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Cytotoxicity of Acridine Compounds for *Leishmania* Promastigotes In Vitro

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The effect of mammalian and bacterial topoisomerase II inhibitors on *Leishmania* promastigotes was studied in vitro. Parasites were incubated with drugs, and cytotoxicity was assessed on the basis of the loss of flagellar motility and cell lysis after 48 h. 9-Aminoacridines, which are structurally related to the known antileishmanial compounds quinacrine and chlorpromazine, showed activity against the parasite at concentrations in the range of 10 to 20 μM. Adriamycin showed far less activity, while etoposide and several quinolones were inactive at 100-μM concentrations. These results demonstrate that a particular structural class of compounds is cytotoxic to *Leishmania* species. The unique structure-activity relationship discovered suggests that leishmanial topoisomerase II could be a useful target for chemotherapy.

Leishmaniasis has traditionally been treated with pentavalent antimonial agents (10). These drugs must be given parenterally over prolonged periods of time, are expensive, and have significant side effects. Furthermore, therapeutic failures with pentavalent antimonial agents have been increasingly recognized (14). A new approach to the chemotherapy of leishmaniasis is needed. Recently, Shapiro and coworkers described the isolation of protein-associated cleaved DNA from trypanosomes treated with antitrypanosomal agents and mammalian topoisomerase II (TPII) inhibitors (12, 13). This cleavable complex was also found in cultured human cells treated with mammalian TPII inhibitors and correlated with cytotoxicity (3, 11). We hypothesize that the leishmanial DNA TPII may represent a potential therapeutic target.

Our studies have focused on the susceptibility of *Leishmania donovani* (MHOM/SU/00/S3) and *Leishmania chagasi* (MHOM/BR/86/L669) to a panel of known mammalian TPII-active agents and gyrase inhibitors (Table 1) (for the structures of some of these compounds, see Fig. 1). Amsacrine was a gift of the National Cancer Institute. The other 9-aminoacridines were synthesized in this laboratory by the method of Cain et al. (2). Chlorpromazine, quinacrine, adriamycin, and etoposide were from Sigma. Pefloxacin, rosoxacin, amifloxacin, nalidixic acid, and norfloxacin were gifts of Sterling Drug Inc., and ofloxacin was a gift of Ortho Pharmaceutical Corp. The effect of drugs was assessed by incubating 5×10^6 promastigotes per ml in medium alone, in medium with dimethyl sulfoxide comparable to that used to solubilize the drugs, and with serial twofold dilutions of drugs ranging from 1.6 to 100 μM for 48 h at 26°C as previously described (8, 9). Experiments were done in duplicate. Promastigote viability was assessed microscopically by counting motile parasites. In general, active drugs resulted in loss of motility and cell lysis. The 90% lethal dose (LD_{90}) of each compound was defined as the concentration of drug that reduced the number of viable promastigotes by 90% in comparison to that of the controls.

Many acridine compounds possessed potent activity against promastigotes (Table 1). For example, the established in vitro antileishmanial compound quinacrine (1) and the closely related *p*-phenol, *p*-methoxyphenyl, and (*N*,*N*-dimethylamino)ethyl 9-aminoacridines resulted in lysis of virtually all parasites at concentrations of ≥25 μM. Interestingly, a hybrid molecule of the known mammalian TPII-active agents chlorpromazine (5) and amsacrine (7) was among the most active agents examined. The sample size was not great enough to assess small differences in the

TABLE 1. Toxicities of agents toward *Leishmania* promastigotes

Drug	LD_{90} (μM)		Application or activity
	L. chagasi	*L. donovani*	
9-Aminoacridines			
Amsacrine[a]	75	50	Antitumor agent via TPII activity
p-Phenol[a]	10	10	Potent mammalian TPII activity
Dimethoxyphenol	12	25	Weak mammalian TPII activity
p-Methoxyphenyl	9	9	Weak mammalian TPII activity
Phenyl	15	25	No mammalian TPII activity
(*N*,*N*-Dimethylamino)ethyl[b]	15	6	Reported antitumor activity (6)
Chlorpromazine	15	22	Antipsychotic, TPII inhibitor (5)
Quinacrine	2	NT[c]	Antimalarial agent
Adriamycin[a]	>100	NT	Antitumor agent via TPII activity
Etoposide[a]	>100	NT	Antitumor agent via TPII activity
Quinolones	>100	>100	Antibacterial via bacterial TPII (gyrase) inhibition

[a] Established activity against human TPII at concentrations below ~10 μM.
[b] A hybrid molecule that incorporates the amsacrine nucleus and the chlorpromazine side chain that was synthesized to assess the relative contributions of the chlorpromazine and amsacrine substructural units.
[c] NT, not tested.

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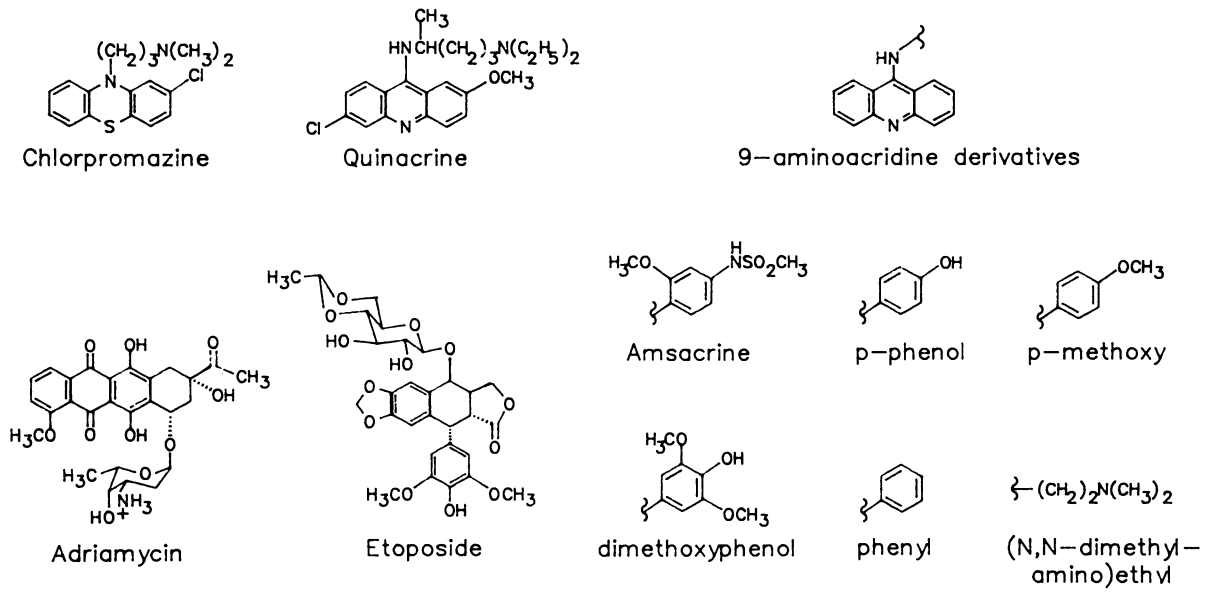

FIG. 1. Structures of compounds tested.

activities of these compounds. Other known mammalian TPII inhibitors, such as adriamycin and etoposide, had little antileishmanial activity. Adriamycin reduced the number of motile organisms at high concentrations (50 and 100 μM), but the LD_{90} was greater than 100 μM. Etoposide resulted in no reduction of motile promastigotes at any concentration. These data suggest that compounds that are active against the leishmanial enzyme but do not inhibit mammalian TPII may be discovered or synthesized. As previously reported, none of the gyrase inhibitors exhibited activity (4). Dimethyl sulfoxide alone had no effect on the parasites.

These data reveal a structural class of compounds with activity against *Leishmania* promastigotes that merit further consideration through animal testing for the treatment of leishmaniasis. The activities against the mammalian enzyme of the compounds investigated in Table 1 provide a basis for the hypothesis that leishmanial TPII may represent a unique therapeutic target. Only through isolation and characterization of the leishmanial TPII enzyme and demonstration of the formation of cleavable complexes with these agents will conclusive support be achieved. Nonetheless, our data suggest that a leishmanial TPII isozyme is more closely related to the mammalian enzyme than to the prokaryotic enzyme (which is sensitive to the quinolones), and it is possible that compounds active against the leishmanial enzyme will exhibit activity against the mammalian enzyme and thus be toxic. However, with the availability of an assay to assess cleavable complex formation of drugs with DNA and mammalian TPII and the knowledge that other classes of mammalian TPII inhibitors do not affect *Leishmania* species, it should be possible to select compounds with antileishmanial activity that have little inhibitory effect on human TPII.

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