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Potential Antitumor Agents. 6. Possible Irreversible Inhibitors of Ribonucleoside Diphosphate Reductase†

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1-Formylisoquinoline thiosemicarbazone, a potent antineoplastic agent, blocks DNA synthesis by inhibiting the enzyme ribonucleoside diphosphate reductase. Several groups potentially capable of alkylation of the enzyme were introduced at the 5 position of the isoquinoline nucleus to design possible irreversible inhibitors of ribonucleoside diphosphate reductase. Two series of compounds were made using either 5-amino or 5-hydroxy derivatives to yield corresponding amides [$-NHSO_2CH_3$, $-NHCOC_6H_4$ (*m*- or *p*-SO_2F)] or esters [$-OSO_2CH_3$, $-OCO_2C_2H_5$, $-OCO_2C_6H_5$, $-OCOC_6H_4$ (*m*- or *p*-SO_2F), $-OSO_2C_6H_4$ (*o*-, *m*-, or *p*-SO_2F)]. In addition, a bis(β-chloroethyl)amino group was introduced at the 5 position by nucleophilic substitution of 5-chloro-1-formylisoquinoline. Although these agents were potent inhibitors of the enzyme *in vitro*, requiring in the range of 10^{-6}–10^{-8} *M* concentration for 50% inhibition of the enzyme, only two derivatives, those containing a $-OCO_2C_2H_5$ or a $-OCO_2C_6H_5$ moiety, were shown to have potent tumor-inhibitory activity in mice bearing Sarcoma 180 ascites cells.

A number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, which exhibit growth-inhibitory activity against a relatively wide spectrum of transplanted rodent tumors,[1-9] spontaneous lymphomas of dogs,[10] and DNA viruses of the Herpes group,[11] have been shown to be potent inhibitors of the activity of the mammalian form of the enzyme ribonucleoside diphosphate reductase.[11-14]

The most active compounds of this series are 80 to 5000 times more potent than hydroxyurea, guanazole, and *meso*-α,β-diphenylsuccinate, the other known inhibitors of this enzyme. Interference with the activity of ribonucleoside diphosphate reductase prevents the conversion of ribonucleotides to deoxyribonucleotides and subsequently results in inhibition of the synthesis of DNA. From studies on the mechanism by which α-(N)-heterocyclic carboxaldehyde thiosemicarbazones inhibit the activity of ribonucleoside diphosphate reductase, it has been postulated that inhibition is due to either the coordination of iron by these compounds in the metal-bound enzyme or that an iron chelate

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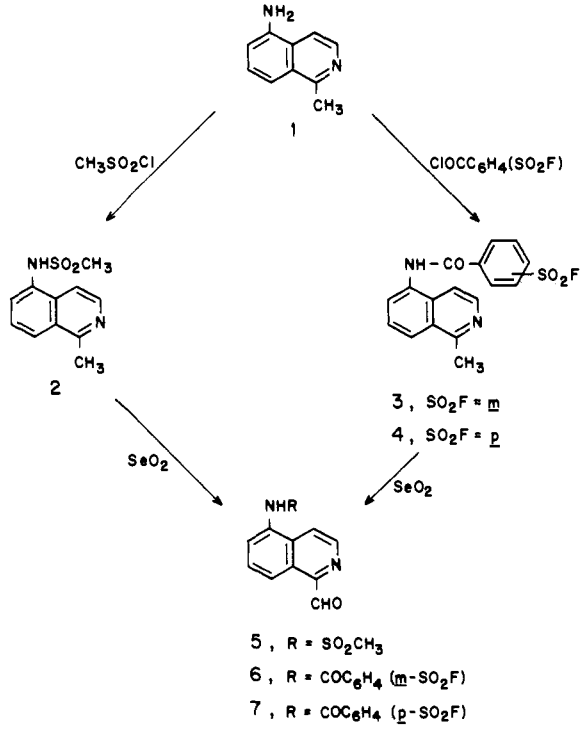
formed *in situ* is the active inhibitory form.[14] These postulates are substantiated by the requirement for biological activity of an intact formyl thiosemicarbazone side chain α to the heteroaromatic N atom, suggesting the involvement of a conjugated N*-N*-S* tridentate ligand system.[5]

Intensive studies of structure-activity relationships in this series, using two of the most potent inhibitors of ribonucleoside diphosphate reductase, 1-formylisoquinoline thiosemicarbazone (IQ–1) and 2-formylpyridine thiosemicarbazone (PT), have delineated[7,14] (a) the bulk tolerance requirements of the enzyme for substituents at various positions of the heterocyclic ring and (b) those changes that favor optimum interaction between the enzyme and the inhibitor. Thus, introduction of a CH_3 group in the 3, 4, or 5 position of PT and the 4 or 5 position of IQ-1 results in derivatives that are effectively bound to the enzyme and, therefore, are potent inhibitors of ribonucleoside diphosphate reductase activity.[14] These studies suggested that an irreversible inhibitor of the enzyme might be synthesized by introducing on the heteroaromatic ring a suitable group that conceivably could produce alkylation at a polar region of the inhibitor-binding site of the enzyme. Accordingly, several groups deemed potentially capable of alkylation of the enzyme were chosen to be introduced initially at the 5 position of the isoquinoline ring.

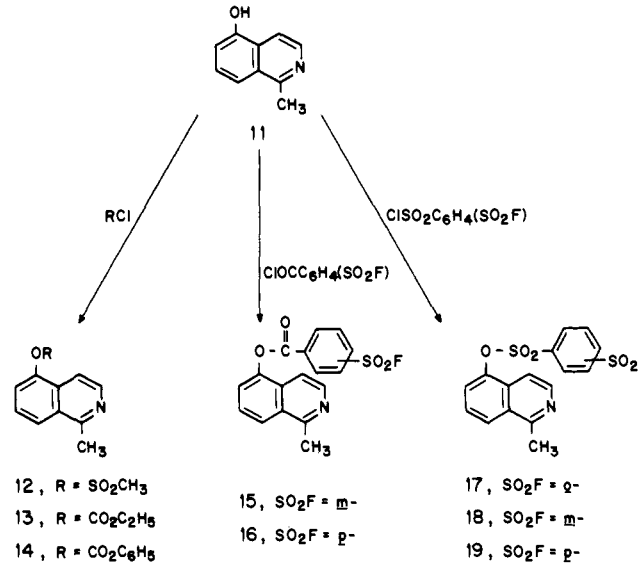
Chemistry. The first series of compounds was synthesized from 1-methyl-5-aminoisoquinoline (**1**) to yield corresponding amides as outlined in Scheme I. The synthesis

Scheme I



of **1** has been reported earlier.[4] Acylation reactions with **1** were carried out in THF using Et_3N as the acid acceptor. The majority of the acid chlorides chosen had a terminal SO_2F group, since compounds containing such groups have been shown to be irreversible enzyme inhibitors.[15,16] 5-Substituted-1-methylisoquinolines (**2**, **3**, and **4**) were then oxidized to the corresponding 1-carboxaldehydes (**5**, **6**, and **7**) with equimolar quantities of SeO_2 in dioxane. The resulting aldehydes were allowed to react with thiosemicarbazide to yield the desired thiosemicarbazones.

Scheme II

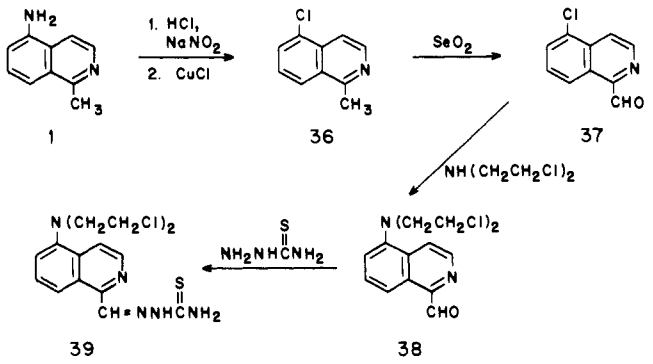


Since these compounds, which contained an amide bridge between the heterocyclic ring and the benzenesulfonyl fluoride moieties, were subsequently found to be generally ineffective in *in vivo* tests for antitumor activity, a second series of derivatives was made containing an ester linkage (Scheme II). The syntheses of these compounds followed the same sequence as with corresponding amides. The esterification reactions were first carried out in DMF due to the relatively low solubility of 1-methyl-5-hydroxyisoquinoline (**11**)[4] in THF. When NaH was employed as the acid acceptor, a mixture of two compounds was obtained in the esterification reactions with $ClOCC_6H_4$ (*m*- or *p*-SO_2F) and $ClO_2SC_6H_4$ (*o*-, *m*-, or *p*-SO_2F). The mixture was best separated by column chromatography using silica gel. The major portion (60–65%) of the mixture was the desired ester; the identity of the second compound was not investigated further. These difficulties in the esterification reactions were satisfactorily overcome using Et_3N as the base; with this acceptor only the desired ester was isolated from the reaction mixture. The 1-methylisoquinoline esters were oxidized with SeO_2 to obtain corresponding 1-carboxaldehydes, which on reaction with thiosemicarbazide yielded the thiosemicarbazones.

The synthesis of 1-formyl-5-bis(β-chloroethyl)aminoisoquinoline thiosemicarbazone, a derivative containing a nitrogen mustard group potentially capable of alkylation of the target enzyme, is shown in Scheme III. Compound **1** was converted to the 5-chloro derivative (**36**) utilizing a Sandmeyer reaction and then was oxidized with SeO_2 to give 1-formyl-5-chloroisoquinoline (**37**).[8] Direct nucleophilic displacement of the 5-Cl in **36** with bis(β-chloroethyl)amine was not successful; however, after oxidation of **36** to **37** it was possible to achieve the nucleophilic substitution to give **38**, which on reaction with thiosemicarbazide yielded the desired compound. The relevant data pertaining to the newly synthesized compounds are listed in Table I.

Biological Results and Discussion. The effects of 5-substituted derivatives of IQ-1 on the survival time of mice bearing Sarcoma 180 ascites cells are shown in Table II. The results indicate that although the 5-NH_2 derivative of IQ-1 exhibits biological potency in increasing the average survival time of tumor-bearing mice equal to the parent compound, its derivatives containing a group potentially able to alkylate, such as **8**, **9**, or **10**, showed essentially no tumor-inhibitory

Scheme III

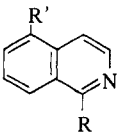


activity. Compounds **9** and **10** were selected as prototypes for introducing a hydrophobic zone between the alkylating moiety and the parent ring structure. It was hypothesized that a hydrophobic bonding area might be available near the inhibitor binding site on the enzyme since insertion of hydrophobic groups such as CH_3 resulted in potent inhibitors of the target enzyme ribonucleoside diphosphate reductase.[14]

The results of tests for inhibition of partially purified ribonucleotide reductase from the Novikoff rat tumor are shown in Table III. Because some variation was observed in the degree of inhibition by IQ-1 of different batches of enzyme, the results are divided into three series to compare the potency of each inhibitor to that of IQ-1 measured simultaneously. Compound **8** was about 25% as effective as IQ-1 as an inhibitor of the enzyme, whereas compounds **9** and **10** were considerably less potent and required about 42 and 140 times, respectively, the concentration of IQ-1 to achieve the same degree of inhibition of reductase activity. The total loss of *in vivo* activity as antitumor agents of compounds possessing an amide linkage (**8**, **9**, or **10**) could possibly be due to relatively poor transport through the cell membranes.[17] The amide bridge would be expected to be hydrogen bonded with water and therefore be poorly transported through the lipid phase of cell membranes. This fact was also substantiated earlier in that acetylation of the 5-NH_2 derivative of IQ-1 to yield the 5-acetylamino derivative resulted in an inactive compound.[4]

In view of these results, substituted esters of the 5-OH derivative of IQ-1 were synthesized, since these derivatives presumably would be better transported through cellular membranes. Interestingly, the esters in general were more lipid soluble than the amides. Compounds **28–35**, therefore, were made and tested for biological activity. Although most of the esters were potent inhibitors of ribonucleoside diphosphate reductase activity (Table III), requiring concentrations of $10^{-6}M$ to $10^{-8}M$ drug for 50% inhibition, only two compounds (**29** and **30**) containing a carboethoxy and a carbophenoxy group, respectively, were found to be very effective antineoplastic agents (Table II). These derivatives increased the average survival of mice to 30 and 31 days, respectively, as compared to untreated tumor-bearing con-

Table I



Compd	R	R'	Method	Crystn solvent	Mp, °C	Yield	Formula	Analyses
2	CH_3	$NHSO_2CH_3$	A	C_6H_6	171-173	72	$C_{11}H_{12}N_2O_2S$	C, H, N
3	CH_3	$NHCOC_6H_4$ (*m*-SO_2F)	A	$CHCl_3 + C_6H_6$	153-155	62	$C_{17}H_{13}FN_2O_3S$	C, H, N
4	CH_3	$NHCOC_6H_4$ (*p*-SO_2F)	A	$CHCl_3 + C_6H_6$	247-248	60	$C_{17}H_{13}FN_2O_3S$	C, H, N
5	CHO	$NHSO_2CH_3$	C	EtOAc	194-196 dec	50	$C_{11}H_{10}N_2O_3S$	C, H, N
6	CHO	$NHCOC_6H_4$ (*m*-SO_2F)	C	EtOAc	205-206 dec	73	$C_{17}H_{11}FN_2O_4S$	C, H, N
7	CHO	$NHCOC_6H_4$ (*p*-SO_2F)	C	THF-hexanes	216-218 dec	67	$C_{17}H_{11}FN_2O_4S$	C, H, N
8	$CH=NNHCSNH_2$	$NHSO_2CH_3$	D	EtOH	232-234 dec	85	$C_{12}H_{13}N_5O_2S_2$	C, H, N, S
9	$CH=NNHCSNH_2$	$NHCOC_6H_4$ (*m*-SO_2F)	D		234-236 dec	90	$C_{18}H_{14}FN_5O_3S_2$	C, H, N, S
10	$CH=NNHCSNH_2$	$NHCOC_6H_4$ (*p*-SO_2F)	D		237-240 dec	90	$C_{18}H_{14}FN_5O_3S_2$	C, H, N, S
12	CH_3	OSO_2CH_3	B	C_6H_6-hexanes	85	65	$C_{11}H_{11}NO_3S$	C, H, N
13	CH_3	$OCO_2C_2H_5$	B	Petr ether	65-66	76	$C_{13}H_{13}NO_3$	C, H, N
14	CH_3	$OCO_2C_6H_5$	B	Petr ether	97-98	80	$C_{17}H_{13}NO_3$	C, H, N
15	CH_3	$OCOC_6H_4$ (*m*-SO_2F)	B	C_6H_6-cyclohexane	98-100	58	$C_{17}H_{12}FNO_4S$	C, H, F
16	CH_3	$OCOC_6H_4$ (*p*-SO_2F)	B	C_6H_6-cyclohexane	148-150	60	$C_{17}H_{12}FNO_4S$	C, H, F
17	CH_3	$OSO_2C_6H_4$ (*o*-SO_2F)	B	THF-hexanes	153-154	70	$C_{16}H_{12}FNO_5S_2$	C, H, F
18	CH_3	$OSO_2C_6H_4$ (*m*-SO_2F)	B	C_6H_6-cyclohexane	123-125	64	$C_{16}H_{12}FNO_5S_2$	C, H, F
19	CH_3	$OSO_2C_6H_4$ (*p*-SO_2F)	B	C_6H_6-cyclohexane	148-150	70	$C_{16}H_{12}FNO_5S_2$	C, H, F
20	CHO	OSO_2CH_3	C	C_6H_6-hexanes	138-139	67	$C_{11}H_9NO_4S$	C, H, N
21	CHO	$OCO_2C_2H_5$	C	Hexanes	88-89	66	$C_{13}H_{11}NO_4$	C, H, N
22	CHO	$OCO_2C_6H_5$	C	Hexanes	123-124	62	$C_{17}H_{11}NO_4$	C, H, N
23	CHO	$OCOC_6H_4$ (*m*-SO_2F)	C	Cyclohexane	112-114	53	$C_{17}H_{10}FNO_5S$	C, H, N
24	CHO	$OCOC_6H_4$ (*p*-SO_2F)	C	EtOAc	177-179	58	$C_{17}H_{10}FNO_5S$	C, H, N
25	CHO	$OSO_2C_6H_4$ (*o*-SO_2F)	C	THF-cyclohexane	169-170	76	$C_{16}H_{10}FNO_6S_2$	N
26	CHO	$OSO_2C_6H_4$ (*m*-SO_2F)	C	THF-cyclohexane	160-162	70	$C_{16}H_{10}FNO_6S_2$	N
27	CHO	$OSO_2C_6H_4$ (*p*-SO_2F)	C	THF-cyclohexane	150-152	78	$C_{16}H_{10}FNO_6S_2$	N
28	$CH=NNHCSNH_2$	OSO_2CH_3	D	EtOH	218-220 dec	90	$C_{12}H_{12}N_4O_3S_2$	C, H, N, S
29	$CH=NNHCSNH_2$	$OCO_2C_2H_5$	D	EtOH	196-197 dec	84	$C_{14}H_{14}N_4O_3S$	C, H, N, S
30	$CH=NNHCSNH_2$	$OCO_2C_6H_5$	D		210-211 dec	95	$C_{18}H_{14}N_4O_3S$	C, H, N, S
31	$CH=NNHCSNH_2$	$OCOC_6H_4$ (*m*-SO_2F)	D	THF-C_6H_6	205-207 dec	86	$C_{18}H_{13}FN_4O_4S_2$	C, H, F, N
32	$CH=NNHCSNH_2$	$OCOC_6H_4$ (*p*-SO_2F)	D	DMF-H_2O	206-208 dec	82	$C_{18}H_{13}FN_4O_4S_2$	C, H, F, N
33	$CH=NNHCSNH_2$	$OSO_2C_6H_4$ (*o*-SO_2F)	D	DMF-EtOH	213-215 dec	80	$C_{17}H_{13}FN_4O_5S_3$	C, H, F, N
34	$CH=NNHCSNH_2$	$OSO_2C_6H_4$ (*m*-SO_2F)	D	THF-C_6H_6	180-182 dec	83	$C_{17}H_{13}FN_4O_5S_3$	C, H, F, N
35	$CH=NNHCSNH_2$	$OSO_2C_6H_4$ (*p*-SO_2F)	D	THF-EtOH	204-206 dec	82	$C_{17}H_{13}FN_4O_5S_3$	C, H, F, N
39	$CH=NNHCSNH_2$	$N(CH_2CH_2Cl)_2$			224-226 dec	34	$C_{15}H_{17}Cl_2N_5S \cdot HCl$	C, H, N, S

Table II. Effect of 5-Substituted-1-formylisoquinoline Thiosemicarbazones on the Survival Time of Mice Bearing S-180 Ascites Cells

Compd	5-Substitution	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival time, days + S.E.
	Control		+12.8	13.9 ± 0.3
	None (IQ-1)	20	−5.1	37.2 ± 5.5 (20)[c]
	NH_2	40	−8.5	37.3 ± 3.2 (30)
8	$NHSO_2CH_3$	60	+5.5	14.2 ± 2.5
9	$NHCOC_6H_4$ (*m*-SO_2F)	20	+5.1	14.4 ± 3.7
10	$NHCOC_6H_4$ (*p*-SO_2F)	60	−3.4	11.8 ± 1.4
	OH	120	+10.4	35.4 ± 3.1 (20)
28	OSO_2CH_3	20	+8.5	15.5 ± 0.6
29	$OCO_2C_2H_5$	60	−4.2	30.0 ± 4.9 (30)
30	$OCO_2C_6H_5$	60	−1.0	31.2 ± 4.0 (20)
31	$OCOC_6H_4$ (*m*-SO_2F)	60	+9.8	17.4 ± 2.8
32	$OCOC_6H_4$ (*p*-SO_2F)	80	+11.2	14.6 ± 1.3
33	$OSO_2C_6H_4$ (*o*-SO_2F)	60	+5.2	11.8 ± 0.5
34	$OSO_2C_6H_4$ (*m*-SO_2F)	40	+4.3	12.4 ± 3.0
35	$OSO_2C_6H_4$ (*p*-SO_2F)	20	+8.3	16.6 ± 4.0
39	$N(CH_2CH_2Cl)_2$	10	+9.6	15.6 ± 0.4

[a] Administered daily for 6 consecutive days, beginning 24 hr after tumor transplantation; each value represents the results obtained with 5–10 animals. [b] Average weight change from onset to termination of drug treatment. [c] The number in parentheses indicates the per cent of tumor bearing animals that survived at least 50 days; these mice were calculated as 50-day survivors in determinations of average survival time.

Table III. Concentration of 5-Substituted-1-formylisoquinoline Thiosemicarbazone Required for 50% Inhibition of Ribonucleoside Diphosphate Reductase of the Novikoff Rat Tumor

Compd	5-Substitution	ID_{50}, *M*	Ratio ID_{50}/ID_{50} (IQ-1)
Series A			
IQ-1	H	1.7×10^{-8}	1.0
8	$NHSO_2CH_3$	6.7×10^{-8}	3.9
9	$NHCOC_6H_4$ (*m*-SO_2F)	7.2×10^{-7}	42
10[a]	$NHCOC_6H_4$ (*p*-SO_2F)	2.4×10^{-6}	140
28	OSO_2CH_3	1.8×10^{-7}	10.6
29	$OCO_2C_2H_5$	7×10^{-8}	4.1
Series B			
IQ-1	H	7×10^{-8}	1.0
IQ-1-5-ol	OH	1.6×10^{-7}	2.3
31	$OCOC_6H_4$ (*m*-SO_2F)	7×10^{-7}	10
Series C			
IQ-1	H	4.3×10^{-8}	1.0
5-Amino-IQ-1	NH_2	4.3×10^{-8}	1.0
30	$OCO_2C_6H_5$	1.5×10^{-6}	35
32	$OCOC_6H_4$ (*p*-SO_2F)	3.7×10^{-6}	86
33	$OSO_2C_6H_4$ (*o*-SO_2F)	5.5×10^{-6}	128
34	$OSO_2C_6H_4$ (*m*-SO_2F)	6.6×10^{-6}	153
35	$OSO_2C_6H_4$ (*p*-SO_2F)	6.9×10^{-6}	160
39	$N(CH_2CH_2Cl)_2$	1.8×10^{-7}	4.2

[a] Only one experiment.

trols, which lived only an average of 13.9 days. Further biochemical studies are in progress with compounds **29** and **30** to determine whether these agents produce irreversible binding of the enzyme. The ineffectiveness of compounds containing the SO_2F moiety in inhibiting tumor growth could not be explained at this time. However, poor transport of these compounds may again be an important factor in their biological inactivity. These esters (**28**, **31**, and **32**) in general were similar to their corresponding amide derivatives (**8**, **9**, and **10**) as inhibitors of the activity of ribonucleoside diphosphate reductase. Another approach that was tried was the introduction of a group with high chemical reactivity (*i.e.*, bis(β-chloroethyl)amino) (**39**). Again, the compound had no appreciable tumor-inhibitory effect, although it inhibited the enzyme effectively *in vitro* requiring only 1.65×10^{-7} *M* for 50% inhibition. This suggests a high probability of nonselective reaction of the nitrogen mustard group with diverse nucleophilic centers available *in vivo*, thus limiting the availability of this drug for the target enzyme.

Experimental Section‡

Antitumor Activity. Experiments were performed on CD-1 mice. Transplantation of Sarcoma 180 ascites cells was carried out using a donor mouse bearing a 7-day tumor growth. The experimental details were described earlier.[4] Mice were weighed during 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 on the prolongation of survival time afforded by the drug treatment.

Enzyme Inhibition. The ribonucleoside diphosphate reductase was partially purified as described[18] from rat Novikoff ascites tumor cells. Several batches of enzyme were used. Reduction of [^{32}P]CDP (0.17 m*M*, 1.6×10^6 cpm/μmole) was assayed as described,[14,18] except that 4×10^{-5} *M* $Fe(NH_4)_2(SO_4)_2$ was used instead of $FeCl_3$. Dithiothreitol or dithioerythritol (6.2 m*M*) 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; IQ-1 was included as a standard inhibitor in each experiment.

Method A. To a soln of **1** (0.79 g, 5 mmoles) in 50 ml of THF, 0.7 ml of Et_3N was added and cooled in an ice bath. Five mmoles of the appropriate acid chloride dissolved in 10 ml of THF was added slowly with stirring. The reaction mixt was stirred at room temp for 8–12 hr and filtered, solvent removed by evapn *in vacuo*, and then the residue crystd from a proper solvent.

Method B. Compd **11** (5 mmoles) was suspended in 50 ml of THF, and 0.7 ml of Et_3N was added. The mixt was cooled, and a soln of appropriate acid chloride (5 mmoles) in 5 ml of THF was added slowly with stirring. The mixt was then heated at 55–60° for 2 hr; the compd slowly went into soln, and a ppt of $Et_3N \cdot HCl$ formed. After the reaction was completed, the insoluble ppt was filtered, the filtrate was evapd to dryness *in vacuo*, and the residue was crystd from a proper solvent.

Method C. The 5-substituted derivative of 1-methylisoquinoline (3 mmoles), prepared according to either method A or B, was dissolved in 25 ml of dioxane. Freshly sublimed SeO_2 (3 mmoles) was added, and the mixt was heated at 100° for 2 hr. The reaction mixt was then cooled and filtered through Celite, the filtrate evapd to dryness *in vacuo*, and the residue extd with cold dil HCl. The acid extracts were neutralized to pH 3.0 with $NaHCO_3$, whereupon some impurities pptd out which were removed by filtration. The clear

‡ Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ir absorption spectra were determined with a Perkin-Elmer Model 257 spectrophotometer and were consistent with the proposed structures. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values.

soln was then neutralized to pH 7.0, yielding a ppt of the desired carboxaldehyde which was filtered, washed with H_2O, dried, and recrystd from an appropriate solvent.

Method D. The thiosemicarbazones were prepd by treating a soln of desired carboxaldehyde in EtOH with an aqueous soln of thiosemicarbazide acidified with a few drops of dil AcOH. In some cases final compounds were sufficiently pure and were not recrystd.

1-Formyl-5-bis(β-chloroethyl)aminoisoquinoline Thiosemicarbazone (39). Compd **37** (0.192 g, 1 mmole) was added to a mixt of 0.18 g of $NH(CH_2CH_2Cl)_2 \cdot HCl$ and 0.28 ml of Et_3N in 25 ml of C_6H_6. The mixt was refluxed for 18 hr and then filtered to remove $Et_3N \cdot HCl$. The solvent was evapd *in vacuo*, and the residue was treated with a soln of thiosemicarbazide in EtOH acidified with concd HCl. The thiosemicarbazone derivative was isolated as the HCl salt.

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Antitumor and Antileukemic Effects of Some Steroids and Other Biologically Interesting Compounds Containing an Alkylating Agent†,[1]

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p-[*N*,*N*-Bis(2-chloroethyl)amino]phenylacetic acid (BCAPAA) esters and amides of some new steroids and other biologically interesting compounds, two steroid esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenylbutyric acid (BCAPBA), and one steroidal nitrosourea were synthesized and tested for antitumor and antileukemic activity.

Two of the best known groups of cancer chemotherapeutic agents are the steroid hormones and the nitrogen mustard class of alkylating agents.[2] The chemical combination of such compounds as a means of obtaining selective distribution at the tumor site and/or reducing the systemic toxicity of the attached alkylating agents was studied as early as 1952.[3] However, until recent reports from a Russian group[4-6] and from our laboratory,[7] this class of compounds had been reported to display only moderate carcinostatic activities.[3,8] We described the synthesis of a series of steroid esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenylacetic acid (BCAPAA) and reported that some of these compounds were excellent inhibitors of DMBA-induced mammary adenocarcinoma (13762).[7] In addition, the steroid BCAPAA esters showed some interesting antileukemic results and appeared to be much less toxic than other commonly used oncolytic agents.[7] Recently the BCAPAA ester of cholesterol (**1a**) has been tested clinically,[9] and the diester of estradiol (**2a**) is being tested similarly. As a rational extension of this work we have now synthesized: (a) the BCAPAA ester or amide derivative of some new steroids; (b) two steroid esters of the highly active antineoplastic agent *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenylbutyric acid (BCAPBA, chlorambucil); (c) one steroidal nitrosourea derivative; and (d) a selected group of BCAPAA esters and amides of nonsteroidal alcohols and amines. The latter were prepared in order to determine whether active, nontoxic compounds in this category were feasible. Biological test results of these compounds as well as new results on previously reported compounds[7] are presented.

Chemistry. The BCAPAA esters **3** and **4** and the BCAPAA amides **5a** and **6** were prepared by direct acylation of the appropriate hydroxy or amino compound with *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenylacetyl chloride (**5b**). In a like manner the acylation of cholesterol (**1b**) and estrone (**2b**) with *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenylbutryl chloride (**5c**) gave the BCAPBA ester **1c** and **2c**, respectively. It was not possible to prepare the bis-BCAPAA ester of 3α,12α-dihydroxycholanic acid (**7a**) in pure form by direct acylation. The steroid acid (**7b**) was first converted to its benzyl ester (**7c**) which was then smoothly acylated with **5b** to give **7d**. Reductive debenzylation of **7d** using palladium hydroxide on carbon catalyst gave the bis-BCAPAA free acid (**7a**). The coupling of BCAPAA with estra-1,3,5(10)-3-ol-17β-ylamine (**2e**) and *tert*-butyl glycinate in the presence of DCC afforded the amides **2d** and **5d**, respectively. Treatment of **5d** with refluxing CF_3CO_2H gave the glycinamide **5e**. The structure assignments were based on the elemental analysis, the ir spectra, which showed typical ester or amide peaks, and the nmr spectra, which showed resonances at δ 3.61–3.67 and 6.10–7.40 ppm characteristic of the chloroethyl and aromatic protons of the

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