9.6 (Na_2CO_3) and extracted with 10 mL of EtOAc. The EtOAc extract was evaporated (N_2) and derivatized with 50 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (60 °C, 15 min) just prior to GC–MS analysis. For comparisons, a standard (50–200 μg of **12**·HCl) was dissolved in 1 mL of H_2O. Sodium bisulfite was added (10 mg), and the sample was immediately extracted with 2 × 5 mL of EtOAc. The combined EtOAc extracts were evaporated (N_2) and derivatized with BSTA as described for the metabolic samples.

The propranolol metabolites were separated with a J&W DB-5 fused silica capillary column (30 m × 0.25 μm film). Separation was achieved with a 5710A Hewlett-Packard gas chromatograph interfaced with the VG-7070 mass spectrometer. Chromatographic conditions were as follows: injector temperature, 250 °C; helium flow rate, 60 mL/min; column head pressure, 15 psi; temperature programmed from 220–300 °C at 5 °C/min. The mass spectrometer was operated at 20 eV, and the ion source was maintained at 200 °C.

Pharmacological Testing. Affinity for β_1- and β_2-adrenoceptors was assessed in guinea pig atria and rat uterus, respectively. Studies in guinea pig atria were performed in Krebs' solution [composition (mM): NaCl, 118; KCl, 4.7; $MgCl_2$, 0.54; $CaCl_2$, 2.5, NaH_2PO_4, 1.0; $NaHCO_3$, 25; glucose, 11], while studies in rat uterus were performed in DeJalon's solution [composition (mM): NaCl, 154, KCl, 5.6, $CaCl_2$, 0.4; $NaHCO_3$, 6; glucose, 2.7]. All solutions were prepared with demineralized water and were continually gassed with a mixture of 95% O_2/5% CO_2. Studies for β_1-adrenoceptor antagonism activity in guinea pig atria were performed at 30 °C with norepinephrine as the agonist. All studies were performed in the presence of 10 nM ICI 118551, a potent β_2-adrenoceptor antagonist.[24] Studies for β_2-adrenoceptor antagonism activity in rat uterus were performed at 25 °C (to reduce spontaneous activity) with isoproterenol as the agonist. All tissues were attached to Grass FT-03 isometric transducers connected to a Beckman R4 Dynograph recorder and allowed to equilibrate for at least 2 h before drug addition. Dose–response curves were obtained by the method of stepwise cumulative addition of the agonist.[27] The concentration of agonist in the muscle chamber was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximum level and remained steady. Drugs were washed from the preparation at regular intervals by the overflow method after completion of a dose–response curve. Consecutive dose–response curves on a given tissue were spaced at least 1 h apart to ensure maximum washout of agonists and to minimize receptor desensitization. In all experiments, at least one uterine strip receiving no antagonist was run in parallel with the experimental strips to correct for time-dependent changes in agonist sensitivity.[28]

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Registry No. **1**, 525-66-6; **3**, 89710-82-7; **4**, 83-72-7; **4** silver(I) salt, 36417-25-1; **5**, 42164-68-1; **6**, 89710-74-7; **7**, 89710-75-8; **8**, 89710-76-9; **9**, 89710-77-0; **10**, 89710-78-1; **11**, 89710-79-2; **12**, 89710-81-6; **12**-HCl, 89710-80-5; $H_2C{=}CHCH_2Br$, 106-95-6.

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Synthesis, Characterization, and Properties of a Group of Platinum(IV) Complexes

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The synthesis, characterization, and antitumor properties of a group of platinum(IV) complexes is presented. The compounds, formed by oxidation of *cis*-dichlorodiammineplatinum(II) (1) or its *cis*-dihydroxo analogue, were characterized by elemental analysis and infrared and ^{195}Pt NMR spectroscopies. EPR studies of aqueous solutions containing the spin trap phenyl-*tert*-butylnitrone and various platinum(IV) complexes revealed that the compounds are incapable of producing radical species which may in turn cause DNA breakage. It appears that the antitumor activity of the compounds is either due to Pt(IV) binding via ligand displacement to important cellular components or through the ability of the compounds to undergo in vivo reduction to platinum(II) species, which in turn exert their cytotoxic effects in a manner analogous to 1. As a group, the platinum(IV) compounds were found to be significantly less active against L-1210 leukemia than the parent platinum(II) complex, 1.

The study of the mechanism of action of the anticancer drug *cis*-dichlorodiammineplatinum(II) (1) and its analogues is under active investigation.[1-3] The bulk of the evidence suggests that 1 exhibits its cytotoxic effects by direct interaction with cellular DNA. Although the interaction is site specific and involves platination at oligoguanine sequences of DNA,[4] 1 does not cleave DNA as does bleomycin[5] and neocarzinostatin.[6]

In addition to square planar platinum(II) compounds, a number of octahedral platinum(IV) complexes are known to be active as antitumor agents.[2,7-11] However, unlike

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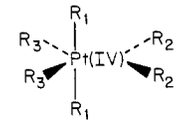
Table I. Structure and Antitumor Properties of the Compounds



				antitumor act. against L1210[a]			
				treatment on day 1 only		treatment on days 1–5	
no.	R_1	R_2	R_3	opt dose, mg/kg ip	max[b] % T/C	opt dose, mg/kg, ip, per injection	max[b] % T/C
1		Cl^-	NH_3	6–8	179–183	1–3	175–186
2[c]	OH^-	Cl^-	$NH_2CH(CH_3)_2$	32	171	16	207
3[d]	OH^-	Cl^-	NH_3	48	150[e]	12	157[f]
4[d]	Br^-	Cl^-	NH_3	12	136	0.75	129
5[g]	Cl^-	Cl^-	NH_3	32	121	4	114
6[g]	OH^-	OH^-	NH_3	32	117	32	133

[a] Mice implanted ip with 10^6 L1210 ascites cells. Treatment groups consisted of six mice. Compound 1 was evaluated with the Pt(IV) compounds. [b] Percent T/C as defined in ref 9. [c] Antitumor data are taken from ref 10, where multiple treatments consisted of once daily injections on days 1 to 9. [d] The test vehicle was water. [e] Reference 9: optimum dose, 120 mg/kg; max % T/C, 164. [f] Reference 9: optimum dose, 20 mg/kg per injection, given on days 1 to 9; max % T/C, 193. [g] The test vehicle was water plus (carboxymethyl)cellulose.

Table II. Infrared and ^{195}Pt NMR Data for the Compounds

	IR, cm^{-1}						^{195}Pt NMR (Me_2SO-d_6)[c]	
no.[a]	OH stretch	NH stretch	M–OH bend	M–N stretch, M–O stretch	M–Cl stretch	other[b]	δ (ppm)	$\Delta\nu_{1/2}$, Hz
2	3535 (s)	3390 (m), 3180 (s)	1050 (m), [1031 (m)]	565 (w), 558 (m), 530 (s)	336 (s)		+956	375
3	3520 (vs)	3265 (s), 3173 (w)	1039 (vs)	550 (s), 450 (m)	345 (m), 330 (sh)	290 (sh), 280 (sh), 270 (m)	*d*, *e*	
4		3260 (s), 3170 (s)		554 (m), 498 (m)	330 (m), 347 (s)		−980	550
5		3345 (w), 3265 (m), 3180		551 (w), 520 (w)	355 (s), 346 (s), 331 (sh)		−124[f]	620
6[d]	3480 (sh)	3364 (mb), 3230 (mb)	1055 (m)	553 (sb), 528 (s),		338 (sh), 307 (sh), 287 (mb)	*d*	

[a] See Table I for structure. [b] Band assignment unknown. [c] Chemical shift reference is K_2PtCl_6 in Me_2SO-d_6. Shifts to the high-field side of the reference are negative. [d] Insufficient solubility in Me_2SO for a ^{195}Pt NMR spectrum. [e] δ, ^{195}Pt (H_2O–H_2O_2) +860 ppm.[15,22] [f] δ, ^{195}Pt (H_2O) −145 ppm.[15]

divalent complexes of platinum, the tetravalent oxidation state of the metal exhibits slow exchange kinetics, and DNA binding involving ligand displacement, as is the case with 1, is less likely for Pt(IV). Thus, it has been have suggested[7,13–15] that the anticancer activity of compounds, such as *cis,trans,cis*-$Pt^{IV}(i\text{-}PrNH_2)_2(OH)_2Cl_2$ (2) is the result of in vivo reduction of platinum(IV) to biologically active platinum(II) species. The concept that platinum (IV) compounds can serve as prodrugs for platinum(II) complexes is supported by the observation that ascorbic acid and cysteine, ubiquitous biological reducing agents, are capable of reducing Pt(IV) to Pt(II).[14,15] However, a second explanation for the antitumor activity of platinum(IV) complexes may lie in the observation that the Pt(IV) complex, 2, can cleave DNA. Mong et al.[16,17] have recently shown that incubation of PM2 DNA with 2 results in the formation of both intrastrand cross-links and the appearance of DNA breakage. They speculated that similar to bleomycin, the compound is capable of producing hydroxyl or superoxide radicals, which in turn cleave DNA. In view of the emerging importance of platinum(IV) compounds, such as 2, as potential second-generation analogues of *cis*-dichlorodiammineplatinum(II) and in light of the uncertainty in the mechanism by which these compounds are able to kill cells, we initiated a study of a series of octahedral Pt(IV) complexes. In this report we describe the synthesis, characterization (using IR and ^{195}Pt NMR spectroscopies), and antitumor properties of a group of platinum(IV) compounds produced by oxidation of 1 or its *cis*-dihydroxo analogue. Using the spin trap phenyl-*tert*-butylnitrone (PBN) and EPR spectroscopy, we further show that the Pt(IV) compounds, including 2, are incapable of producing a radical species that could potentially lead to DNA damage.

Results and Discussion

Synthesis and Characterization of the Compounds. The structures of the compounds employed in this study and their antitumor data are given in Table I. Subjecting 1 to the strong oxidizing agents hydrogen peroxide, bro-

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mine, or chlorine converts the drug into the *trans*-dihyroxo, *trans*-dibromo, and *trans*-dichloro complexes, **3–5**. Dissolution of **1** in strongly basic media results in loss of the chloride ligands and the formation of the corresponding *cis*-dihydroxo analogue.[1] Addition of hydrogen peroxide to the basic solution containing *cis*-$Pt^{II}(NH_3)_2(OH)_2$ produces the tetrahydroxyplatinum(IV) species, **6**. Attempts to use *tert*-butyl hydroperoxide, benzoyl peroxide, and iodine in aqueous media to form, respectively, the corresponding *trans*-hydroxo, *tert*-butoxide, *trans*-hydroxobenzoate and *trans*-diiodido by oxidation of **1** were unsuccessful. Although *tert*-butyl hydroperoxide affected oxidation, as was evidenced by a color change in the solution, workup revealed the product to be the *trans*-dihydroxo compound **3**.

The infrared and ^{195}Pt NMR spectral data for the complexes are given in Table II. Compounds **2** and **3** exhibit a single strong absorption for the OH stretch at ~3500 cm^{-1}. Since the absorption in the spectrum of **3** at 1039 cm^{-1} does not occur in the spectrum of either the *trans*-dichloro or the *trans*-dibromo analogues, **5** and **4**, the band has been assigned to the Pt–O–H bending mode of the compound.[15,18,19] The relatively rich infrared spectrum of **2** precluded definitive assignment of the OH bending mode for the compound, but it appears to be at either 1050 or 1031 cm^{-1} (Table II). The IR spectrum of the tetrahydroxo analogue **6** exhibits broad featureless bands indicative of hydrogen bonding in the solid state. The Pt–OH bending mode occurs as a broadened band at 1055 cm^{-1}.

Two earlier studies[20,21] have disagreed on the number of Pt–N stretching modes possessed by *cis*-$Pt^{IV}(NH_3)_2Cl_4$ (**5**). However, synthesis of the compound in the manner described by Kauffman[22] and the synthetic approach used in this study, as well as the two earlier investigations,[20,21] shows that **5** possess *two* Pt–N stretching modes (551 and 520 cm^{-1}, Table II) and not *one* as claimed by James and Nolan.[20] Point group analysis predicts *two* IR active Pt–N stretching modes (A_1 and B_1) for the compound.[18] Three of the four Pt–Cl stretching modes for **5** can be found in the region 360–330 cm^{-1} (Table II). However, due to the limitations of the spectrometer, the fourth Pt–Cl stretching mode for **5**, at ~200 cm^{-1},[20,21] as well as the Pt–Br stretches of **4** <250 cm^{-1}, could not be observed.

The ^{195}Pt NMR data for **2**, **4**, and **5** in Me_2SO solution are given in Table II. In each case, the spectrum the complex shows a single, broad, resonance line. Studies involving ^{15}N-labeled platinum compounds[23,24] have shown that the width of the resonance line (600 Hz, 8 ppm) in such systems is due to coupling to and quadrapolar broadening by ^{14}N present in the ligands directly bound to the platinum ion. In view of the broad nature of the line, the presence of other platinum-containing species within ~5 ppm of the major signal cannot be excluded.

The ^{195}Pt NMR chemical shift is known to be sensitive to the nature of the groups attached to the metal ion,[25] and, in this case, substitution of *trans*-hydroxyo groups for *trans*-bromo ligands results in a ~2000-ppm shift of the resonance (Table II). Although the origin of ^{195}Pt NMR chemical shifts is not known with certainty,[25] the ability of the trans groups to deshield the platinum nucleus for the three compounds studied is in the order $OH^- > Cl^- > Br^-$.[26] The low solubility of compounds **3** and **6** in Me_2SO precluded determination of their ^{195}Pt NMR spectra in the sulfoxide.

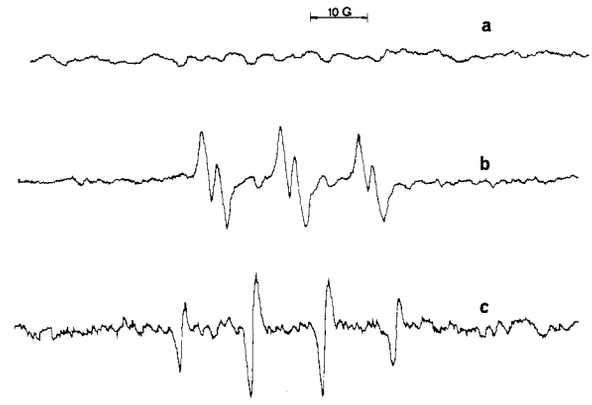

Figure 1. (a) The EPR spectrum of 0.05 M aqueous PBN heated to 100 °C for 2 min is shown; receiver gain 2.5 × 10^4. (b) The EPR spectrum of trapped hydroxyl radical is shown. The radical was generated by heating an aqueous solution (100 °C, 2 min) containing 0.05 M PBN and 0.07 M H_2O_2. Receiver gain 2.0 × 10^4. (c) The EPR spectrum of *tert*-butyl hydronitroxide is shown. The radical was generated by heating (100 °C, 2 min) an aqueous solution containing 0.05 M PBN and 0.02 M **2**. Receiver gain 4.0 × 10^4.

Studies Pertaining to the Mechanism of Action of the Pt(IV) Complexes. In view of the evidence that compound **2** is able to cleave DNA and in light of the suggestion that radicals are involved in the breakage mechanism,[16] we examined the ability of various platinum(IV) complexes to produce radicals that could be detected using a spin trap. The results of these experiments are summarized in Figure 1. Heating a 0.05 M aqueous unbuffered solution of the spin trap, phenyl-*tert*-butylnitrone (PBN) for 2 min at 100 °C, followed by cooling and EPR analysis, did not result in an EPR spectrum (Figure 1a). However, if H_2O_2 is added to the solution of PBN followed by heating, the radical spectrum shown in Figure 1b results. The spectrum, a triplet of doublets with EPR parameters of $g = 2.005$, $a^N = 15.9$ G, and $a^H = 3.6$ G, is that of a nitroxide produced by reaction of a hydroxyl radical at the β-position of the spin trap.[27,28] The same radical (·OH) is trapped by PBN in solutions containing the DNA cleaving antibiotic ferrous bleomycin.[29] Attempts to trap hydroxy radical or any other radical, which may be produced by the thermal decomposition of the hydroxy analogues **2**, **3**, and **6**, were unsuccessful. However, subjecting the solutions containing the complexes and the spin trap to forcing conditions (100 °C) resulted in the EPR spectrum shown in Figure 1c. The spectrum, centered at $g = 2.005$ and having relative signal intensities of 1:2:2:1, appears to be a double of triplets resulting from the coupling of the unpaired electron spin with two nuclei having nuclear spins of $I = {}^1/_2$ and $I = 1$. The hyperfine

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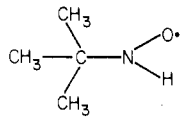
coupling constant associated with either nucleus is 14.6 G. Since the same EPR spectrum can be obtained by heating the spin trap in the presence of *cis,trans*-bis(isopropylamine)dihydroxomalonatoplatinum(IV) (7) and *trans*-[1,1-bis(aminomethyl)cyclobutane]dichlorosulfatoplatinum(IV) (8), the radical appears to be *independent* of the structure of the platinum complex present in solution with the spin trap. In fact, the EPR parameters for the radical indicate that it is *tert*-butyl hydronitroxide (9)



9

($a^N = a^H = 14.6$ G), which has been previously reported[30] to arise from the decomposition of the spin trap, PBN. Thus, heating a solution containing the spin trap and a platinum(IV) complex causes facile hydrolysis of PBN, initially forming *tert*-butylhydroxylamine, which is in turn air-oxidized to radical 9.[31] Since no conditions were found (varying temperature, time, or [Pt]/[PBN]) that produced a radical other than 9, we conclude that the platinum compounds are thermally stable and do not produce radicals, e.g., ·OH, which can be detected with the spin trap PBN.[32]

Biological Data for the Complexes. The in vivo antitumor activity of the compounds against L-1210 leukemia are summarized in Table I. The antitumor data of 2, given for comparison purposes in Table I, have been previously discussed and analyzed.[10] Among compounds 3–6, only two are active (% T/C > 125) in single injection treatment, while multiple injection treatment brought the tetrahydroxo analogue 6 from inactive to modestly active (T/C = 133). The chlorine oxidation product of 1, compound 5, was inactive when either single or multiple injection treatment was used. If in vivo reduction to platinum(II) is important in the mechanism of action of the Pt(IV) compounds, compound 4, the *trans*-dibromo analogue, would be expected to be the most active of the complexes studied. Relative to Cl^- and OH^-, bromide ion would have the greatest destabilization effect on Pt(IV),[33] and thus 4 should be the easiest of the analogues to reduce to Pt(II). However, the antitumor data for the complexes, though very limited, do not appear to support this hypothesis (Table I).

In summary, a group of Pt(IV) complexes produced by oxidation of 1 or its *cis*-dihydroxo analogue have been synthesized and characterized by infrared and ^{195}Pt NMR spectroscopy. EPR studies show that 2 and related hydroxo complexes of Pt(IV) are incapable of producing hydroxyl radical or other radicals that can be trapped by the spin trap PBN. Thus, the cytotoxic activity of the platinum(IV) compounds is very likely due to either their susceptibility to in vivo reduction to active Pt(II) species or possibly to their ability to directly interact via ligand displacement without reduction with important cellular components, e.g., DNA. As a group, the Pt(IV) compounds were found to be significantly less active as antitumor agents against L-1210 leukemia than was the parent compound *cis*-dichlorodiammineplatinum(II).

Experimental Section

Infrared spectra (4000–250 cm^{-1}) of Nujol mulls of the compounds between KBr disks were determined on a Beckman 4220 IR spectrometer. The ^{195}Pt NMR spectral data (at 77.25 MHz) were collected on ~50 mM Me_2SO-d_6 solutions of the complexes (25 °C) on a Bruker WM-360 wide bore NMR spectrometer. The chemical shift data are reported relative to external K_2PtCl_6 dissolved in Me_2SO-d_6. The spectra of the compounds were determined immediately after dissolution in Me_2SO-d_6. No evidence for reaction of the platinum complexes with Me_2SO-d_6 was observed.

Spin trapping experiments were carried out with the spin trap phenyl-*tert*-butylnitrone (PBN; Aldrich Chemical Co). Aqueous unbuffered solutions of PBN (50 mM) and either aqueous H_2O_2 or an aqueous solution (suspension of a platinum(IV) complex were mixed and placed in a standard flat cell. The solutions were heated (40–100 °C) and cooled, and their EPR spectra were recorded with a Varian E-9 EPR spectrometer operating at X-band frequency.

The antitumor evaluations were carried out by Bristol-Myers Co. Chemical analyses were done by Galbraith Laboratories, Knoxville, TN.

The platinum complexes *cis,trans,cis*-Pt^{IV} (*i*-$PrNH_2)_2(OH)_2Cl_2$ (JM-9, CHIP, 2), *cis*-$Pt^{II}(NH_3)_2Cl_2$ (CDDP, 1), *trans*-bis(isopropylamine)dihydroxomalonatoplatinum(IV) (JM-28, 7), and [1,1-bis(aminomethyl)cyclobutane]dichlorosulfatoplatinum(IV) (TNO-27, 8) were supplied by Bristol-Myers Co. The complex *cis*-Pt(IV)$(NH_3)_2Cl_4$ (5) was prepared as previously described.[20-22]

***cis,trans,cis*-$Pt^{IV}(NH_3)_2(OH)_2Cl_2$ (3).** This compound was synthesized in a manner analogous to that of Babaeva.[34] To a stirred suspension of 1.05 g (3.5 mmol) of *cis*-diamminedichloroplatinum(II) in 30 mL of water (50 °C) was dropwise added 50 mL of 30% aqueous hydrogen peroxide. After 1 h, the light yellow solution was cooled, and its volume was reduced in vacuo to induce crystallization of a pale yellow solid. X-ray crystallographic analyses of the solid showed it to be a perhydrate complex.[35] Recrystallization of the solid from water afforded 0.6 g (51%) of bright yellow 3. Anal. ($H_8Cl_2N_2O_2Pt$) H, Cl, N, Pt.

***cis,trans,cis*-$Pt^{IV}(NH_3)_2Br_2Cl_2$ (4).** To 1.23 g (4.1 mmol) of *cis*-diamminedichloroplatinum(II) suspended in 200 mL of water (60 °C) was dropwise added over a 2-h period 106 mL (6.2 mg/mL, 4.1 mmol) of an aqueous solution of bromine. After the addition of bromine, the lemon-yellow solution was cooled, and its volume was reduced in vacuo to 25 mL. The orange solid, 4, was recovered by filtration, washed with a few milliliters of cold water, and air-dried (1.9 g, 63%). Anal. ($H_6Br_2Cl_2N_2Pt$) H, Br, Cl, N.

***cis*-$Pt^{IV}(NH_3)_2(OH)_4$ (6).** A suspension of 0.5 g (1.67 mmol) of *cis*-$Pt^{II}(NH_3)_2Cl_2$ in 30 mL of distilled water was heated with stirring to 90 °C. Excess sodium hydroxide, 0.267 g (6.68 mmol) dissolved in 25 mL of water, was added dropwise to the suspension of 1. The temperature of the reaction mixture was raised to 100 °C, and, after refluxing for 1 h, 25 mL of 30% H_2O_2 was dropwise added to the suspension. The reaction was allowed to reflux for 1 additional hour after peroxide addition and then cooled slowly to room temperature. Adjustment of the pH of the solution to pH 7 with a small amount of HCl (conc) resulted in the precipitation of a white solid. The white crystalline compound was recovered by filtration and recrystallized from water–methanol (0.4 g, 80%). Anal. ($H_{10}N_2O_4Pt$) H, N, Pt.

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Registry No. 1, 15663-27-1; 2, 75213-35-3; 4, 90130-06-6; 5, 16893-05-3; 6, 90065-14-8; *cis*-$Pt(NH_3)_2(OH)_2$, 63700-88-9; H_2O_2, 7722-84-1; Cl, 7782-50-5; Br, 7726-95-6.

Identification of an Acridine Photoaffinity Probe for Trypanocidal Action

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Twenty-four acridine derivatives were screened for trypanocidal activity in *Trypanosoma brucei* in order to determine which structural features of the acridine molecule confer maximal antiparasitic activity. The syntheses of several new azidoacridine derivatives are also reported as well as an assessment of their value as possible photoaffinity probes for the study of acridine trypanocidal action. The most effective and selective acridine trypanocides, with and without irradiation, were the 3-amino-10-methylacridinium salt derivatives. With brief irradiation, one azidoacridine, 3-amino-6-azido-10-methylacridinium chloride, showed considerable trypanocidal activity at very limiting drug concentrations (10^{-7} M) and warrants consideration as a possible photoaffinity probe.

Trypanosomes and their close relatives are flagellated protozoa that cause major human diseases, such as African trypanosomiasis, Chagas' disease, and leishmaniasis. Acridines possess considerable trypanocidal activity but their use in humans has been limited due to the toxicity of these compounds. Acriflavine (Trypaflavin) induces trypanosomes to lose their kinetoplast[1,2] and can be used to cure mice of trypanosomiasis.[3] In addition, trypanosomes treated with acriflavine in vitro become photosensitized and can be killed upon prolonged irradiation with visible light.[1,2] Structure–activity studies for a few acridine derivatives have demonstrated the effectiveness of acridinium quaternary salts in the treatment of trypanosomiasis in mice.[4] However, these studies were limited in the number of acridines tested, and they did not adequately assess the contribution of substituents to the overall trypanocidal activity of the compound. Given the potential importance of the acridines to the development of new chemotherapeutic agents for the treatment of trypanosomiasis, we have, therefore, undertaken the synthesis and testing for trypanocidal activity of a series of acridine derivatives in order to (a) determine which structural features were required for maximal trypanocidal activity and (b) develop an appropriate photoaffinity probe with which to study the subcellular distribution of acridines in the trypanosome. Here we report the synthesis of new azidoacridine compounds and an assessment of their value as photoaffinity probes. In addition, structure–activity studies are included for a selected number of acridine derivatives.

Synthesis. The syntheses of previously reported compounds are not presented here but are referred to within the Experimental Section where they serve as the starting material. The azidoacridine compounds were prepared by diazotization of the corresponding primary aminoacridine derivatives, followed by simple Sandmeyer-type substitution with sodium azide. In general, these reactions proceeded readily with yields between 60 and 90%. Since 9-aminoacridine does not undergo the diazonium reaction, those acridines that required an azido group in the "9"-position were prepared by refluxing the 9-chloro or 9-*N*-pyridyl derivative in methanol to which sodium azide had been added. In most cases, the crude reaction product could be effectively purified by chromatography on cellulose cation-exchange columns. Homogeneity for all compounds was further confirmed by chromatography on silica gel plates with an ethanol–butanol–chloroform–ammonium hydroxide mixture (3:3:5:1) or a methanol–benzene mixture (1:4), or both. In addition, high resolution NMR analysis of both the precursor and azido derivatives indicated that the azido compounds were better than 98% free of contaminating precursor. Elemental analysis for the azido compounds was not attempted because in our experience such analysis could not be performed reliably under mandatory dark conditions. The nonvolatile nature of the azido acridinium salts precluded mass spectral analysis. Confirmation of structure and position assignments were determined by high-resolution NMR spectroscopy. Previously reported precursor compounds were used as standards in these analyses, and the assignments were made with a high level of confidence. The presence of an azido group was easily detected by its strong 2100-cm^{-1} absorption in the infrared. Due to the reactivity of the azido group, the azidoacridines invariably decompose before melting. Where decomposition occurred over a short range, decomposition temperatures are given. Polymerization upon heating results in tarlike compounds with very high melting points (~350 °C). Prior to use in biological testing, a UV–visible spectrum was determined for each compound in order to monitor decomposition.

Compounds Tested. Acriflavine was purchased from Aldrich Chemical Co. and was separated from the contaminating proflavine according to the procedure of Gupta.[5] Proflavine was purchased from Sigma Chemical

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