compounds, as well as Drs. Doyle and Rose (also of Bristol-Myers) for helpful discussions pertaining to the chemistry and antitumor properties of the compounds. Thanks also to Professor Janzen and Dr. DuBose (University of Guelph-Waterloo) for their comments on the spin-trapping experiments. Discussions with Dr. Freedman (Syracuse University) were helpful in interpreting the IR data of the compounds. This work was supported by a grant from Bristol-Myers Co.

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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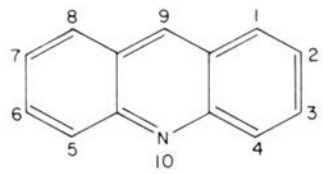
Table I. Dose–Response of *Trypanosoma brucei* in Swiss Mice[a]

no. parasites injected	survival ± SD, days	no. parasites injected	survival ± SD, days
10^1	8.5 ± 1.4 (5)	10^4	5.7 ± 1.8 (5)
10^2	7.5 ± 1.9 (5)	10^5	5.3 ± 1.5 (5)
10^3	7.0 ± 0.7 (5)	10^6	4.2 ± 1.0 (5)

[a] The results show the dose–response of untreated parasites injected into mice. The data are presented as the mean of the days survival postinjection with the standard deviation and the number of mice treated for each determination in parentheses. The experimental details are given under Biological Procedures.

Chart I. Structure and Numbering System for Acridine



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Co. and was used without further purification. The syntheses of the following compounds have been reported previously: 3-aminoacridine[6,7] 9-amino-1-nitroacridine,[8] 1-nitro-9-[[3–(dimethylamino)propyl]amino]acridine (Ledakrin),[9] 9-amino-10-methylacridinium chloride,[10] 3,9-diaminoacridine,[11] 1,9-diaminoacridine,[12] 1-amino-10-methylacridinium chloride,[10] and 3-amino-8-azido-5-ethyl-6-phenylphenanthridinium chloride (monoazido derivative of ethidium bromide).[13,14] Berenil was purchased from Calbiochem Co. and was used without further purification.

Biological Evaluation. The structural requisites of a series of acridines for trypanocidal activity against *Trypanosoma brucei* were examined by exposing the parasites directly to drug prior to injection into mice. The total volume of inoculated parasites in drug solution was 0.1 mL, and the drug concentration was reduced by at least three orders of magnitude upon injection. The days of survival following inoculation was monitored, and control groups of mice receiving the same number of parasites which had not been exposed to drugs were included with experimental groups. The dose–response for parasite killing was obtained by injecting groups of five mice each with isolated untreated parasites in doses ranging from 10^1 to 10^6 parasites/mouse (Table I). "Cures" were defined as 30 days survival after inoculation with no detectable parasites in the blood. The data are summarized in Tables II and III for non-azido- and azidoacridines, respectively, and the structural formula and numbering system are given in Chart I. None of the acridines were effective at 10^{-6} M *without light activation. For a reference, berenil* (4,4′-diamidinodiazoaminobenzene), a non-acridine triazene used clinically as a trypanocide, was found to be slightly active at 10^{-6} M, prolonging the life of parasite-infected animals 6 days beyond the controls. However, even at 10^{-5} M berenil did not eliminate parasite infection completely, prolonging survival only 15 days beyond the controls. In contrast, many of the acridines effectively eliminated parasite viability at 10^{-5} M without light activation; they

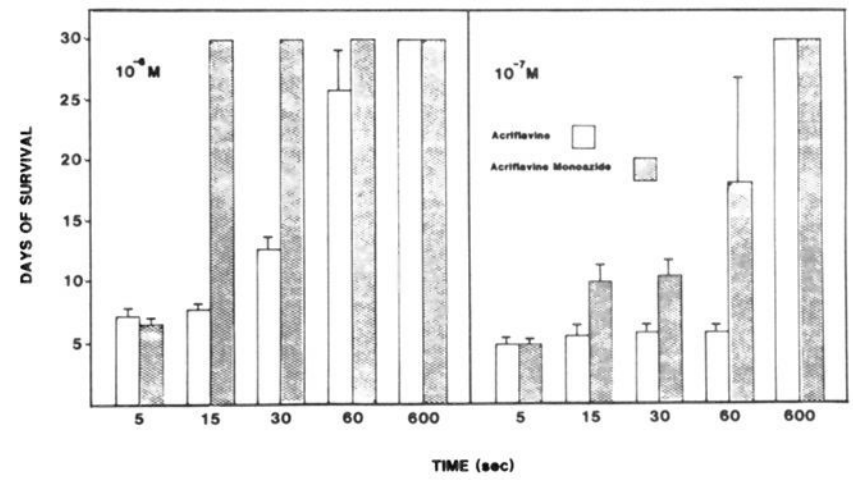

Figure 1. The effect of irradiation time on the trypanocidal action of acriflavine and 3-amino-6-azido-10-methylacridinium chloride (acriflavine monoazide). Drug treatments were as described under *Biological Procedures and in the legends of Tables I–III. The* standard deviations are included. Five mice were used for each experimental determination. The controls for the acriflavine experiments survived 4.0 ± 0 days after inoculation, and the controls for the 3-amino-6-azido-10-methylacridinium chloride survived 5.6 ± 1.1 days: (□) acriflavine; (▩) 3-amino-6-azido-10-methylacridinium chloride.

were 9-azidoacridine (*13*), 3,6-diamino-10-methylacridinium chloride (acriflavine), and 9-amino-1-nitroacridine.

The effect of irradiation on drug action was examined, since azidoacridines are capable of covalent adduct formation upon irradiation, and many non-azidoacridines are known to possess "photodynamic" actions. Irradiation significantly enhanced the trypanocidal activities of 9-azidoacridine (**13**) and 3-amino-6-azido-10-methylacridinium chloride (**1**), producing "cures" at 10^{-6} M. The non-azidoacridines, 3-amino-10-methylacridinium chloride, acriflavine, ledakrin, proflavine, and 3,9-diaminoacridine, also demonstrated enhanced activity at 10^{-6} M with irradiation. When the acridine concentration was reduced to 10^{-7} M, only 3-amino-6-azido-10-methylacridinium chloride (**1**) could consistently effect a "cure" with irradiation, while 3-amino-10-methylacridinium chloride and acriflavine were slightly less active.

Experiments designed to test whether the "photo" effects of the acridines could be eliminated by preirradiating the drugs prior to exposure to the parasite were also conducted. In this respect, acridines were irradiated for 1 h with visible light prior to incubation with parasites. Following incubation, samples were then either irradiated again or left in the dark. In the case of the azidoacridines, preirradiation abolished the light-enhanced activity of these compounds (9-azidoacridine and 3-amino-6-azido-10-methylacridinium chloride), while for the non-azidoacridines, 3-amino-10-methylacridinium chloride, and acriflavine, trypanocidal activity was only slightly reduced. However, these non-azido compounds required an additional post-irradiation in the presence of parasites for maximum activity. These results indicate that (1) light activation must occur in the presence of the parasite in order to be effective and that (2) azidoacridine activation is immediate and irreversible, while non-azidoacridine activation is prolonged.

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Table II. Trypanocidal Activity of Non-Azido Acridines and Non-Acridines[a]

acridine	control	10^{-4} M		10^{-5} M		10^{-5} M (pre)		10^{-6} M		10^{-6} M (pre)		10^{-7} M	
		dark	light	dark	light	dark	light	dark	light	dark	light	dark	light
3-amino-	5.9 ± 2.5 (15)	6.9 ± 1.7 (10)	30.0 ± 0 (15)	6.6 ± 1.9 (15)	30.0 ± 0.9 (15)	6.7 ± 2.4 (10)	30.0 ± 0 (10)	4.9 ± 2.0 (10)	5.7 ± 2.3 (10)				
3-amino-10-methyl-	4.8 ± 1.5 (10)	30.0 ± 0 (5)		14.2 ± 12.4 (10)	30.0 ± 0 (5)		30.0 ± 0 (5)	6.9 ± 2.8 (10)	30.0 ± 0 (5)		15.5 ± 9.9 (5)	4.6 ± 1.2 (10)	12.4 ± 4.6 (10)
3,6-diamino- (proflavine)	5.6 ± 3.0 (20)	30.0 ± 0 (10)	30.0 ± 0 (10)	12.0 ± 11.8 (20)	30.0 ± 0 (10)			5.5 ± 2.1 (10)	30.0 ± 0 (25)	7.2 ± 0.4 (5)	20.6 ± 12.5 (5)	6.3 ± 4.6 (25)	8.1 ± 0.7 (10)
3,6-diamino-10-methyl (acriflavine)	5.2 ± 1.9 (25)	30.0 ± 0 (5)		30.0 ± 0 (25)	30.0 ± 0 (25)			6.9 ± 2.4 (20)	30.0 ± 0 (25)	7.8 ± 1.1 (5)	30.0 ± 0 (10)	6.3 ± 3.0 (15)	17.8 ± 11.9 (25)
9-(*p*-aminoanilino)-	4.4 ± 0.9 (5)	30.0 ± 0	30.0 ± 0 (5)	5.0 ± 0 (5)	6.4 ± 0.5 (5)			5.4 ± 2.1 (5)	4.4 ± 1.5 (5)				
9-amino-1-nitro-	4.8 ± 0.4 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	25.4 ± 6.4 (5)	30.0 ± 0 (5)			4.4 ± 0.5 (5)	5.8 ± 2.2 (5)				
1-nitro-9-[[3-(dimethylamino)-propyl]amino]- (Ledakrin)	4.7 ± 1.0 (10)	30.0 ± 0 (10)	30.0 ± 0 (10)	5.7 ± 1.2 (10)	30.0 ± 0 (15)			5.7 ± 1.9 (15)	22.9 ± 11.2 (15)		10.4 ± 6.0 (15)	6.0 ± 2.1 (10)	6.6 ± 3.1 (5)
9-amino-10-methyl-	4.4 ± 0.5 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	5.4 ± 0.9 (5)	21.0 ± 8.3 (5)			4.2 ± 0.8 (5)	5.8 ± 1.3 (5)		6.2 ± 1.9 (5)		
3,9-diamino-	5.2 ± 1.3 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	8.1 ± 1.1 (5)	30.0 ± 0 (5)			5.0 ± 0 (5)	17.4 ± 7.6 (5)		27.8 ± 4.9 (5)	5.2 ± 1.6 (5)	15.0 ± 8.5 (5)
1,9-diamino-	5.2 ± 1.6 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	4.6 ± 1.5	5.6 ± 0.5 (5)			5.4 ± 1.5 (5)	4.6 ± 0.5 (5)				
1-amino-10-methyl-	4.4 ± 0.9 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	4.6 ± 2.1 (5)	6.4 ± 2.4 (5)			4.0 ± 0 (5)	4.2 ± 0.4 (5)				
2-amino-10-methyl-	3.8 ± 1.1 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	5.8 ± 0.8 (5)	6.8 ± 2.3 (5)			3.6 ± 1.1 (5)	6.0 ± 3.1 (5)				
4,4′-diamidinodiazo-aminobenzene (Berenil)	4.2 ± 2.0 (5)			19.2 ± 12.9 (5)				10.8 ± 1.1 (5)				5.0 ± 1.4 (5)	
3-amino-8-azido-5-ethyl-6-phenyl-phenanthridinium chloride	4.6 ± 1.3 (5)							4.60 ± 1.3 (5)	30.0 ± 0 (5)			4.0 ± 0 (5)	27.4 ± 5.8 (5)

[a] The results presented in the table reflect the curative effect of the compound. Light activation involved a 10-min irradiation of the compound with the parasites after a 10-min incubation. Dark conditions omitted any exposure to light, and preirradiation (pre) included a 1-h irradiation of the compound prior to its exposure to the parasites. Control groups of mice, injected with a sample of parasites not exposed to compound, were included for every parasite preparation. The data in the table include the mean of the days survival postinoculation plus or minus the standard deviation, with the number of mice treated for each determination in parentheses. Experimental details are included under Biological Procedures.

Table III. Trypanocidal Activity of Azido Acridines[a]

acridine	control	10^{-4} M		10^{-5} M		10^{-5} M (pre)		10^{-6} M		10^{-6} M (pre)		10^{-7} M	
		dark	light	dark	light	dark	light	dark	light	dark	light	dark	light
9-(*p*-azidoanilino)-	4.4 ± 0.9 (5)	4.8 ± 1.9 (5)	30.0 ± 0 (5)	4.0 ± 0 (5)	30.0 ± 0 (5)			4.4 ± 0.5 (5)	5.8 ± 1.3 (5)				
9-amino-2-azido-	5.5 ± 1.7 (10)	9.8 ± 1.3 (5)	30.0 ± 0 (5)	5.4 ± 1.5 (10)	30.0 ± 0 (10)			4.8 ± 1.8 (10)	11.6 ± 7.8 (10)	4.6 ± 1.3 (10)	4.6 ± 1.5 (5)	6.0 ± 1.0 (5)
9-azido-1-nitro-	4.6 ± 1.5 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	10.0 ± 1.4 (5)	30.0 ± 0 (5)			4.2 ± 1.8 (5)	6.0 ± 1.7 (5)	4.0 ± 0 (5)	5.4 ± 1.3 (5)	5.0 ± 2.0 (5)
9-azido-10-methyl-	4.2 ± 1.8 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	3.6 ± 1.1 (5)	20.0 ± 13.7 (5)			5.0 ± 1.7 (5)	6.8 ± 3.3 (5)	8.6 ± 1.5 (5)	5.2 ± 1.6 (5)	6.2 ± 1.3 (5)
9-azido-	4.7 ± 0.9 (10)	30.0 ± 0 (10)	30.0 ± 0 (10)	30.0 ± 0 (10)	30.0 ± 0 (10)			5.0 ± 0.9 (10)	30.0 ± 0 (10)	4.2 ± 1.8 (10)	4.6 ± 0.9 (5)	4.0 ± 0.7 (5)
9-amino-3-azido-	4.8 ± 0.9 (10)	25.3 ± 8.4 (10)	26.8 ± 6.7 (10)	6.3 ± 0.7 (10)	14.2 ± 11.1 (10)	5.2 ± 0.4 (5)		5.8 ± 1.8 (5)	7.6 ± 1.7 (5)				
2-azido-10-methyl-	4.6 ± 1.3 (10)	30.0 ± 0 (5)	30.0 ± 0 (5)	14.5 ± 11.9 (10)	13.9 ± 11.6 (10)			4.4 ± 0.7 (10)	5.5 ± 2.5 (10)	4.8 ± 0.8 (5)	4.8 ± 1.0 (5)	4.2 ± 0.4 (5)
1-azido-10-methyl-	4.4 ± 0.9 (5)	4.4 ± 0.9 (5)	4.8 ± 1.1 (5)	6.4 ± 2.2 (5)	4.8 ± 1.1 (5)								
3-azido-10-methyl-	5.2 ± 2.4 (10)	30.0 ± 0 (5)	30.0 ± 0 (5)	6.6 ± 1.5 (5)	30.0 ± 0 (10)	4.4 ± 0.9 (5)	5.8 ± 2.2 (5)	5.4 ± 1.4 (10)	8.6 ± 2.2 (10)	4.7 ± 1.2	5.0 ± 1.6 (5)		
3-amino-6-azido-10-methyl-	4.4 ± 0.7 (25)	30.0 ± 0 (5)	30.0 ± 0 (5)	18.2 ± 14.6 (5)	30.0 ± 0 (5)			6.9 ± 4.2 (30)	30.0 ± 0 (25)	6.3 ± 3.7 (20)	5.5 ± 1.8 (10)	6.4 ± 3.5 (20)	30.0 ± 0 (15)
3-amino-6-azido-	5.7 ± 2.9 (15)	8.3 ± 1.8 (10)	30.0 ± 0 (10)	5.5 ± 1.0 (10)	12.7 ± 12.5 (10)	5.2 ± 2.6 (5)	5.2 ± 2.6 (5)	4.6 ± 1.0 (10)	11.2 ± 6.7 (15)		5.4 ± 1.3 (10)	5.7 ± 1.6 (10)	5.9 ± 1.8 (10)
3,6-diazido-10-methyl-	5.6 ± 0.9 (5)	30.0 ± 0 (5)	30.0 ± 0 (5)	6.0 ± 1.2 (6)	26.6 ± 7.6 (5)			5.8 ± 1.1 (5)	8.2 ± 0.8 (5)		5.4 ± 0.5 (5)	5.4 ± 0.5 (5)	5.6 ± 0.5 (5)
3,6-diazido-	6.9 ± 4.6 (20)	6.0 ± 1.6 (15)	5.5 ± 1.6 (10)										

[a] Refer to the legend of Table II and the Biological Procedures for explanations of results and experimental methods.

By examining a large number of substituted acridines, we have attempted to define those structural features of the acridine ring system conferring maximal trypanocidal activity. The results indicate that quaternary acridinium salts with amino groups at either the 3- or 6-position demonstrate effective trypanocidal activity. In addition, 9-azido- and 1-nitro-substituted acridines also show significant trypanocidal activity. However, the specificity of action of these latter compounds is in question, since they are also toxic in bacterial tester systems[15,16] and have been observed to cause massive granular clumping in the trypanosome (unpublished results). In contrast, the 3-amino-10-methylacridinium salts selectively stained the nuclear and kinetoplast regions of the trypanosome (unpublished results) and were not toxic in bacterial tester systems.[15,16]

In order for a compound to be considered as a photoaffinity probe, it must be able to bind to the same sites as the parent compound in the dark. In addition, it should be capable of covalent adduct formation with its receptor when photoactivated. These studies have identified 3-amino-6-azido-10-methylacridinium chloride as a possible photoaffinity probe for the parent compound acriflavine. In the dark, both the azido probe and acriflavine demonstrated the same trypanocidal activity. However, with minimal photoactivation (less than 2 min) (Figure 1) the azido probe shows a 100-fold increase in trypanocidal activity, presumably due to covalent adduct formation. In addition, fluorescent microscopic examination of parasites exposed to the lowest effective concentration of the probe showed selective staining of the kinetoplast and nucleus (unpublished results). These results indicate that the azido probe mimics the parent compound in binding specificity and biological activity and, thus, deserves consideration as a photoaffinity probe.

Interestingly, the parent compound itself may be a natural photoaffinity probe, since it demonstrates enhanced biological activity with prolonged irradiation. In general, short irradiation times are preferred, however, since nonspecific effects are possible with increasing exposure to light. Photodynamic action is thought to involve the production of oxygen free radicals which may migrate prior to reacting, resulting in long-range effects. In contrast, photoaffinity labeling, mediated through photoreactive azido substituents, results in a direct covalent interaction of the probe with its biological targets.

Finally, a comparison of the acridines most effective as trypanocides with those most effective in producing mitochondrial damage in yeast[17] shows these acridines to be the same. Thus, it is suggested that the target for the trypanocidal action of the acridines may be the large specialized mitochondrion of the trypanosome called the kinetoplast.

Experimental Section

General Methodology and Procedures. IR spectra were performed on a Perkin-Elmer Model 221 grating infrared spectrometer; absorption spectra were obtained on a Varian Cary 219 spectrophotometer (included in Supplementary Material), and melting points were determined on an Electrothermal melting point apparatus. Thin-layer chromatography was carried out on 10 × 80 mm glass slides by using silica gel "G" (Merck), and column chromatography was performed with 40 × 2.5 cm glass

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columns packed with cation-exchange (carboxymethyl)cellulose (Cellex CM, Bio-Rad). Visualization of compounds during and after chromatograpy was effected by UV and visible light. All syntheses and handling procedures were carried out under subdued lighting, except for the azido compounds, which were under photographic safelight conditions. All samples were stored desiccated in the dark. The ^1H NMR data were obtained on a Nicolet NMC-300/WB Fourier transform NMR spectrometer and are included as Supplementary Material (see paragraph at the end of paper concerning Supplementary Material). In general, the azidoacridines (except 9-azido deratives) were prepared in the following way. A solution of the aminoacridine in 1 N HCl was cooled at 5 °C in an ice bath and diazotized by the dropwise addition of a cold (5 °C) solution of sodium nitrite in water (5 mL). The reaction was allowed to continue for 5 min in the cold, at which time a cold solution (5 °C) of sodium azide in water (5 mL) was added. After 10–15 min at 5 °C, the acridine free base was precipitated by the addition of 10 N NaOH. The precipitate was filtered and the cake washed once in cold dilute NaOH. Alternatively, if the azidoacridine formed a precipitate during the course of the reaction, the precipitate was filtered and washed with cold 1 N HCl. The washed cake was purified in most cases by column chromatography using (carboxymethyl)cellulose cation exchanger (BioRad Cellex-CM) eluted with a water–HCl gradient (pH 5–2.2). The desired acridine was eluted from the column and lyophilized to dryness.

3-Amino-6-azido-10-methylacridinium Chloride (1) and 3,6-Diazido-10-methylacridinium Chloride (2). Purified acriflavine chloride (396 mg, 1.53 mmol) was diazotized with 316 mg of sodium nitrate (4.59 mmol), followed by the addition of 500 mg of sodium azide (7.70 mmol). A yellow precipitate formed immediately. The mixture was stirred in the cold for an additional 10 min and then filtered. The filter cake was washed twice with cold 1 N HCl and redissolved in water, and the pH was adjusted to 6.0 with 1 N NaOH. Chromotography with (carboxymethyl)cellulose columns yielded three major homogeneous bands detectable by UV and visible light. A yellow band, **2**, was first to elute, followed by **1**, which appeared as a bright yellow band that fluoresced a brilliant yellow. The last band to elute was a yellow band, which fluoresced brilliant yellow–green and was determined to be unreacted acriflavine. Each band was collected separately and lyophilized to dryness. Homogeneity for each band was further affirmed by thin-layer chromatography on silica gel G with an acetone–ethanol–ammonium hydroxide–water mixture (7:2:1:2) as the eluent, R_f values for acriflavine, and monoazide (1), and the diazide (2) were, respectively, 0.3, 0.32, and 0.5. Samples of acriflavine and 1 gave a cerise-colored solution when diazotized and coupled to β-naphthol, indicating the presence of a primary amino group; IR (KBr) of 1 and 2 showed strong absorption at 2100 cm^{-1} indicative of the azido group. The yields of compounds 1 and 2 were 238 (60%) and 80 mg (20%), respectively.

9-(*p*-Azidoanilino)acridine (4). 9-(*p*-Aminoanilino)acridine (3) was prepared by a modification of the Atwell procedure[18] in which 9-chloroacridine was reacted with *p*-phenylenediamine (Sigma Chemical Co.) in aqueous ethanol. A solution of **3** (200 mg, 0.71 mmol) was diazotized with sodium nitrite (500 mg, 7.20 mmol), followed by the addition of sodium azide (700 mg, 10.80 mmol). The crude product from the reaction mixture was recrystallized as the hydrochloride from 1 N HCl to give pale-yellow needles: mp 175–177 °C dec; yield 146 mg (68%); homogeneous by TLC (benzene–methanol, 5:2); IR (KBr) 2100 cm^{-1}.

9-Amino-2-azidoacridine (7). The starting material, 2,9-diaminoacridine (**6**), was prepared according to the procedure of Albert and Royer.[19] A solution of **6** (490 mg, 2.40 mmol) was diazotized with sodium nitrite (820 mg, 12.60 mmol). After 5 min, sulfamic acid (93 mg) was added to destroy excess nitrous acid. Sodium azide (820 mg, 12.10 mmol) was then added. The yellow precipitate that formed was purified by column chromotography: yield 440 mg (78%); homogeneous by TLC (benzene–methanol, 5:2); IR (KBr) 2100 cm^{-1}.

2-Azido-10-methylacridinium Chloride (9). The starting material, 2-amino-10-methylacridinium bromide (**8**), was prepared according to the procedure of Albert and Ritchie.[10] A solution of **8** (298 mg, 1.22 mmol) was diazotized with sodium nitrite (200 mg, 2.90 mmol), followed by the addition of sodium azide (325 mg, 5.00 mmol). The carbinol base was redissolved in 1 N HCl (50 mL) and then evaporated to dryness. The product was chromatographed to give a major band, which was bright yellow and fluoresced yellow–green: yield 224 mg (83%); homogeneous by TLC (benzene–methanol, 5:2); IR (KBr) 2100 cm^{-1}.

3-Amino-10-methylacridinium Chloride (10). Purified acriflavine chloride (Aldrich Chemical Co.) (500 mg, 1.92 mmol) was diazotized with sodium nitrite (600 mg, 8.70 mmol). After 1 min, 1.0 mL of 48% hypophosphorous acid (12 mmol) was added, and the reaction was allowed to stand for 48 h at 5 °C. The acridine carbinol bases were neutralized with 1 N HCl and chromatographed. Three major bands could be distinguished by fluorescence: a pale-yellow band with green–blue fluorescence, 10-methylacridinium chloride, was first to elute, followed by **10**, which fluoresced bright yellow; unreacted acriflavine was last to elute and appeared as an orange band with a yellow-green fluorescence. The three bands were collected separately and lyophilized to dryness. Purity was confirmed by TLC (benzene–methanol, 5:2), and 10-methylacridinium chloride, purchased commercially (Eastman Kodak Co.), appeared identical with the first band to elute by TLC, mp, and IR. Methylation of the 3-acetamidoacridine by refluxing with methyl iodide, followed by deacetylation, gave **10**. A cerise-colored solution was observed when **10** was diazotized and coupled to β-naphthol: yield 253 mg (54%) mp 264–266 °C. Anal. ($C_{14}H_{13}N_2Cl$) C, H, N.

3-Azido-10-methylacridinium Chloride (11). A solution of **10** (203 mg, 0.83 mmol) was diazotized by the addition of sodium nitrite (600 mg, 8.70 mmol), followed by sodium azide (780 mg, 12.00 mmol). Only one band (yellow) could be detected by column chromatography which was collected and lyophilized to dryness to yield 150 mg (67%): mp 142–148 °C dec; homogeneous by TLC (ethanol–butanol–chloroform–ammonium hydroxide, 3:3:5:1).

9-Azido-1-nitroacridine (12). The starting material, *N*-(1-nitroacridin-9-yl-pyridinium chloride (**5**) was prepared according to the procedure of Ledochowski.[9] In a 100-mL round-bottom flask **5** (300 mg, 0.89 mmol) was added to anhydrous methanol (30 mL), and the solution was filtered. The filtrate was placed in a water bath and heated until boiling began. Sodium azide (500 mg, 7.69 mmol) dissolved in methanol (30 mL) was added, and the solution was refluxed gently for 1 h. Cooling and dilution with water precipitated the azidoacridine. The yellow–green powder was purified by chromatography on thick-layer silica gel plates with 20% methanol–benzene as eluent: yield 193 mg (84%); mp 119–123 °C dec; IR (KBr) 2100 cm^{-1}.

9-Azido-10-methylacridinium Iodide (14). 9-azidoacridine (**13**) was prepared according to the procedure of Reynolds.[20] To a solution of **13** (500 mg, 2.27 mmol) dissolved in warm anhydrous methanol (50 mL) was added 2 mL of methyl iodide (39.45 mmol), and the solution was refluxed for 2 h. The reaction mixture was then evaporated to dryness, and the crude product was chromatographed on thick-layer silica gel plates eluted with 20% methanol–benzene: yield 256 mg (42%); mp 144 °C dec; IR (KBr) 2100 cm^{-1}.

1-Azido-10-methylacridinium Chloride (16). The starting material, 1-amino-10-methylacridinium chloride (**15**), was prepared according to the procedure of Albert and Ritchie.[10] A solution of **15** (196 mg, 0.80 mmol) was diazotized by the addition of sodium nitrite (112 mg, 1.60 mmol), followed by sodium azide (167 mg, 2.40 mmol). Purification by column chromatography yielded two major bands that could be detected by visible and UV light: a yellow band with orange fluorescence (**15**) was first to elute, followed by a purple band with no fluorscence, the unreacted 1-amino-10-methylacridinium chloride. The yellow band was collected and lyophilized to dryness to yield 103 mg (48%): mp 30 °C dec; IR (KBr) 2100 cm^{-1}; homogeneous by TLC (20% methanol–benzene).

9-Amino-3-azidoacridine (18). The starting material, 3,9-

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diaminoacridine (17), was prepared according to the method of Albert and Gledhill.[11] The diazotization of 17 was effected by the same procedure given above for the preparation of 16: yield 440 mg (78%); homogenous by TLC (benzene–methanol, 5:2); IR (KBr) 2100 cm^{-1}. The fluorescence of 18 in aqueous solution is pale blue. When irradiated for 2 min with UV light, the fluorescence changed to bright yellow-green.

Biological Procedures. *Trypanosoma brucei* (EATRO 110) was kindly provided by Dr. M. Rifkin at Rockefeller University. The parasites were propagated in young adult male CD-1 Swiss mice (Charles River Co.) by intraperitoneal injections of 0.1 mL of whole blood containing about 1×10^7 parasites/mL. Larger quantities of parasites were obtained by injecting heavily parasitized mouse blood intraperitoneally into Long Evans rats followed by cardiac puncture in 3 days. Stocks of infected heparinized blood were stored at -70 °C in the presence of 12% glycerol. *T. brucei* was separated from blood cells by passage through a DEAE-cellulose (DE52 Whatman) column with Tris-buffered saline–glucose (TSG) (I = 0.129) as described by Lanham and Godfrey.[21] The parasites were pelleted by centrifugation at 1000g for 10 min and washed twice with TSG buffer. Survival following injection was scored for each group, and the mean of the number of days of survival was determined for each parasite dose.

Drug screening for trypanocidal activity was performed as described previously.[22] Parasites at a concentration of 5×10^6/mL in TSG and aliquots of drug diluted in TSG were added to obtain the appropriate final concentration. Each drug, whether photoreactive or not, was tested both with and without light activation. Light activation was carried out for 10 min in 9 × 50 mm plastic petri dishes (Falcon), using a GE 30W fluorescent lamp placed 4 cm above the surface of the sample. All nonphotoactivated (dark) experiments were carried out in the dark, with a red photographic safelight until after the mice were injected with parasites. Preirradiation conditions included a 1-h irradiation of the compound prior to its exposure to the parasites. The drug was mixed with parasites by gentle rotation for 10 min. Parasite viability was checked following all manipulations by monitoring motility. No differences in motility were noted between control and drug-treated parasites, and all preparations used for injection showed greater than 97% motility immediately prior to injection. The dose of parasites indicates the total number (motile plus nonmotile) of parasites injected. Mice were injected intraperitoneally with 0.1 mL containing 5×10^5 drug-treated parasites, and control groups of mice which received 5×10^5 untreated parasites were included for every parasite preparation. The numbers of surviving mice were scored each day over a period of 30 days. Trypanocidal activity is reported as the mean number of days of survival postinjection. The standard deviations are included, and the number of mice included in the determinations are given in parentheses (Tables II and III).

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Registry No. 1, 78276-17-2; 2, 17784-47-3; 3, 58658-11-0; 4, 89873-24-5; 4·HCl, 89873-25-6; 5, 20141-88-2; 6, 23043-62-1; 7, 78276-18-3; 8, 89873-26-7; 9, 78276-14-9; 10, 75586-70-8; 11, 78276-15-0; 12, 78276-10-5; 13, 21330-56-3; 14, 89873-27-8; 15, 75605-59-3; 16, 78276-12-7; 17, 951-80-4; 18, 78276-06-9; 9-chloroacridine, 1207-69-8; *p*-phenylenediamine, 106-50-3; 3-aminoacridine, 581-29-3; acriflavine, 86-40-8; proflavine, 92-62-6; 9-amino-1-nitroacridine, 21914-54-5; ledakrin, 6514-85-8; 9-amino-10-methylacridinium chloride, 5776-38-5; 1,9-diaminoacridine, 23043-60-9; berenil, 908-54-3; 3-amino-8-azido-5-ethyl-6-phenylphenanthridinium chloride, 65282-35-1; 3-amino-6-azidoacridine, 78276-16-1; 3,6-diazidoacridine, 57459-61-7.

Supplementary Material Available: Three tables containing the absorption characteristics and the ^1H NMR chemical shifts and coupling constants for the acridine compounds (6 pages). Ordering information is given on any current masthead page.

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Tumor Inhibitory Triazenes.[1] 3. Dealkylation within an Homologous Series and Its Relation to Antitumor Activity

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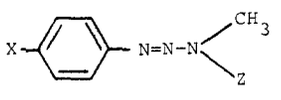
The in vivo antitumor activity and in vitro metabolic dealkylation have been measured for an homologous series of 3-alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes and have been compared with their partition coefficients. This investigation has shown that the extent of oxidative metabolism in vitro and the antitumor activity in vivo of these compounds are dependent upon hydrophobicity. These findings provide confirmation for the relationship between metabolism and antitumor activity for aryldialkyltriazenes.

As part of our investigation of potential clinically less toxic second-generation analogues of the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, Dacarbazine), we have recently reported[1] on an homologous series of 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes (Ia). In this series there was a slight



Ia X = COOH

Ib X = $CONH_2$

improvement in antitumor activity against the TLX/5 lymphoma as the alkyl chain length increased from methyl to pentyl. At greater chain lengths, however, the activity rapidly diminished, the heptyl derivative being totally inactive. A number of possible reasons for this marked change in activity were suggested, and we now report on our investigations related to one of these.

The antitumor activity of the dialkyltriazenes is thought to be dependent on oxidative metabolism.[2,3] We therefore set out to determine whether there is any alteration in the type or extent of metabolism that might account for the sudden change in activity referred to above. Unfortunately, from this point of view, the 1-(4-carboxyphenyl)-triazenes do not undergo measurable in vitro dealkylation,[4] an observation that is currently under investigation. We therefore turned our attention to a similar series of 3-al-

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