test compound indicated above for 15 min. Experimental and control tissues are subjected to five bath changes during the incubation interval. Changes in bath fluid during the incubation period are helpful in ensuring the reproducibility of tissue responses to the agonist. Control tissues are incubated with test compound vehicle (if any). The same concentration of the agonist is reapplied in the presence of the test compounds, and the response is registered and compared with controls. Percent inhibition produced by the test compound is calculated by subtracting the mean percentage change in control tissue from the mean percentage change in tissues exposed to the test compound. Additional compounds are then evaluated as long as the tissue remains reporducibly responsive to the agonist. Six tissues obtained from six animals are used simultaneously—three controls and three experimental. Partially purified guinea pig SRS-A was prepared and purified as described.[29] FPL-55712 was used as a reference for each compound tested.

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Synthesis and Antitumor Activity of New Platinum Complexes

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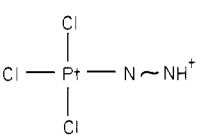
A new type of antitumor platinum complex has been prepared and examined for antitumor activity against L1210 leukemia both in vitro and in vivo. The coordination environment of platinum in these complexes consists of three anionic chloride ions and a positively charged amine. The positive charge is introduced by monoprotonation or monoalkylation of a diamine. Platinum(IV) derivatives have been prepared for several of the complexes, and a water-soluble sulfate derivative has been prepared for one of them. Several of these complexes exhibit significant in vitro activity, and trichloro(3-aminoquinuclidinium)platinum(II) (QTP) exhibits significant in vivo activity as well. An increase in life span of approximately 40% has been observed using QTP. QTP is toxic at doses slightly in excess of effective doses.

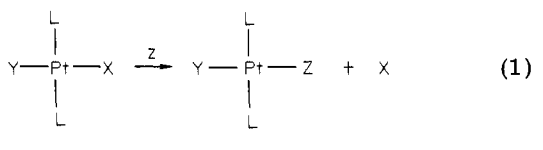
The effectiveness of transition-metal complexes, particularly platinum complexes, as experimental antitumor agents has been demonstrated repeatedly in recent years.[1] *cis*-Dichlorodiammineplatinum(II) (PDD, cisplatin) is the prototype metal complex with antitumor activity and is currently available for clinical use in testicular tumors, ovarian carcinoma, and other tumor types.[2] A large number of platinum complexes have been evaluated in an effort to identify compounds with a broader spectrum of clinical applicability than PDD and lower toxicity than PDD. The vast majority of platinum complexes that have been examined are of the general form *cis*-A_2PtX_2, where X is an anionic ligand, typically chloride, and A is an amine (or A_2 a chelating diamine).

The mechanistic details of the reaction(s) of platinum complexes with cellular macromolecules are still not established satisfactorily, but it is generally agreed that cytostatic activity results from substitution reactions involving displacement of anionic ligands from the metal complex.[3] The rates of substitution reactions at square-planar platinum(II) are dominated by the trans effect.[4] All other factors being comparable, the relative rate of displacement of a particular ligand, X, is dependent on the nature of the ligand, Y, occupying the position trans to it in the complex (eq 1). PDD has a leaving group, the chloride ion, that is situated trans to ammonia. Amines are at the lower end of the trans effect series; consequently, the substitution reactions of Cl^- in PDD are relatively sluggish. The reactivity of PDD, and possibly its biological activity, could be enhanced by replacing ammonia with ligands that are higher in the trans-effect series. Replacing ammonia with anionic ligands would produce negatively charged complexes that might not penetrate target cells readily. However, the desired complex neutrality could be obtained if one ammonia were replaced by an anion and the other ammonia were replaced by a cationic ligand. We have chosen to introduce the chloride ion, which lies above ammonia in the trans-effect series, as the new anionic ligand and to use monoprotonated (or monoalkylated) diamines as the cationic ligands. This results in a series of compounds having the general structure I, where N~



I

NH^+ represents a protonated diamine. The trans effect dictates that a chloride ion lying trans to another chloride will be substitutionally labile, and the symmetry of the complex provides two activated chloride ions that can serve as the initial leaving group. This report describes the synthesis and biological activity of a series of complexes of type I.



(1)

Discussion

Synthesis and Characterization of Complexes. There have been a few reports of platinum complexes with positively charged amine ligands, but in most instances the complexes were prepared only as precursors for studies of

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(2) Einhorn, L. H.; Williams, S. D. *N. Engl. J. Med.* **1979**, *300*, 289.
(3) Rosenberg, B. *Cancer Treat. Rep.* **1979**, *63*, 1433.
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0022-2623/82/1825-0952$01.25/0

Table I. Chemical and Biological Data for Complexes of the Type L^+HPtCl_3

no.	L	formula	ν_{Pt-Cl}, cm^{-1}	anal.[a]	ID_{50},[b] μg/mL L1210/0	ID_{50},[b] μg/mL L1210/PDD
1	3-(aminomethyl)pyridine	$C_6H_9N_2Cl_3Pt$	315, 290	C, H	>10 (>10)	>10 (5)
2	3-[(methylamino)methyl]pyridine	$C_7H_{11}N_2Cl_3Pt$	300, 290	C, H		
3	2-[β-(methylamino)ethyl]pyridine	$C_8H_{13}N_2Cl_3Pt$	300, 290	C, H		
4	4-(2-piperidinoethyl)pyridine	$C_{12}H_{19}N_2Cl_3Pt$	300, 285	H; C[c]		
5	2-(aminoethyl)pyridine	$C_7H_{11}N_2Cl_3Pt$	310, 290	H; C[d]		
6	1,2-bis(4-pyridyl)ethane	$C_{12}H_{13}N_2Cl_3Pt$	290	C, H		
7	nicotine	$C_{10}H_{15}N_2Cl_3Pt$	310, 290	C, H	>10 (4)	>10 (5)
8	1,4-diazabicyclo[2.2.2]octane (dabco)	$C_6H_{13}N_2Cl_3Pt$	308, 290	H, N; C[e]		
9	piperazine	$C_4H_{11}N_2Cl_3Pt$	310, 290	C, H, N		
10	*N*-methylpiperazine	$C_5H_{13}N_2Cl_3Pt$	302, 285	C, H, N	9	>10
11	3-aminoquinuclidine	$C_7H_{15}N_2Cl_3Pt$	310, 300, 280	C, H, N	2 (>10)	5[f] (>10)
12	*N*-aminopiperidine	$C_5H_{13}N_2Cl_3Pt$	300, 285	C, H, N	4.2	>10
13	2,5-dimethylpiperazine	$C_6H_{15}N_2Cl_3Pt$	305, 285	C, H; N[g]	10	>10
14	3-aminopyridine	$C_5H_7N_2Cl_3Pt$	305, 290	C, H		

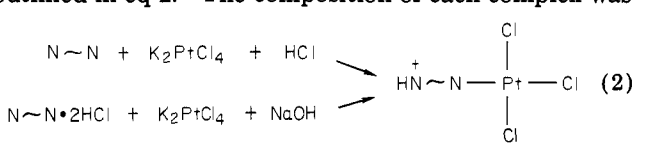
[a] Analytical values were all within ±0.4%, except where noted otherwise. In all cases, chloride analyses were also obtained and confirm the formulations given. [b] Figures in parentheses obtained with drug solubilized in Me_2SO; where no figures are entered for a compound this signifies that the ID_{50} exceeds 10 μg/mL. [c] C: calcd, 29.27; found, 28.58. [d] C: calcd, 19.80; found, 19.14. [e] C: calcd, 17.39; found, 16.65. [f] Average of values for five independent determinations on four different preparations. [g] N: calcd, 6.73; found, 6.22.

Table II. Chemical Data for Platinum(IV) Complexes of the Type $L^+PtCl_3X_2$[a]

no.	L	X	formula	ν_{Pt-Cl}, cm^{-1}	method of preparation
15	*N*-methyldabco	OH	$C_7H_{17}N_2O_2Cl_3Pt$	300	D
10	3-aminoquinuclidine	OH	$C_7H_{17}N_2O_2Cl_3Pt$	312	D
17	3-aminoquinuclidine	Cl	$C_7H_{15}N_2Cl_5Pt$	310, 285	E
18	nicotine	OH	$C_{10}H_{17}N_2O_2Cl_3Pt$	310, 280	D
19	3-[(methylamino)methyl]pyridine	OH	$C_7H_{13}N_2O_2Cl_3Pt$	310, 280	D
20	3-[(methylamino)methyl]pyridine	Cl	$C_7H_{11}N_2Cl_5Pt$	310, 290	E
21	1,1,4-trimethylpiperazine	OH	$C_7H_{19}N_2O_2Cl_3Pt$		D

[a] All of the compounds in this table were inactive when evaluated against L1210 in vitro, except for compounds 16 and 17 (see Table III).

the kinetics of ring closure upon deprotonation. To our knowledge, only three complexes having structure I have been characterized. Terzis[5] prepared and determined the crystal structure of trichloro(9-methyladeninium)platinum(II), Maresca et al.[6] prepared trichloro(tetramethylethylenediammonium)platinum(II), and Adeyemo et al.[7] prepared trichloro(4-amino-2,6-dimethylpyrimidine)platinum(II). We find that a large number of complexes of type I can be prepared using the two general reactions outlined in eq 2. The composition of each complex was



(2)

verified by C, H, and (generally) N microanalysis (Table I). Under the preparative conditions employed, a variety of products or impurities [e.g., $(N\sim N)_2PtCl_2$, $(N\sim NH^+)_2PtCl_4$, etc.] are plausible and might not be readily detected by C, H, and N analysis. However, these alternative formulations differ significantly in chloride ion content, and we have consequently obtained Cl analyses for all complexes prepared. These data, combined with the other analytical data, demonstrate unequivocally a ratio of 3Cl/diamine/Pt. Although the analytical data for a few of the compounds do not fall within generally accepted limits, purification was generally impossible due to both low solubility and potentially interfering hydrolysis reactions.

Several derivatives of structure I, desirable because of enhanced aqueous solubility and because they possessed characteristics useful for possible structure–activity studies, were prepared by the reactions summarized in eq 3. These

$$LPtCl_3 + Ag_2SO_4 \rightarrow LPtCl(SO_4)\cdot H_2O + 2AgCl$$

$$LPtCl_3 + 2H_2O_2 \rightarrow LPt^{IV}Cl_3(OH)_2 + 2H_2O$$

$$LPtCl_3 + H_2O_2 + 2HCl \rightarrow LPt^{IV}Cl_5 + 2H_2O \quad (3)$$

last two reactions, which represent oxidation to Pt(IV), are difficult to control. Furthermore, the Pt(IV) complexes are somewhat unstable and subject to an apparent hydrolysis reaction. Consequently, analytical results for some of them are not totally satisfactory. Again, however, the combination of C, H, N, and Cl analyses (obtained for all of these complexes) are adequate to establish the ratio of 3(or 5)Cl/diamine/Pt/2(or 0)OH.

Certain diamines could not be induced to form complexes of type I. Powerful chelators, such as ethylenediamine, apparently do not provide a stable complex that is coordinated at only one nitrogen. Amines containing two equivalent but electronically remote nitrogen atoms, such as 1,2-bis(4-pyridyl)ethylene, generally protonate simultaneously and lead to tetrachloroplatinate salts, $(LH_2^{2+})PtCl_4$. Finally, some amines fail to produce the desired complexes for reasons that are not readily identified.

We have examined infrared spectra of all of these complexes. The spectra are, in general, complex and are not open to simple interpretation. In general, however, these compounds show two absorption bands in the platinum–

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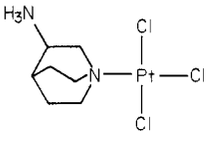
(6) Maresca, L.; Natile, G.; Rizzardi, G. *Inorg. Chim. Acta* **1980**, *38*, 137.
(7) Adeyemo, A.; Teklu, Y.; Williams, T. *Inorg. Chim. Acta* **1981**, *51*, 19.

chlorine stretching region (Tables I and II). Three infrared active modes ($2A_1 + B_1$) are expected on the basis of symmetry considerations, but only two distinct absorptions are usually observed in the related ions $(LPtCl_3)^-$.[8]

It is a difficult problem to determine which of the two nitrogen sites of the diamine is coordinated to platinum and, consequently, which nitrogen site is protonated. It is probable that the specific isomeric form that is produced is a consequence of some complex interplay of both kinetic and thermodynamic factors. In particular, its identity will depend upon the Lewis basicity of each nitrogen donor site toward the Lewis acid platinum in the form $PtCl_3^-$. Since knowledge of that Lewis basicity does not exist, we have no predictive ability. In the case of compound 11, we have tried to resolve the structural question by examination of infrared spectra in the N–H stretching region. Comparison of spectra for 3-aminoquinuclidine, its mono- and diprotonated hydrochlorides, and compound 11 suggests that in the platinum complex it is the NH_2 group which is protonated. Specifically, a broad and intense band at ca. 2800 cm^{-1}, which can be assigned to the NH^+ group in the mono- and dihydrochloride, is absent from compound 11. Infrared spectra thus suggest structure II for compound



II

11. Although we were able to prepare single crystals of compound 11, the crystals proved to be twinned and, consequently, not usable for X-ray structure determination purposes.

Biological Studies. All compounds reported have been evaluated as inhibitors of the growth of murine leukemic cells (L1210) and a PDD-resistant subline (L1210/PDD). We have chosen a 50% inhibitory dose (ID_{50}) of 10 μg/mL as our upper-limit criterion for a significant level of activity. Compounds that satisfy the criterion are evaluated further for ability to prolong the life span of mice inoculated with L1210 cells.

It must be stressed that there is no direct evidence concerning the forms of the compounds in Table I under conditions of biological evaluation. (Indeed, to some degree the same statement applies to PDD.) The acid–base (eq 4) and hydrolysis (eq 5) equilibria will be established in

$$(HN\sim N)PtCl_3 \rightleftharpoons H^+ + [(N\sim N)PtCl_3]^- \quad (4)$$

$$[(N\sim N)PtCl_3]^- + H_2O \rightleftharpoons (N\sim N)PtCl_2(H_2O) + Cl^- \quad (5)$$

solution, and the dominant species at physiological pH will be a function of the pK of the coordinated amine, the chloride ion concentration, and time. For the majority of the diamines employed in our studies, the pK's are such that the protonated form should predominate. (For example, we have measured the two pK_a values for protonated 3-aminoquinuclidine as 8.6 and ca. 11.) It is of course possible that coordination of one nitrogen center to platinum will alter the pK of the other nitrogen center, although we do not expect this effect to be large.

We also must emphasize that the water solubility of the compounds in Table I is not high; the poor activity observed for several of the compounds may reflect poor solubility rather than inherent inactivity of the molecule. Dipolar aprotic solvents, such as dimethyl sulfoxide, are frequently used to solubilize compounds for biological studies, and, as indicated in Table I, parallel experiments were carried out, in vitro, on some of the compounds dissolved in Me_2SO. In several instances, the complexes solubilized in Me_2SO exhibited enhanced activity over that observed when water was used as the administration vehicle; in the case of the trichloro(3-aminoquinuclidinium)platinum(II) (compound 11), activity was lower when the complex was solubilized with Me_2SO. It is difficult to interpret the results of studies obtained in the presence of Me_2SO, since Me_2SO can react with complexes of this type[9] by sequential substitution for chloride ions. Thus, a mixture of Me_2SO-containing species may be present in the cell culture experiments.

Most of the compounds that we have prepared have relatively little activity as inhibitors of the growth of L1210 and L1210/PDD cells in vitro (Table I). The most interesting compound in terms of biological activity is compound 11 with 3-aminoquinuclidine as the ligand, especially in view of its ability to produce roughly equivalent inhibition of the growth of both L1210 and L1210/PDD cells. Several compounds related to compound 11 were evaluated in vitro. These compounds (Table III) had activity against the "standard" L1210 cells comparable to that of compound 11 and were less effective against L1210 cells resistant to PDD.

Compound 11 was administered intraperitoneally to mice with L1210 leukemia. Studies in our laboratory re-

Table III. Activity of 3-Aminoquinuclidinium Complexes against L1210 Cells in Vitro

compd	ID_{50}, μg/mL L1210/0	ID_{50}, μg/mL L1210/PDD
$LPtCl_3$ (11)	2	5
$LPtCl_5$ (17)	4.4	>10
$LPtCl_3(OH)_2$ (16)	4.5	>10
$LPtCl(SO_4)$	4.6	>10

Table IV. Activity of Trichloro(3-aminoquinuclidinium)platinum(II) against L1210 Cells in Vivo[a]

no.	dose, mg/kg	schedule	% T/C
1[b]	5	1	123
	10	1	125
	50	1	toxic
	1	1, 2, 5, 6, 9	100
	5	1, 2, 5, 6, 9	138
2[c]	0.62	1, 5, 9	110
	1.25	1, 5, 9	122
	2.50	1, 5, 9	126
	5	1, 5, 9	120
	10	1, 5, 9	130
	20	1, 5, 9	133
3	2.5	1, 5, 9	119
	5	1, 5, 9	122
	10	1, 5, 9	139
	20	1, 5, 9	140
	40	1, 5, 9	148

[a] Because of the low solubility of this compound, data from different laboratories may not be directly comparable due to different sonification/suspension/solubilization procedures. [b] Data obtained at the University of Vermont. [c] Experiments 2 and 3 represent data obtained from the NCI utilizing two different screening contractors.

(8) Goodfellow, R. J.; Goggin, P. L.; Duddell, D. A. *J. Chem. Soc. A* **1968**, 504.

(9) Tobe, M. L.; Khokhar, A. R. *J. Clin. Hematol. Oncol.* **1977**, *7*, 114.

vealed that the compound has significant antileukemic activity. For example, when given as a single dose (10 mg/kg; day 1) the compound gave a percent T/C value of 125%, and the percent T/C value was increased somewhat (to 138) when a more intensive schedule of administration was employed (5 mg/kg; days 1, 2, 5, 6, and 9). Our results were confirmed by independent studies carried out under the auspices of the Developmental Therapeutics Program, National Cancer Institute (Table IV). We also evaluated the soluble sulfato derivative of compound 11 in vivo, but we found it to be more toxic than compound 11 and to be ineffective against murine leukemia at a nontoxic dose (5 mg/kg). The biological activity of compound 11 is not shared by the ligand per se since 3-aminoquinuclidine hydrochloride was found to exhibit no cytotoxicity in vitro under conditions in which the complex was quite active.

We have examined several other compounds for in vivo activity. A complex which satisfies our cell culture criterion for activity (compound 10), one which fails to satisfy our criterion (compound 8), and an N-alkylated diamine complex [trichloro(N,N,N^1-trimethylpiperazinium)platinum-(II)] all have been studied in mice, but in none of these cases was significant activity observed at any dose.

The activity of compound 11 in the experimental leukemia system prompted us to carry out toxicological studies on this complex. When administered as a single intraperitoneal injection, compound 11 caused no immediate signs of toxicity, but within 48 h, mice became lethargic, developed piloerection, and displayed signs of tetany. The toxic effects progressed until paralysis of the hind limbs was observed. Deaths occurred 3 to 8 days following treatment. Gross necropsy revealed signs of renal toxicity, including pale renal cortex, friability, and exaggerated corticomedullary border. Other grossly observable signs of toxicity were sharply reduced spleen size and distention of the small intestine. The calculated LD_{10}, LD_{50}, and LD_{90} values were 22, 42, and 83 mg/kg, respectively. Toxic signs and gross necropsy findings were similar in mice given compound 11 as five daily intraperitoneal injections. The LD_{10}, LD_{50}, and LD_{90} values for the latter regimen were 10, 25, and 48 mg/kg, respectively.

Our studies of the biological activity of compound 11 indicate that complexes of the type $LPtCl_3$ represent a new class of antitumor platinum coordination compounds. We are currently engaged in studies of the preparation and evaluation of new examples of this class of compound that may be more effective and less toxic than compound 11.

Experimental Section

Chemistry. Most of the complexes were obtained by the representative methods described in the following paragraphs; those that required special procedures are discussed individually. All the ligand (diamines) were purchased from Aldrich Chemical Co. and used without further purification. K_2PtCl_4 was obtained from Matthey Bishop, Inc. 1,1,4-Trimethylpiperazinum chloride and N-methyldabconium chloride were prepared by established methods.[10,11] Infrared spectra of the complexes (as KBr pellets) were recorded on a Beckman IR-20A spectrophotometer (4000–250 cm^{-1}); Pt–Cl stretching frequencies are listed in Tables I and II. Elemental analyses were performed by Integral Microanalytical Laboratories, Inc., Raleigh, NC.

Preparation of Complexes. Method A. [Trichloro(N-dabconium)platinum(II)] (8). 1,4-Diazabicyclo[2.2.2]octane (Dabco; 0.112 g, 1 mmol) was dissolved in water (5 mL), and 3 M HCl (0.33 mL) was added. The resulting solution was added to a filtered aqueous solution of K_2PtCl_4 (0.415 g, 1 mmol), and the reaction mixture was stirred at room temperature for 3 to 4 h. The orange-yellow product was filtered off and then washed with water, ethanol, acetone, and finally ether. The product was dried over P_2O_5 under vacuum: yield 65%.

Method B. Trichloro(3-aminoquinuclidinium)platinum-(II) (11). 3-Aminoquinuclidine dihydrochloride (0.199 g, 1 mmol) was dissolved in water (5 mL) and NaOH (0.04 g, 1 mmol) was added. The resulting solution was added to a filtered aqueous solution of K_2PtCl_4 (0.415 g, 1 mmol), and the reaction mixture was stirred at room temperature for 24 h. The pale orange-yellow product was filtered off, washed with water, ethanol, and then acetone, and finally dried over P_2O_5 under vacuum: yield 72%.

Method C. Trichloro(N-methyldabconium)platinum(II). K_2PtCl_4 (0.415 g, 1 mmol) was dissolved in water (5 mL), and the filtered solution was treated with N-methyldabconium chloride (0.162 g, 1 mmol) in water (5 mL). The reaction mixture was stirred at room temperature for 4 to 5 h. The orange-colored product was filtered off and washed with an excess of water, ethanol, and acetone. Finally, the product was dried under vacuum: yield 72%. Trichloro(1,1,4-trimethylpiperazinium)-platinum(II) was also prepared by this method. Neither of these compounds exhibited significant cytotoxicity.

Method D. Trichlorodihydroxo(3-aminoquinuclidinium)platinum(IV) (16). Hydrogen peroxide (10 mL, 30% aqueous) was added to a heated and stirred suspension of trichloro(3-aminoquinuclidium)platinum(II) (0.05 g) in water. There was a vigorous effervescence, and the product was obtained in a quantitative yield by evaporating the resulting solution to dryness. The brown product was dried over P_2O_5 under vacuum.

Method E. Pentachloro(3-aminoquinuclidinium)platinum(IV) (17). $PtCl_3(OH)_2$(3-aminoquinuclidinium) was prepared as in method D but not separated from the reaction mixture. When effervescence had ceased, concentrated hydrochloric acid was added, and the mixture was heated further for a few minutes. On cooling, a nearly quantitative yield of the yellow precipitate was filtered off and washed with water, then acetone, and finally ether. The compound was dried under vacuum.

Method F. (3-Aminoquinuclidinium)monochlorosulfatoplatinum(II). Trichloro(3-aminoquinuclidinium)platinum(II) (1.0 g, 2.3 mmol) was suspended in water (20 mL), and a solution of Ag_2SO_4 (0.725 g, 2.3 mmol) in water (100 mL) was added. The reaction mixture was stirred at room temperature for several hours in the dark. The precipitated AgCl was filtered off, and the yellow filtrate was evaporated to dryness using a rotary evaporator. A quantitative yield of brown-yellow product was obtained and dried over P_2O_5 under vacuum: IR ν 1140 (SO_4) cm^{-1}.

Biological Studies. Mouse leukemic L1210 cell lines studied at Vermont were obtained from Dr. Burchenal's laboratory at Sloan-Kettering Institute (Rye, NY) and were maintained in McCoy's 5A medium containing 10% fetal calf serum (Grand Island Biological Co., Grand Island, NY). L1210/0 cells were highly sensitive (ID_{50} = 0.5 μg/mL) to PDD.[12] PDD controls were periodically run to verify the continued viability of the screen. Compounds to be tested were dissolved in distilled water or dimethyl sulfoxide (Me_2SO); compounds that were poorly soluble in water were also prepared in suspension. Stock solutions were prepared at constant ratios up to 500 times that required in the growth medium, so that 10 μL of stock solution could be added to 5 mL of inoculated growth medium; the maximum concentration of Me_2SO in incubation media was 0.2%, a concentration that had no effect on cell growth or viability. Cells were inoculated into media at a concentration of approximately 5×10^4 cells/mL and allowed to grow for 96 h in 5% CO_2 at 37 °C. Control cells grew to a density of 1.2×10^6 cells/mL; cell density was measured by use of an electronic cell counter (Coulter Counter, Model ZF).

To evaluate the activity of compounds against L1210 in vivo, we employed the following procedure. L1210 cells (1×10^6 suspended in 0.1 mL of physiological saline solution) were inoculated intraperitoneally into BDF_1 mice (20–22 g) and drug treatment (ip) initiated 24 h after inoculation of leukemic cells. Drugs were dissolved or suspended in 0.3% hydroxypropylcellulose in saline ("Klucel", obtained from the National Cancer Institute,

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(12) Burchenal, J. H.; Kalaher, K.; Dew, K.; Lokys, L. *Cancer Treat. Rep.* **1979**, *16*, 1493.

NIH, Bethesda, MD). Animals that received no drug treatment died between 7 and 9 days after inoculation of L1210 cells. All animals were housed in central animal facilities having controlled temperature, relative humidity, and photoperiods.

Toxicological studies were done in male CD_1 albino mice (18–25 g). Compound 11 was administered intraperitoneally as a suspension in Klucel such that 0.1 mL of suspension/10 g of body weight delivered the desired dose. Two treatment schedules were evaluated, i.e., a single intraperitoneal injection or five daily intraperitoneal injections. Mice were observed daily for 14 days after the final injection. The LD_{10}, LD_{50}, and LD_{90} values were calculated for each treatment schedule using the probit analysis method of Finney.[13] Gross necropsy examination was performed on all mice that died during the observation period as well as those mice sacrificed at the completion of the study.

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Affinity Therapeutics. 1. Selective Incorporation of 2-Thiouracil Derivatives in Murine Melanomas. Cytostatic Activity of 2-Thiouracil Arotinoids, 2-Thiouracil Retinoids, Arotinoids, and Retinoids

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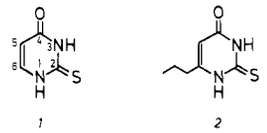
The incorporation of 2-[^{35}S]thiouracil and two of its derivatives into murine melanomas, in vivo, was studied. It was confirmed [J. R. Whittaker, *J. Biol. Chem.*, **246**, 6217–6226 (1971)] that 2-thiouracil has a marked affinity for melanin-producing tissue and that an affinity for such tissue could be sustained by 5-substituted 2-thiouracils. A series of derivatives of arotinoids and retinoids, with or without a 2-thiouracil group as a potential carrier to obtain affinity for melanomas, was examined for cytostatic activity, in vitro. None of these showed significant activity against murine melanomas.

The lack of tissue selectivity of the presently used cancer chemotherapeutic drugs constitutes a major problem. Consequently, the construction of chemotherapeutics that show specific affinity for, in casu, melanoma tissue would constitute an important improvement in such drugs.

It is known that 2-thiouracil (1) and 6-propyl-2-thiouracil



1 2

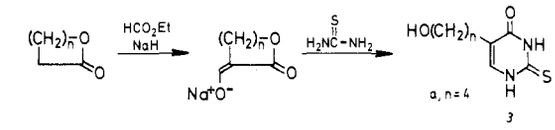
(2) exhibit marked affinities for melanin-producing tissue in vitro,[1] and 1 a similar affinity in vivo,[2] where they presumably act as false precursors for melanin.[1,2]

This affinity for melanin-producing tissue offers a possibility for preparing new potent drugs against malignant melanoma, where primary tumors and metastases often show a very high rate of melanin synthesis.

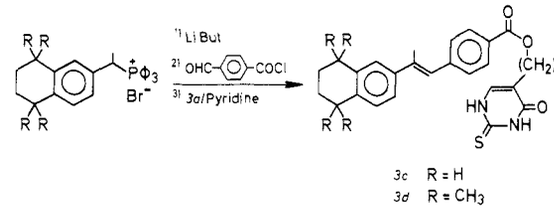
The necessary prerequisites for the development of an anticancer drug based on a carrier capacity of 2-thiouracil are (1) that 2-thiouracil can act as a carrier of substituents into the target tissue, i.e., that the affinity of the thiouracil moiety is of such a character that it is sustained in variously substituted derivatives, and (2) that substituents with, for example cytostatic properties or substituents capable of releasing, for example cytostatic drugs can be inserted into the thiouracil nucleus, etc.

We are attempting to develop such drugs, and our initial strategy was prompted by early reports that indicated that retinoids might cause regression of prenoplastic lesions and malignant skin lesions.[3–5] Furthermore, studies, in vitro, have shown that retinoids can inhibit cell proliferation and, idependently of this, stimulate melanogenesis.[6] This, combined with the fact that the incorporation of thiouracil was known to be related to the rate of melanin synthesis, constituted the basis for the present work, which indicates that it is possible to design derivatives of 2-thiouracil with affinity for murine melanomas.

Scheme I



Scheme II



Results

Chemistry. From a biochemical point of view, it appears most obvious to introduce substitutents in the 5- or

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(1) J. R. Whittaker, *J. Biol. Chem.*, **246**, 6217–6226 (1971).
(2) L. Dencker, B. Larsson, K. Olander, S. Ullberg, and M. Yokota, *Br. J. Cancer*, **39**, 449–452 (1979).
(3) W. Bollag, *Eur. J. Cancer*, **10**, 731–737 (1974).
(4) R. Lotan, G. Neumann, and D. Lotan, *Cancer Res.*, **40**, 1097–1102 (1980).
(5) W. Bollag and A. Matter, *Ann. N.Y. Acad. Sci.*, **359**, 9–23 (1981).
(6) R. Lotan and D. Lotan, *Cancer Res.*, **40**, 3345–3350 (1980).

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