5 ml, 0.029 mol), and EtOH (50 ml) was refluxed for 7 hr. Et_2O and excess ethanolic HCl were added to afford a precipitate of the **2**·HCl salt of the product (6.9 g, 84%). It had mp 289–290° after crystallization from EtOH–EtOAc. *Anal.* ($C_{15}H_{22}Cl_2N_4$) C, H, N.

4,6-Dihydro-8-methylpyrrolo[4,3,2-*de*][1,2,4]triazolo[3,4-*a*]isoquinoline (39). A mixture of acetylhydrazine (5.0 g, 0.0674 mol) and **35** (9.0 g, 0.0448 mol) was refluxed in anhydrous EtOH (140 ml) for 48 hr under N_2. The EtOH was partially removed and an ether–hexane mixture was added. The resultant solid (9.3 g) was crystallized from MeOH to afford the product (7.3 g, 77%), mp 314–315°. *Anal.* ($C_{12}H_{10}N_4$) C, H, N. By refluxing a mixture of **38**·HCl and Ac_2O for 2 hr and then pouring it into aqueous NaOH solution, compound **39** is also obtained.

5-(Isopropylidinehydrazino)-1,3-dihydropyrrolo[4,3,2-*de*]isoquinoline (40). The hydrazino derivative **38** (4.5 g, 0.0242 mol) was refluxed in Me_2CO (100 ml) for 10 min. Removal of Me_2CO and crystallization of the residue from a C_6H_6–MeOH–Et_2O mixture gave the product (3.0 g, 55%), mp 200–201°. *Anal.* ($C_{13}H_{14}N_4$) C, H, N.

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Potential Antitumor Agents. 10. Synthesis and Biochemical Properties of 5-*N*-Alkylamino-, *N,N*-Dialkylamino-, and *N*-Alkylacetamido-1-formylisoquinoline Thiosemicarbazones†

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In an attempt to exploit a postulated hydrophobic bonding region at the inhibitor binding site of ribonucleoside diphosphate reductase, several 5-substituted monoalkylamino, dialkylamino, and *N*-alkylacetamido derivatives of 1-formylisoquinoline thiosemicarbazone were prepared. Two of the derivatives demonstrated impressive antitumor activity against Sarcoma 180 ascites cells and several were potent inhibitors of the target enzyme, requiring concentrations in the range of 10^{-6}–10^{-8} *M* for 50% inhibition. 5-Methylamino-1-formylisoquinoline thiosemicarbazone, which was the most effective of the newly synthesized compounds, required a concentration of 3×10^{-8} *M* for 50% inhibition of reductase activity and increased the life span of tumor-bearing mice over untreated animals by a factor of 2.5 at an optimal daily dose of 40 mg/kg. This agent, at a therapeutic dosage level, caused almost complete inhibition of the incorporation of thymidine-^3H into the DNA of Sarcoma 180 cells *in vivo* which was maintained for up to 24 hr after exposure of the cells to the drug; slight but prolonged inhibition of RNA synthesis was also produced as measured by incorporation of uridine-^3H.

Several α-(N)-heterocyclic carboxaldehyde thiosemicarbazones have demonstrated inhibitory activity against transplanted rodent neoplasms,[1-9] spontaneous lymphomas of dogs,[10] and DNA viruses of the Herpes family.[11] The activity of these compounds is apparently due to inhibition of the biosynthesis of DNA[12-17] with the metabolic lesion occurring at the level of reduction of ribonucleotides to deoxyribonucleotides by the enzyme ribonucleoside diphosphate reductase.[11,13,15] From studies on the mechanism by which members of this class inhibit the activity of ribonucleoside diphosphate reductase, it has been postulated that inhibition is due to the coordination of iron by these compounds either by a preformed iron complex binding to the enzyme or by the free ligand complexing with the iron-charged enzyme.[18]

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,[19] because of the water solubility of its sodium salt, as well as its relatively great therapeutic index against animal tumors.[9,10,14] Unfortunately, 5-HP failed to achieve the impressive antineoplastic activity in man that it exhibited in laboratory animals. The reasons for this inactivity in man appear to be in part (a) the relatively low inhibitory potency for the target reductase enzyme [5-HP is approximately 100 times less active at the enzymatic level than

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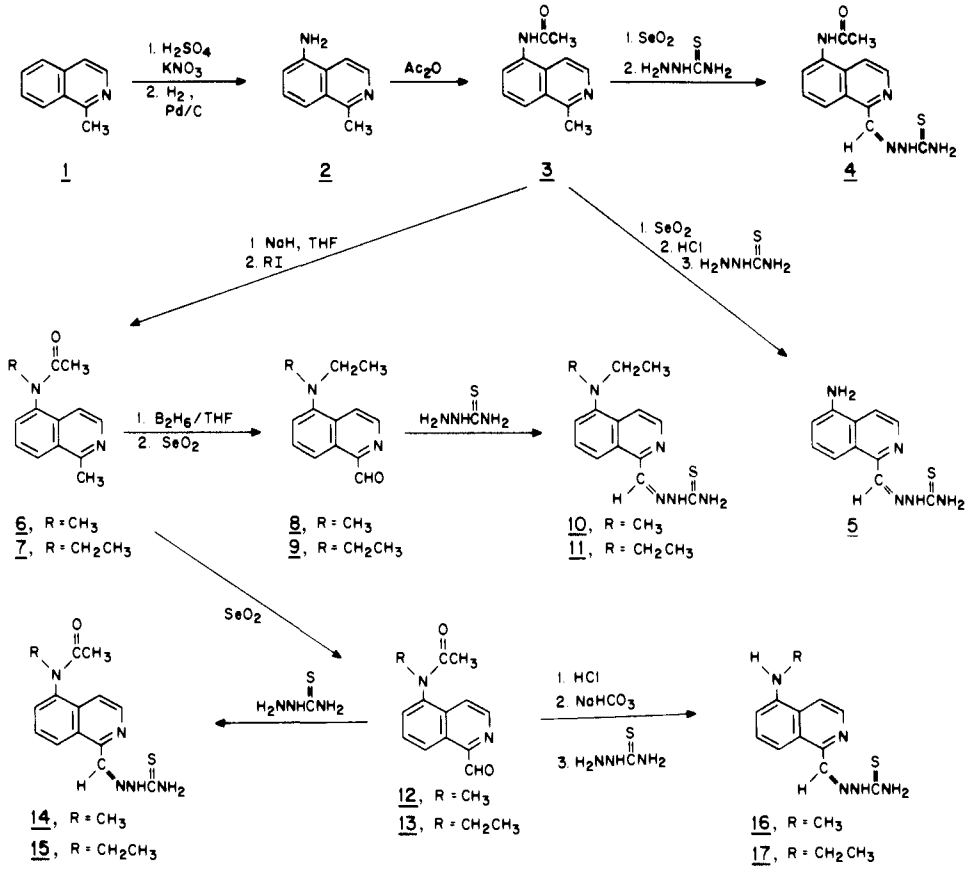
Scheme I



the most active member of this series, 1-formylisoquinoline thiosemicarbazone (IQ-1)], and (b) the relatively short-lived activity of 5-HP in man due to its rapid metabolism to an *O*-glucuronide[19] and excretion from the host (about 2 to 3.5 times faster than that occurring in the mouse).

To determine structural features compatible with increased inhibitory potency toward the target enzyme, 2-formylpyridine thiosemicarbazone (PT) was extensively modified. Introduction of a methyl group at the 3, 4, or 5 position of PT enhanced inhibitory activity.[18] In addition, IQ-1, which can be visualized as PT with a benzene ring fused at positions 3 and 4 of the pyridine ring, was approximately 2.5-fold more inhibitory toward ribonucleoside diphosphate reductase than was PT. These findings suggested the existence of a hydrophobic bonding zone adjacent to the inhibitor binding site on the enzyme;[18] this was further explored by the preparation of a series of meta-substituted phenylpyridine derivatives.[20]

Further modification of IQ-1 produced 5-amino-1-formylisoquinoline thiosemicarbazone (5-NH_2-IQ-1, 5), which was essentially equal to IQ-1 in antitumor activity[4] and could be prepared as a soluble acidic salt to facilitate parenteral administration. In addition to its desirable solubilizing capability, the amino function is obviously not susceptible to inactivation by *O*-glucuronide formation.

The present investigation was undertaken in an effort to develop a more potent inhibitor of ribonucleoside diphosphate reductase through exploration and exploitation of the apparent hydrophobic bonding zone of the enzyme. Alkylation of the 5-NH_2 function was carried out, since this substitution would increase the lipophilicity of the amino group and possibly increase the affinity of the inhibitor for the enzyme by more effective interaction with the proposed hydrophobic region of the enzyme. In addition, alkylation of the NH_2 function would be expected to provide some degree of protection of the NH_2 group from enzymatic modification.

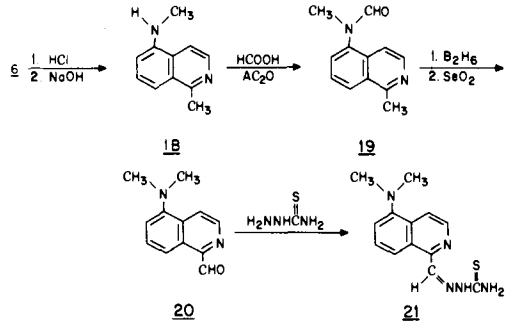
Chemistry. 1-Methylisoquinoline was allowed to react with KNO_3 in concentrated H_2SO_4 to give exclusively the 5-nitro derivative[4] which was reduced catalytically in ethanol over palladium on carbon to yield 5-amino-1-methylisoquinoline[4] (2, Scheme I). Alkylation of the 5-NH_2 derivative by an alkyl iodide in THF or DMF using NaH as base afforded only small amounts of the desired alkylamino derivative; large yields of high-melting, ethanol- and H_2O-soluble, I-containing solids were obtained that were not characterized but were presumably quarternary salts. However, alkylation was readily effected by first activating the amino function by acetylation (3),[4] then reacting 3 with NaH in anhydrous THF, followed by the necessary alkyl iodide. The *N*-alkylacetamido derivatives 6 and 7 were oxidized with SeO_2 to the corresponding 1-carboxaldehydes 12 and 13. Direct condensation of 12 and 13 with thiosemicarbazide or acid hydrolysis followed by condensation gave the alkylacetamido (14 and 15) and the monoalkylamino (16 and 17) thiosemicarbazones, respectively.

The *N*-alkyl-*N*-ethylamino thiosemicarbazones (10 and 11) were prepared by diborane reduction of the appropriate 5-*N*-alkylacetamido-1-methylisoquinoline (6 or 7), followed by oxidation with SeO_2 to the aldehyde and condensation with thiosemicarbazide.

5-*N*,*N*-Dimethylamino-1-formylisoquinoline thiosemicarbazone (21) was prepared (Scheme II) by formylation of 5-methylamino-1-methylisoquinoline (18), obtained from acid hydrolysis of 6, with formic acid in acetic anhydride, followed by reduction with diborane, SeO_2 oxidation, and condensation with thiosemicarbazide.

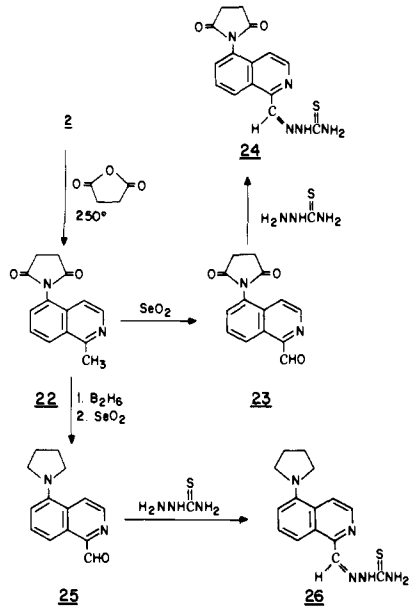
Fusion of 5-amino-1-methylisoquinoline (2) with succinic anhydride (Scheme III) at 250° yielded the succinimido

Scheme II



derivative **22**, which was either directly oxidized to the corresponding aldehyde **23** or was first reduced with diborane to the pyrrolidinyl derivative and then oxidized with SeO_2 to yield the respective aldehyde **25**. Both aldehydes **23** and **25** were then allowed to react with thiosemicarbazide to yield the desired thiosemicarbazones **24** and **26**, respectively.

Scheme III



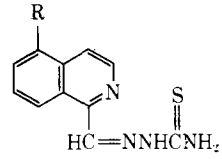
Biological Results and Discussion. The antitumor activities of 5-*N*-monoalkylamino-, *N,N*-dialkylamino-, and *N*-alkylacetamido-1-formylisoquinoline thiosemicarbazones against Sarcoma 180 ascites cells are shown in Table I. These results indicate that at least two of the new derivatives (**15** and **16**) are as effective as the unsubstituted parent **5** against this neoplasm. It is especially interesting that **14** and **15** display activity, since the 5-*N*-acetamido derivative **4** has previously been shown to be inactive in this test system.[4]

The results of tests for inhibition of partially purified ribonucleoside diphosphate reductase from the Novikoff rat tumor are shown in Table II. Compound **16**, like **5**, was equal to IQ-1 in enzyme inhibitory potency. The loss of potency with further increase in substituent size indicates either that the enzyme is sensitive to steric factors in this particular region, and the substituent bulk offsets any enhancement of affinity due to increased lipophilicity of the molecule, or that the hydrophobic bonding region of the enzyme, upon formation of the EI complex, is not in proximity to carbon-5 of the isoquinoline nucleus.

Essentially complete inhibition of the incorporation of thymidine-^3H into DNA of Sarcoma 180 ascites cells occurred when the radioactive precursor was injected intraperitoneally into mice bearing 6-day accumulations of neoplastic cells 15 min after the administration of IQ-1 at a dose level of 25 mg/kg and was allowed 1 hr to be utilized. This degree of blockade persisted for 12 hr after IQ-1, but by 24-hr inhibition was completely relieved.[12] The data in Table III demonstrate that molar equivalent levels of **5** and **16** caused essentially complete inhibition of thymidine-^3H incorporation into DNA at 6 hr. Twelve hours after drug, however, inhibition by **5** was decreased, with blockade by this agent being terminated by 24 hr. However, 24 hr after exposure of neoplastic cells to **16**, an extremely high degree of inhibition (85%) was still exhibited. Like other members of this class, both **5** and **16** caused only a slight decrease in the incorporation of uridine-^3H into RNA. Maximum inhibition of RNA synthesis was produced by IQ-1 3 hr after administration of the drug, and the level of inhibition decreased slowly thereafter.[12] Under essentially the same conditions, **5** and **16** inhibited the synthesis of RNA to the same degree at 12 hr after administration of the drug. After 18 hr, however, inhibition by **5** decreased while that produced by **16** was essentially unchanged.

Thus, substitution of a methyl group onto the amino function of **5** produced a drug **16** that was equipotent to **5** as an inhibitor of ribonucleoside diphosphate reductase and produced a significantly longer lasting inhibition of thymidine-^3H incorporation into DNA of the neoplastic cells than did the parent compound **5** when administered to tumor-bearing animals *in vivo*. The phenomenon might be the result of less rapid inactivation and/or elimination of the methylated compound. The finding that **15** is significantly less effective than **16** as an inhibitor of the activity of ribonucleoside diphosphate reductase (probably the result of steric factors), yet remains almost as effective as **16** in prolonging the survival time of tumor-bearing mice, suggests that either (a) a second biochemical site of action

Table I. Effect of 5-N-Substituted Monoalkylamino-, Dialkylamino-, and Alkylacetamido-1-formylisoquinoline Thiosemicarbazones on the Survival Time of Mice Bearing Sarcoma 180 Ascitic Cells



Drug	R	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival, days ± S.E.
None			+17.3	12.5 ± 0.5
5	NH_2	20	−7.8	27.4 ± 2.9
10	$N(CH_3)CH_2CH_3$	20	−5.4	10.4 ± 1.5
11	$N(CH_2CH_3)_2$	10	+21.6	18.6 ± 6.8
14	$N(CH_3)COCH_3$	60	−1.3	20.6 ± 2.5
15	$N(CH_2CH_3)COCH_3$	60	+2.6	29.0 ± 5.2
16	$NHCH_3$	40	−7.9	32.0 ± 5.4
17	$NHCH_2CH_3$	10	+15.3	14.4 ± 3.4
21	$N(CH_3)_2$	10	+13.3	13.6 ± 0.7
24	*N*-Succinimido	20	+6.0	16.0 ± 6.8
26	*N*-Pyrrolidinyl	10	−8.9	9.6 ± 0.6

[a] Administered once daily for 6 consecutive days, beginning 24 hr after tumor transplantation; each value represents the results obtained with 5-20 animals. [b] Average weight change from onset to termination of drug treatment.

Table II. Concentration of 5-N-Substituted Monoalkylamino-, Dialkylamino-, and Alkylacetamido-1-formylisoquinoline Thiosemicarbazones Required for 50% Inhibition of Ribonucleoside Diphosphate Reductase of the Novikoff Rat Tumor[a]

Compd	R	ID_{50}, μM
IQ-1	H	0.04
4	$NHCOCH_3$	0.11
5	NH_2	0.03
10	$N(CH_3)CH_2CH_3$	0.94
11	$N(CH_2CH_3)_2$	8.5
14	$N(CH_3)COCH_3$	1.8
15	$N(CH_2CH_3)COCH_3$	2.0
16	$NHCH_3$	0.03
17	$NHCH_2CH_3$	0.13
21	$N(CH_3)_2$	0.23
24	*N*-Succinimido	5.0
26	*N*-Pyrrolidinyl	8.6

[a] The ID_{50} is the concentration of drug required to reduce by 50% the observed activity of the enzyme. The values were estimated from graphs summarizing the results of at least two experiments with four concentrations of each compound.

may be operative, (b) biological modification *in vivo* activates 15, or (c) the enzyme structure *in situ* may be slightly different in its ability to accommodate bulk at the 5 position of IQ-1.

Experimental Section

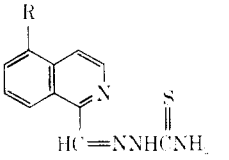
Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are corrected. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and by the Baron Consulting Co., Analytical Services, Orange, Conn. Analyses indicated by element symbols agreed with calculated values within ±0.4%. The ir absorption spectra were obtained with a Perkin-Elmer Model 257 spectrometer and nmr spectra were determined with a Varian A-60A spectrometer. Chemical shifts (δ) are given in parts per million downfield from TMS which was used as an internal standard. The spectral data were as expected, and therefore only representative findings are included. Pertinent data for the compounds synthesized are listed in Table IV; yields are based on immediate precursor.

Antitumor Screening. Experiments were performed on female CD-1 mice 9 to 11 weeks of age. 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.[4] Mice were weighed throughout the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents was based upon the prolongation of survival time afforded by the drug treatment.

Enzyme Inhibition. The ribonucleoside diphosphate reductase was partially purified from rat Novikoff ascites tumor cells as previously described.[21] Reduction of ^{32}P-CDP was assayed as reported,[21] except that $Fe(NH_4)_2(SO_4)_2$ was used instead of $FeCl_3$. Dithioerythritol was the reducing substrate. The enzyme was added to the ice-cold mixture of substrates and inhibitors, immediately warmed to 37°, and incubated 30 min. Inhibitors were dissolved in DMSO; the maximum concentration of DMSO in the incubation mixture was 1% and was not inhibitory. Each inhibitor was tested at four concentrations in at least two separate experiments.

Thymidine-^3H and Uridine-^3H Incorporation into Nucleic Acids. The effect of inhibitors on the synthesis of RNA and DNA was determined by injecting either 200 μg of uridine-^3H (1.3×10^4 cpm/μg) or 200 μg of thymidine-^3H (2.2×10^4 cpm/μg) intraperitoneally into mice bearing 6-day accumulations of Sarcoma 180 ascites cells that had previously been treated for various periods of time with a single intraperitoneal injection of drug at a level equimolar to 25 mg of IQ-1/kg. The radioactive tracers were allowed 1 hr to be incorporated into either RNA or DNA and the specific radioactivity of the nucleic acids was then measured by previously described methodology.[12]

Table III. Incorporation of Thymidine-^3H and Uridine-^3H into DNA and RNA, Respectively, of Sarcoma 180 Cells Treated with Either 5-Amino-1-formylisoquinoline Thiosemicarbazone or 5-Methylamino-1-formylisoquinoline Thiosemicarbazone



Drug	R	Dose, mg/kg	Pretreatment (hr) before radioactive precursor	% inhibition[a] Thymidine-^3H into DNA	% inhibition[a] Uridine-^3H into RNA
5	NH_2	26.6	6	98	
			12	70	34
			18	16	15
			24	0	
16	$NH(CH_3)$	28.2	6	98	
			12	92	35
			18	99	44
			24	85	

[a] Control animals received the necessary radioactive precursor without drug pretreatment. The specific activities of control DNA thymine and RNA pentose, respectively, were 22.2 ± 1.5 cpm/nmol and 9.8 ± 0.5 cpm/nmol. These values represent the mean (± standard error) of results obtained with 16 and 8 mice, respectively. Drug-treated groups each consisted of material from at least four animals analyzed separately.

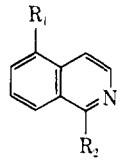
5-Amino-1-methylisoquinoline (2). 5-Nitro-1-methylisoquinoline[4] (9.04 g, 0.05 mol) was dissolved in 200 ml of EtOH and 300 mg of Pd/C (10% catalyst) was added. After the mixture was hydrogenated for 1 hr (40 psi) at room temperature, the solution was filtered and the solvent removed. Recrystallization (EtOH–H_2O, Darco) gave 6.58 g (83.5%) of amine, mp 212.5–213° (lit.[4] 213°).

5-*N*-Alkylacetamido-1-methylisoquinoline (6, 7). Compound 3 (0.01 mol) was dissolved in 300 ml of refluxing anhydrous THF distilled from $LiAlH_4$. NaH (0.042 g of 57% oil dispersion, 0.01 mol) suspended in 60 ml of anhydrous THF was carefully added; after effervescence ceased, the resulting solution was refluxed for 30 min. Alkyl iodide (0.01 mol) in 20 ml of anhydrous THF was added and the solution refluxed for 3 hr. The solvent was removed and the oily residue extracted with hot $CHCl_3$ (3 × 50 ml). The combined extracts were washed with H_2O (3 × 50 ml) and dried (Na_2SO_4), and the solvent was removed. The oil was then extracted with boiling hexane (4 × 50 ml), the hot combined extracts were decolorized (Darco), and the volume was reduced by half. Upon cooling, colorless star-like clusters were formed. Additional yields were obtained by further reducing the volume of the filtrate.

5-*N*-Methylamino-1-methylisoquinoline (18). Compound 6 (2.14 g, 0.01 mol) was dissolved in 20 ml of 6 *N* HCl and refluxed 2 hr. The solution was cooled and made strongly basic with NaOH. The precipitate that formed was filtered and recrystallized (EtOAc–hexane) to give 1.34 g (78%) of pale yellow prisms, mp 161.5–162.5°.

5-*N*-Methylformamido-1-methylisoquinoline (19). Compound 18 (1.72 g, 0.01 mol) was dissolved in 10 ml of 88% formic acid. The mixture was heated to 60° and Ac_2O (6 ml) was added

Table IV



Compd	R_1	R_2	Recrystn solvent	Yield, %	Mp, °C	Formula	Analyses
6	$N(CH_3)COCH_3$	CH_3	Hexanes	45	106–107	$C_{13}H_{14}N_2O$	C, H
7	$N(CH_2CH_3)COCH_3$	CH_3	Hexanes	61	94–94.5	$C_{14}H_{16}N_2O$	C, H
10	$N(CH_3)CH_2CH_3$	$CH{=}NNHCSNH_2$	*a*	43	200–200.5 dec	$C_{14}H_{17}N_5S$	C, H, N, S
11	$N(CH_2CH_3)_2$	$CH{=}NNHCSHN_2$	*a*	80	224–224.5 dec	$C_{15}H_{19}N_5S$	C, H, N, S
12	$N(CH_3)COCH_3$	CHO	Hexanes	50	115–116	$C_{13}H_{12}N_2O_2$	C, H, N
13	$N(CH_2CH_3)COCH_3$	CHO	Hexanes	49	109–110	$C_{14}H_{14}N_2O_2$	C, H, N
14	$N(CH_3)COCH_3$	$CH{=}NNHCSNH_2$	EtOAc	92	240–241.5 dec	$C_{14}H_{15}N_5OS$	C, H, N, S
15	$N(CH_2CH_3)COCH_3$	$CH{=}NNHCSNH_2$	*b*	83	241.5–242 dec	$C_{15}H_{17}N_5OS$	C, H, N, S
16	$NHCH_3$	$CH{=}NNHCSNH_2$	*a*	46	221.5–222 dec	$C_{12}H_{13}N_5S\cdot{}^2/_3H_2O$	C, H, N
17	$NHCH_2CH_3$	$CH{=}NNHCSNH_2$	*b*	40	207.5–208 dec	$C_{13}H_{15}N_5S$	C, H, N
18	$NHCH_3$	CH_3	EtOAc–hexanes	78	161.5–163	$C_{11}H_{12}N_2$	C, H, N
19	$N(CH_3)CHO$	CH_3	*b*	78	76–78	$C_{12}H_{12}N_2O$	C, H, N
21	$N(CH_3)_2$	$CH{=}NNHCSNH_2$	*a*	69	193–194 dec	$C_{13}H_{15}N_5S$	C, H, N
22	*N*-Succinimido	CH_3	Water	78	205.5–206.5	$C_{14}H_{12}N_2O_2$	C, H, N
23	*N*-Succinimido	CHO	Hexanes	60	205–205.5	$C_{14}H_{10}N_2O_3$	C, H, N
24	*N*-Succinimido	$CH{=}NNHCSNH_2$	*b*	94	220.5–221 dec	$C_{15}H_{13}N_5O_2S$	C, H, N, S
26	*N*-Pyrrolidinyl	$CH{=}NNHCSNH_2$	*b*	80	204–204.5 dec	$C_{15}H_{17}N_5S$	C, H, N, S

[a] Purified as described in the Experimental Section. [b] Further purification was not necessary.

to the stirred solution at a rate sufficiently slow to maintain the temperature below 70°. The reaction was stirred at room temperature for 2 hr, whereupon 20 ml of ice-water was added. The solvent was removed under vacuum; the yellow oil was diluted with 5 ml of H_2O and then made basic with 10% NaOH. The tan oil that formed was collected, dissolved in warm ether, and dried (Na_2SO_4) and the ether removed. The remaining oil solidified when allowed to stand overnight to give 1.55 g (77.5%) of product, mp 76–78°.

5-*N*-Alkyl-*N*-ethylamino-1-methylisoquinolines. The proper amide (**6** or **7**) (0.01 mol) was dissolved in 50 ml of anhydrous THF and this solution added to 25 ml (0.025 mol) of 1 *M* B_2H_6–THF (prepared according to published procedure[22]) diluted with 25 ml of anhydrous THF. The reaction was kept under N_2 and refluxed for 3 hr. The solution was allowed to cool and EtOH (5 ml) was added, followed by H_2O (10 ml), and finally by 10 ml of concentrated HCl. The acidic mixture was warmed for 5 min on a steam bath, then stirred 30 min at room temperature, followed by removal of THF under vacuum. The aqueous residue was made basic with NaOH, cooled, and extracted with ether (3 × 50 ml). After drying the combined extracts (Na_2SO_4), the ether was removed and the oil allowed to stand overnight, whereupon a fine precipitate occasionally formed that was filtered off and discarded. The crude ethylmethylamino and diethylamino derivatives were obtained in 84 and 53% yields, respectively. They were effectively purified by chromatography on a column of silica gel (50 g of SilicAR cc-7, Mallinckrodt) using EtOAc or EtOAc–petroleum ether (1:1, v/v) as eluent. Characterization of reduction products was by ir (loss of C=O absorption, 1640-1660 cm^{-1}) and nmr (quartet, δ 3.01-3.16, 2 H or 4 H).

5-*N*,*N*-Dimethylamino-1-methylisoquinoline. Compound **19** was reduced with diborane and the amine obtained as described previously was distilled: bp 95–98° (0.05 mm); yield 81%. Identification was by ir (loss of C=O absorption, 1660-1680 cm^{-1}) and by nmr (loss of CHO, singlet δ 3.83, 1 H; singlet, δ 2.60, 6 H). The amine was used without further purification.

5-*N*-Succinimido-1-methylisoquinoline (22). Compound **2** (1.58 g, 0.01 mol) was mixed with succinic anhydride (1.00 g, 0.01 mol), then immersed in an oil bath preheated to 250°, and maintained at this temperature for 10 min after effervescence ceased. The reaction was cooled to room temperature and the solid brown mass that formed was dissolved in hot acetone, filtered, decolorized (Darco), and refiltered, and the solvent was removed. The solid residue was washed several times with saturated $NaHCO_3$ solution and air-dried to give 1.78 g (78%) of a tan solid. Recrystallization (H_2O) yielded fine tan prisms, mp 205.5–206.5°.

5-*N*-Pyrrolidinyl-1-methylisoquinoline. Compound **22** was reduced with diborane as previously described: yield 92%. The crude amine thus obtained was used without further purification. Identification was by ir (loss of C=O, 1700-1730 cm^{-1}) and nmr (multiplet, δ 3.18-3.92, 8 H).

5-*N*-Alkylacetamido-1-formylisoquinolines (12 and 13). A suspension of SeO_2 (1.11 g, 0.01 mol) in dioxane (30 ml) was added over 30 min to a refluxing solution of 5-*N*-alkylacetamido-1-methylisoquinoline (0.01 mol) in dioxane (25 ml) and the mixture then refluxed for 3 hr. The hot solution was filtered (Celite Analytical Filter Aid), the solvent removed, and the remaining red oil extracted with boiling hexane (8–10 × 25 ml). The combined extracts were repeatedly treated with carbon (Darco) to rid the product of the yellow color of Se impurities, and the volume was reduced to 100 ml. Upon standing overnight, clusters of colorless crystals formed. Yields averaged around 50%.

5-*N*-Alkylamino-1-formylisoquinoline. Compound **12** or **13** (0.005 mol) was dissolved in 10 ml of concentrated HCl and refluxed 2 hr, after which time the solution was made slightly basic with $NaHCO_3$. The solution was repeatedly extracted with $CHCl_3$ until the extracts were nearly clear, the combined extracts were dried (Na_2SO_4), and the solvent was removed. The residue was extracted with boiling ether until the extracts were nearly colorless. The ether extracts were treated with charcoal and dried (Na_2SO_4), and the solvent was removed to leave the aldehyde which was identified by ir (appearance of N-H absorbance, 3420 cm^{-1}). The aldehydes were used without further purification.

5-*N*,*N*-Dialkylamino-1-formylisoquinolines. The dialkylamino-1-methylisoquinolines were oxidized with SeO_2 as described above. However, the aldehyde was extracted from the residual red oil with $CHCl_3$ (4 × 30 ml) rather than hexanes. The $CHCl_3$ was removed and the residue extracted with ether (4 × 50 ml). The combined ether extracts were dried (Na_2SO_4) and the solvent was removed. The red oil was then purified by chromatography on 50 g of SilicAR cc-7 (Mallinckrodt) (**8**, EtOAc; **9**, petroleum ether (bp 30–60°)–EtOAc, 1:1, v/v; **20**, $CHCl_3$; **24**, EtOAc). Characterization was by ir (appearance of C=O, 1710 cm^{-1}), nmr (loss of singlet, δ 2.83-2.93, 3 H; appearance of singlet, δ 10.36-10.45, 1 H), and derivatization.

5-*N*-Pyrrolidinyl-1-formylisoquinoline (25). 5-*N*-Pyrrolidinyl-1-methylisoquinoline, obtained from the diborane reduction of **22**, was oxidized with SeO_2 in dioxane and the crude aldehyde was

obtained as described for the dialkylamino aldehydes. The crude aldehyde was purified by column chromatography on 50 g of silica gel (SilicAR cc-7, Mallinckrodt), using EtOAc as eluent, and then characterized by ir (appearance of C=O, 1708 cm^{-1}), nmr (loss of CH_3, δ 3.18; appearance of singlet, δ 10.33, 1 H, CHO), and derivatization.

Thiosemicarbazones. A solution of thiosemicarbazide (0.091 g, 1 mmol) dissolved in hot H_2O (6–7 ml) plus AcOH (1 drop) was added to a solution of appropriate aldehyde (1 mmol) dissolved in EtOH (2 ml). The solution was stirred and refluxed for 1 hr and upon cooling the derivative precipitated. Purification was effected by recrystallizing or by dissolving in warm 10% HCl, treating with charcoal, and reprecipitating with $NaHCO_3$.

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Bis-Basic-Substituted Polycyclic Aromatic Compounds. A New Class of Antiviral Agents.[1,2] 6. Bis-Basic-Substituted Fluoranthenes

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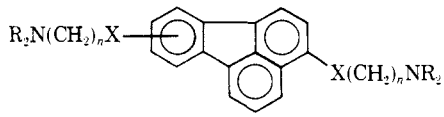
A series of bis-basic esters, ketones, ethers, and alkanes of fluoranthene was synthesized and evaluated for antiviral activity. Compounds from each group were found to have potent antiviral activity when administered subcutaneously to mice infected with encephalomyocarditis virus. Bis-basic ketones of fluoranthene were the most potent antiviral agents when administered orally. Structural modifications included variation of the alkylene chain and amine substituents within each group. Position of attachment of the basic side chain to the fluoranthene nucleus was varied for bis-basic esters of fluoranthene. 3,9-Fluoranthenedicarboxylic acid bis[3-(diethylamino)propyl]ester dihydrochloride (**9**, RMI 9563DA) and 1,1′-(3,9-fluoranthenediyl)bis[2-(dimethylamino)ethanone] dihydrochloride (**24**, RMI 11,645DA) were selected for further biological evaluation.

In this paper we will discuss results obtained from testing a series of bis-basic-substituted fluoranthene derivatives for antiviral activity. The fluoranthene nucleus, containing four fused rings, was the first example of an aromatic hydrocarbon that differed from the general structure for tricyclic aromatic hydrocarbons previously reported.[3-7] A number of esters I, ketones II, ethers III, and alkanes IV were synthesized for the purpose of comparing antiviral activity with the corresponding fluorene and fluorenone derivatives.[3-5] In general, the SAR pattern for the



I. X = CO_2
II. X = CO
III. X = O
IV. X = CH_2

fluoranthene series corresponded to that previously reported for the fluorene(one) series. Representative fluoranthene derivatives were shown to induce interferon,[8-10] an activity consistent with the possible mode of action for tilorone and other fluorenone compounds.

Chemistry. The general method of synthesis of the bis-basic-substituted fluoranthenes is outlined in Scheme I. Bromination of fluoranthene with 2 equiv of bromine gave 3,8-dibromofluoranthene which was converted to the dinitrile followed by hydrolysis to the dicarboxylic acid.[11] The diacid chloride when treated with the appropriate aminoalkanols gave the 3,8-bis-basic esters **2–6** (Table I). The 3,9-bis-basic esters **7–18** (Table I) were synthesized from the diacid,[12] obtained by the haloform reaction on 3,9-diacetylfluoranthene.

The 3,9-bis-basic ketones **24–49** (Table II) were prepared by amination of 3,9-bis(ω-chloroalkanoyl)fluoranthenes obtained by Friedel-Crafts diacylation of fluoranthene. Two general procedures were used for the amination reaction. The amine was allowed to react with the appropri-