Synthesis and *in Vitro* Evaluation of 9-Anilino-3,6-diaminoacridines Active Against a Multidrug-Resistant Strain of the Malaria Parasite *Plasmodium falciparum*

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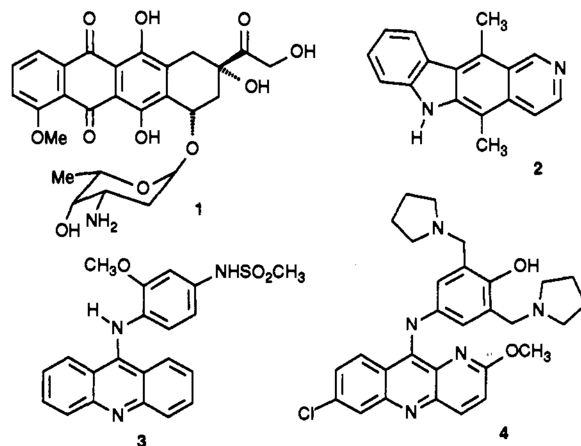
A series of 9-anilinoacridines have been prepared and evaluated for their activity against a multidrug-resistant K1 strain of the malaria parasite *Plasmodium falciparum* in erythrocyte suspensions. 3,6-Diamino substitution on the acridine ring resulted in lower mammalian cell cytotoxicity and higher antiparasitic activity than other substitution patterns, providing compounds with the highest *in vitro* therapeutic indices. A new synthesis of 3,6-diamino-9-anilinoacridines, via reduction of the corresponding diazides, gives much higher yields than traditional methods. Within the subset of 3,6-diamino-9-anilinoacridines, there was considerable tolerance to substitution at the 1′-anilino position. In a sharp divergence with structure–activity relationships for high mammalian cell toxicity and anticancer effects, derivatives bearing electron-withdrawing 1′-substituents (e.g., SO_2-NHR and CONHR) showed the most potent antimalarial activity (IC_{50} values of 10–20 nM). Representative compounds were shown to be potent inhibitors of the DNA strand-passing activity of human topoisomerase II and of the DNA decatenation activity of the corresponding parasite enzyme. The 1′-SO_2NH_2 derivative **7n** completely inhibited strand passage by Jurkat topoisomerase II at 20 μM, and an increase in linear DNA (indicative of inhibition of religation) was seen at or above 1 μM. It also inhibited the decatenating activity of the parasite topoisomerase II at 6 μM and above. In contrast, the analogous compound without the 3,6-diamino substituent was inactive in both assays up to 100 μM. Overall, there was a positive relationship between the ability of the drugs to inhibit parasite growth in culture and their ability to inhibit parasite topoisomerase II activity in an isolated enzyme assay. The 1′-SO_2NH_2 derivative **7n** showed a high IVTI (1000) and was a potent inhibitor of both *P. falciparum in vitro* (IC_{50} 20 nM) and *P. falciparum*-derived topoisomerase II. However, the compound was inactive against *Plasmodium berghei* in mice; reasons may include rapid metabolic inactivation (possibly by N-acetylation) and/or poor distribution.

Malaria, caused in humans primarily by the parasites *Plasmodium falciparum* and *Plasmodium vivax*, is currently the most widespread infectious human disease in the world, including South-East Asia and Oceania,[1] with an annual death toll of over 2 million people.[2] This is due largely to the widespread emergence of strains of *P. falciparum* which are resistant to the presently available drugs, including chloroquine, primaquine, pyrimethamine, and mefloquine.[3] The development of vaccines against malaria has proven extremely difficult, due to the complex life cycle of the parasite.[4,5] This has sparked a renewed search for new types of drugs with novel targets; these include a wide variety of oxidant compounds,[2,3,6] inhibitors of glutathione reductase,[7] and cationic DNA minor-groove-binding agents.[8]

The topoisomerase II enzyme of the parasite has also been considered as a target. This enzyme is responsible for the topological control of DNA in mammalian and bacterial cells[9,10] and in protozoa.[11] Riou and co-workers[12] showed that *Plasmodium berghei* topoisomerases I and II were sensitive to a variety of anticancer drugs and mammalian topoisomerase II inhibitors (e.g., adriamycin (1), ellipticine (2), and amsacrine (3)) but only at high drug concentrations. Their conclusion from this work was that these enzymes were not good targets for antimalarial drugs. However, the 9-anilinoazaacridine derivative pyronaridine (4) shows potent activity against multidrug-resistant *P. falciparum* strains[13] and is in clinical trial in China,[14] and we have recently shown[15] that this compound does inhibit *P. falciparum* topoisomerase II.



Substituted 9-anilinoacridines have been used successfully to treat different forms of leukemia.[16] The intracellular target for these drugs in mammalian cells has been identified as mammalian DNA topoisomerase II, and their mode of action has been shown to involve stabilization of

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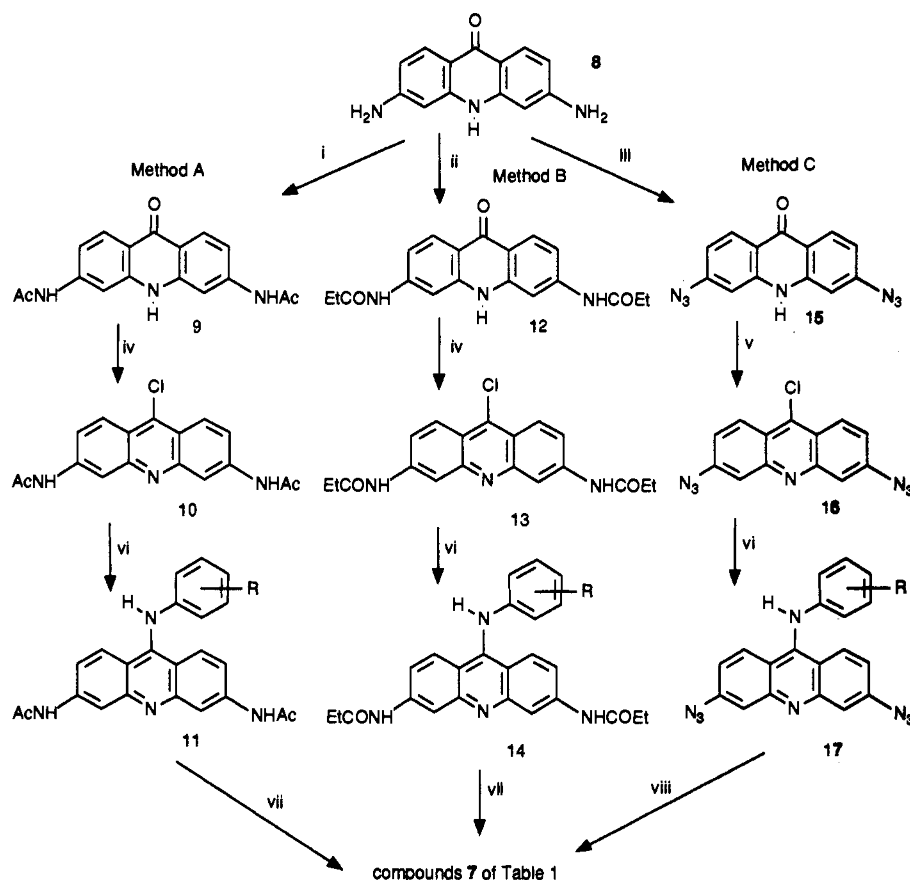
Scheme 1 [a]



[a] (i) Ac_2O/AcOH; (ii) Pr_2O/PrOH; (iii) $NaNO_2$/HCl, then NaN_3; (iv) $POCl_3$/Δ; (v) $SOCl_2$/DMF/Δ; (vi) R-$ArNH_2$; (vii) 2 N HCl/EtOH/Δ; (viii) Pt–C/H_2/MeOH.

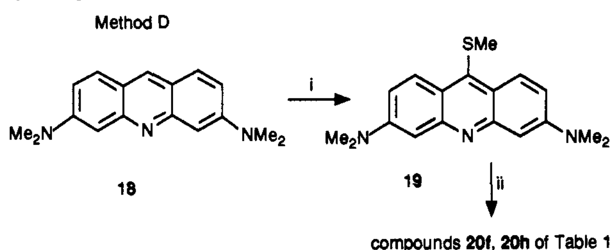
a covalently linked topoisomerase II–DNA intermediate during the DNA strand-passing reaction catalyzed by the enzyme.[17] The available evidence supports models where the acridine moiety intercalates into the DNA and the 9-anilino moiety projects into the DNA minor groove where it interacts with the enzyme.[18] The nature of the substituents on the 9-anilino moiety dramatically alters the antitumor activity of 9-anilinoacridines, supposedly by altering contacts with the enzyme; thus, moving the 3′-OMe group of the clinical antileukemic drug amsacrine (**3**) to the 2′-position abolishes activity.[19] Further, analogues of amsacrine with altered aniline substituents are active in cells which exhibit topoisomerase-mediated drug resistance by expression of the topoisomerase IIα isozyme.[20] These observations led us to seek 9-anilinoacridines with specificity for topoisomerases in other organisms, by modifying the substituents on the anilino moiety. Consistent with this idea, a series of 9-anilinoacridines with varied anilino substituents showed differences in structure–activity relationships against *P. falciparum* and human Jurkat leukemia cells and inhibited *P. falciparum* topoisomerase II.[21] A later study[15] also identified 3,6-diamino substitution on the acridine ring as greatly increasing drug potency against the parasite *in vitro*. In this paper, we report the synthesis of a series of 9-anilino-3,6-diaminoacridines and the evaluation of these and other analogues against multidrug-resistant *P. falciparum* parasites in erythrocyte cultures.

Chemistry

The 9-anilino- (**5**) and 9-anilino-3-amino (**6**) compounds listed in Table 1 were available from previous work or were prepared by reported methods.[22,23] The 9-anilino-3,6-diaminoacridines (**7a–t**) were synthesized from the key intermediate 9-oxo-3,6-diaminoacridan (**8**), which was prepared from diphenylmethane as reported previously.[24] The standard synthesis[22] of 9-anilino-3,6-diaminoacridines from **8** (method A of Scheme 1) employs an *N*-acetyl protecting group, and several of the compounds in Table 1 were prepared in this way. However, the products were contaminated with impurities which were difficult to remove, resulting in poor to modest yields of pure material. A major problem is the insolubility of the intermediate *N*-acetates **9** and **10**. Thus, conversion of **9** to the 9-chloro compound **10** with $POCl_3$ is a heterogeneous reaction, neutralization of excess $POCl_3$ is difficult, and the product must be used directly to form the 9-anilinodiacetamides **11**, since it is too insoluble to purify by crystallization. Use of an *N*-propionamide protecting group (method B of Scheme 1) was preferable, since the better solubility characteristics of **12** and **13** permitted a homogeneous activation reaction and purification of the latter 9-chloro compound by crystallization.

However, hydrolytic deprotection of the resulting 9-anilinodipropionamides **14** still provided low yields in some instances, and a method involving milder deprotection was sought. We have shown previously[25] that 9-oxo-3,6-diazidoacridan (**15**) can be readily prepared from **8** by diazotization and activated to give the 9-chloro derivative **16** (method C of Scheme 1). Coupling of this with anilines gave the corresponding 9-anilino compounds **17**, which underwent rapid catalytic hydrogenation to cleanly form the desired 3,6-diamino derivatives **7** in high yield.

Scheme 2 [a]



[a] (i) S/200–210 °C, then EtONa/EtOH, then MeI; (ii) R-ArNH$_2$/phenol.

Direct conversion of 3,6-bis(dimethylamino)acridine (**18**) to 9-alkyl derivatives via the 9-methylthio derivative **19** (method D, Scheme 2) has been reported previously.[26] While this method also proved suitable for the synthesis of analogous 9-anilino-3,6-bis(dimethylamino)acridine compounds (e.g., **7r,s**), activation of 3,6-diaminoacridine by this route was not successful. 3,6-Dimethoxyacridone[24] and 3,6-dichloroacridone[27] for the preparation of **21n** and **22n**, respectively, were made by the regioselective piperidide ring-closure method.[19]

Results and Discussion

The 9-anilinoacridines described here are listed in Table 1. Drug concentrations required to inhibit the growth of a human leukemia cell line (Jurkat) by 50% (IC_{50}[J] values) were determined as a measure of toxicity toward mammalian cells. Activity against a chloroquine- and pyrimethamine-resistant K1 strain of *P. falciparum* was measured by incubating parasite-infected erythrocyte suspensions and determining the concentration of drug required to inhibit the incorporation of [^{3}H]hypoxanthine by 50% (IC_{50}[P]). The ratio of these two values is also recorded, as a form of *in vitro* therapeutic index (IVTI = IC_{50}[J]/IC_{50}[P]), to provide a method of comparing structure–activity relationships within the series.

Previous results suggested that the high hydrophilicity, high pK_a, and 3,6-diamino substitution increased the antimalarial activity of 9-anilinoacridines, with no apparent correlation between DNA binding and antimalarial activity.[15,21] These studies focused on derivatives with electron-donating substituents (NH_2, NHMe, NMe_2, and CH_2NMe_2) at the 1′-position (compounds **5a–d**, **6a–d**, and **7a–d**) because such substituents are known to be an absolute requirement for biological activity in 9-anilinoacridines.[23,28] In all cases, the 3,6-diamino compounds showed both lower mammalian cell cytotoxicity and higher antiparasitic activity than did the corresponding unsubstituted and 3-amino derivatives, resulting in much higher therapeutic indices. The reason for the large enhancement of antiparasitic activity by the 3,6-diamino substitution pattern on the acridine nucleus is unclear but may be related in part to uptake of the drugs into erythrocytes or into malarial parasites, since some data suggest that charged molecules are more readily sequestered by infected erythrocytes.[29]

As noted above, the 1′-$NHSO_2Me$, 3′-OMe derivative amsacrine (**3**) is an effective antileukemic drug and a potent inhibitor of the religation activity of mammalian topoisomerase II. Removal of the OMe group lowers mammalian cytotoxicity, but shifting this group to the 2′-position abolishes both anticancer activity and interaction with the mammalian enzyme.[19,30] These compounds, and their corresponding 3-amino and 3,6-diamino analogues (**5e–g**, **6e–g**, and **7e–g**), were also studied to see whether the marked effects seen against mammalian cells were paralleled in the malaria parasites. This appeared to be the case for the parent compounds, with activity being in the order **5f** > **5e** > **5g**. While 3,6-diamino substitution again greatly increased potency against *P. falciparum*, the generally high mammalian cytotoxicity of these compounds resulted in relatively low IVTIs and showed that a 1′-$NHSO_2Me$ substitution pattern, while superior for mammalian cytotoxicity, is not suitable for potential antimalarial activity.

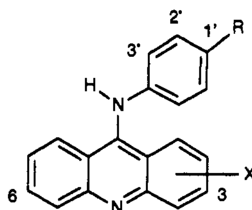
Although electron-donating 1′-NHR substituents favor biological activity, such compounds are also known to undergo facile oxidative metabolism to quinonediimines followed by hydrolysis and reaction with thiols, notably glutathione.[31,32] Since the intermediate quinone oxidation products are unlikely to be responsible for the cytotoxicity of amsacrine against tumor cells in culture,[32] such metabolism is liable to reduce the effectiveness of related 9-anilinoacridines as antimalarial agents *in vivo*. To prevent inactivation by this route, we explored two routes to 1′-substituted 9-anilinoacridines in which quinonediimine formation was not possible.

In the first, electron-donating substituents incapable of undergoing quinoneimine formation were used (although methyleneimine formation is possible with the 1′-CH_2NMe_2 compounds **5d–7d**, it was considered unlikely). Some of these compounds (**5c,d**, **6c,d**, and **7c,d**) were evaluated previously, and because 3,6-diamino analogues were the most effective, with the 1′-CH_2NMe_2, 3,6-diamino derivative **7d** in particular showing high potency and selectivity (IC_{50}[P] 0.04 μM, IVTI >500),[21] studies were confined to these analogues. Increasing the length of the alkyl side chain of **7d** (and the lipophilicity) to give **7l** did not improve the activity against *P. falciparum* or reduce the toxicity to Jurkat cells. The 1′-unsubstituted compound **7h** showed moderate activity, as did the 1′-OMe derivative **7j**. The 1′-OH compound **7i** was more potent *in vitro* (IC_{50}[P] 0.05 μM) but could undergo rapid oxidation to the quinoneimine *in vivo*.

In the second approach, 1′-electron-withdrawing groups were used. While it was considered that such compounds would be less susceptible to electrophilic metabolism, 9-anilinoacridines containing such substituents invariably show low mammalian cytotoxicity,[33] as demonstrated here by the low potency of the 1′-SO_2NH_2 derivative **7n** for Jurkat cells (IC_{50}[J] 20 μM). However, **7n** shows very high potency against *P. falciparum* (IC_{50}[P] 0.02 μM), leading to an IVTI of 1000-fold. This was a particularly interesting result, and a series of related compounds were studied. The corresponding 1′-SO_2NHMe analogue **7o** had similar antiparasitic potency (IC_{50}[P] 0.014 μM) but higher mammalian cytotoxicity. The *N,N*-disubstituted analogues **7p,q** were significantly less effective. The 1′-$CONH_2$ and -CONHMe derivatives **7r,s** also showed high antiparasitic potency, without being quite as effective, with the disubstituted 1′-$CONMe_2$ compound **7t** again being significantly less effective.

In order to explore further the role of the 3,6-diamino groups in conferring high antiparasitic activity, a small series of compounds containing other substituents at these positions were evaluated. The poor activity of the 3,6-bis(dimethylamino) compounds **20f,h** shows that even this group, which retains the electronic and hydrogen-bond-acceptor capabilities of the amines, is not favorable. The

Table 1. Biological Properties of Antimalarial 3-Amino- and 3,6-Diamino-9-anilinoacridines



no.	X	R	Jurkat[a] IC_{50}[J] (μM)	*P. falciparum*[b] IC_{50}[P] (μM)	IVTI[c]
			growth inhibition data		
5a	H	NH_2	0.75	1.5	0.5
6a	3-NH_2	NH_2	0.75	0.1	7.5
7a	3,6-diNH_2	NH_2	15	0.025	600
5b	H	NHMe	1.5	0.4	3.8
6b	3-NH_2	NHMe	0.5	0.32	1.6
7b	3,6-diNH_2	NHMe	11.5	0.16	72
5c	H	NMe_2	<1[d]	0.47	<2
6c	3-NH_2	NMe_2	<1	0.29	<3.5
7c	3,6-diNH_2	NMe_2	6	0.034	176
5d	H	CH_2NMe_2	7.5	0.15	50
6d	3-NH_2	CH_2NMe_2	2.5	0.25	10
7d	3,6-diNH_2	CH_2NMe_2	>20	0.04	>500
5e	H	$NHSO_2Me$	<1	3	<0.3
6e	3-NH_2	$NHSO_2Me$	2	3	0.7
7e	3,6-diNH_2	$NHSO_2Me$	1	0.03	33
3	H	$NHSO_2Me$[e]	<1	0.6	<1.5
6f	3-NH_2	$NHSO_2Me$[e]	<1	0.2	<5
7f	3,6-diNH_2	$NHSO_2Me$[e]	0.8	0.1	8
5g	H	$NHSO_2Me$[f]	10	26	0.4
6g	3-NH_2	$NHSO_2Me$[f]	<1	2.7	<0.4
7g	3,6-diNH_2	$NHSO_2Me$[f]	8.5	0.5	17
7h	3,6-diNH_2	H	15	0.3	50
7i	3,6-diNH_2	OH	10	0.05	200
7j	3,6-diNH_2	OMe	4	0.15	27
7k	3,6-diNH_2	Me	3	0.15	20
7l	3,6-diNH_2	$CH_2NH(CH_2)_4Me$	16	0.6	27
7m	3,6-diNH_2	$CH_2N(CH_2)_5$[g]	>20	0.2	>100
7n	3,6-diNH_2	SO_2NH_2	20	0.02	1000
7o	3,6-diNH_2	SO_2NHMe	2	0.014	143
7p	3,6-diNH_2	SO_2NMe_2	5	0.16	31
7q	3,6-diNH_2	$SO_2N(CH_2)_5$[g]	14	1.4	10
7r	3,6-diNH_2	$CONH_2$	>20	0.07	>285
7s	3,6-diNH_2	CONHMe	>20	0.04	>500
7t	3,6-diNH_2	$CONMe_2$	>20	0.6	33
17n	3,6-diN_3	SO_2NH_2	2.5	0.03	83
17r	3,6-diN_3	$CONH_2$	1.5	0.06	25
20f	3,6-diNMe_2	$NHSO_2Me$[e,h]	<1	0.4	<2.5
20h	3,6-diNMe_2	H	1.4	2.4	0.6
21n	3,6-diOMe	SO_2NH_2	3.1	>10	<3.2
22d	3,6-diCl	CH_2NMe_2	5	0.25	20
22n	3,6-diCl	SO_2NH_2	>20	>10	
4	pyronaridine		4	0.0027	1480

[a] IC_{50}[J], concentration of drug (μM) to reduce growth of human Jurkat leukemia cells to 50% of control cultures, using a 72 h continuous exposure. Values are averages of at least two independent determinations; variation was typically ±15%. [b] IC_{50}[P], concentration of drug (μM) to reduce the incorporation of [^{3}H]hypoxanthine by *P. falciparum* K1 to 50% of controls, using a 24-h drug exposure. [c] IVTI, *in vitro* therapeutic index = IC_{50}[J]/IC_{50}[P]. [d] Accurate IC_{50}[J] values were not determined for compounds showing high mammalian cell toxicity. [e] 3'-OMe (amsacrine analogue). [f] 2'-OMe (*o*-AMSA analogue). [g] $N(CH_2)_5$ = piperidinyl. [h] We thank the Warner-Lambert/Parke-Davis Co. for a sample of this compound.

synthetic route to these compounds (method D, Scheme 2) had limited flexibility, so that other derivatives were not prepared. The 3,6-diOMe and 3,6-diCl analogues **21n** and **22n** of the highly active 1'-SO_2NH_2 derivative **7n** were devoid of antimalarial activity. Finally, the 3,6-diazides **17n,r** showed similar antimalarial activity to the corresponding diamines **7n,r** but were more potent mammalian toxins, thus lowering their IVTIs. It is possible that the diazides serve as prodrugs for the diamines, to which they are reduced in the cells. If so, it appears that some intermediary metabolites of this process are more toxic to mammalian cells than to malaria parasites.

Overall, in this series of compounds, there are no obvious structure–activity relationships observable for different 1'-substituents, apart from an indication that H-bonding capability increases antimalarial activity (e.g., NH_2, SO_2NH_2, OH, and CH_2NMe_2 substituents). In contrast, the results clearly indicate a requirement for 3,6-diNH_2 substitution in the acridine ring for high *in vitro* potency against *P. falciparum*. The reason for this is not clear.

We have previously suggested[15] that low lipophilicity and high basicity are important determinants for the *in vitro* antimalarial activity of 9-anilinoacridines. The clinically used antimalarial drug pyronaridine (4), which has a 1'-OH and two pyrrolidine residues on an anilino ring attached to a benzonaphthyridine nucleus, has high overall basicity (albeit not in the chromophore) and shows very high *in vitro* antimalarial activity.[34] Addition of a

Table 2. Inhibitory Effects of 9-Anilinoacridines Against Jurkat and *P. falciparum* DNA Topoisomerase II

no.	Jurkat topo II MIC[a] (μM)	*P. falciparum* topo II MIC[b] (μM)
7a	20–30	10
7c	20	12.5
7d	20	50
7e	10	6
7i	20	6
7j	20	6
7n	20	6
7o	20	25
7r	20	6
17r	>100	50
21n	>100	>100
22n	>100	100

[a] MIC, concentration of drug (μM) to completely inhibit topoisomerase II activity from Jurkat human leukemia cells, using a P4 DNA unknotting assay; see refs 35 and 44. [b] MIC, concentration of drug (μM) to completely inhibit topoisomerase II activity from *P. falciparum* cells, using a P4 DNA decatenation assay; see ref 15.

basic piperazine unit in a series of indolo[3,2-*c*]quinolines is also reported to greatly increase antimalarial activity.[35] However, basicity is not the only effect, since the 3,6-$diNH_2$ group was clearly superior to either a single 3-NH_2 group or 3,6-$diNMe_2$ group substitution, yet all three substitution patterns result in very similar acridine pK_as.[23] Published pK_a values[23] for these and related 9-anilinoacridines suggest that all the compounds (with the possible exception of the 3,6-diazides) will be fully ionized at physiological pH.

A number of the 3,6-diamino compounds which showed potent antimalarial activity and high therapeutic indices were evaluated for their ability to inhibit topoisomerase II in extracts from both Jurkat cells and the parasite (Table 2). The proteinase K method of Robinson and Osheroff[36] was used to detect inhibition of strand passing and/or religation during DNA unknotting of phage P4 DNA by the Jurkat topoisomerase, and a kinetoplast decatenation assay was used to assess inhibition of the *P. falciparum* topoisomerase.[21] The 1′-SO_2NH_2 derivative **7n** completely inhibited strand passage by Jurkat topoisomerase II at 20 μM, and an increase in linear DNA (indicative of inhibition of religation) was seen at or above 1 μM. It also inhibited the decatenating activity of the parasite topoisomerase II at 6 μM and above. In contrast, the analogous compound without the 3,6-diamino substituents was inactive in both assays up to 100 μM (data not shown). The compounds (**7c,d**) bearing electron-donating 1′-substituents also inhibited strand passing by Jurkat topoisomerase II at concentrations of 20 μM and above but did not inhibit religation at concentrations up to 100 μM. They were slightly less potent than **7n** against the parasite topoisomerase II, with MICs of 10 and 50 μM, respectively, for inhibition of decatenating activity.

The assay measuring topoisomerase II strand passing is not very discriminatory (Table 2), possibly because only a partially purified enzyme preparation was used (both human and parasite enzymes were purified to a similar level). Nevertheless, the data do indicate a relationship between ability to inhibit parasite growth in culture and ability to inhibit parasite topoisomerase II activity in the assay. In particular, the 1′-SO_2NH_2 derivative **7n**, which has a high IVTI (1000) and is a potent inhibitor of the parasite (IC_{50}[P] 20 nM), is a very effective inhibitor of *P. falciparum* topoisomerase II. Recent studies[37] show that these compounds stimulate topo II-mediated DNA breakage in Jurkat cells in culture, with this activity correlating well with their cytotoxicity in this cell line. However, it is recognized that inhibition of parasite topo II may not be the only mechanism of action of these compounds. The 3,6-diamino derivatives do show ca. 4-fold higher levels of DNA binding than the corresponding 3-amino compounds,[15] and this may be a contributing factor.

Table 3. *In Vitro* Activity (IC_{50} values in nM) of Selected 9-Anilino-3,6-Diaminoacridines and Other Antimalarial Drugs Against Different Strains of *P. falciparum*, Using the Infected Erythrocyte Assay

compound	*P. falciparum* strain[a] W2 (Indochina)[b]	D6 (African)[c]	K1 (Thailand)[d]
7a	37	33	25
7c	118	63	34
7d	277	113	40
pyronaridine (4)	0.8	1	27
chloroquine	144	4.8	250
mefloquine	8	17	70[e]
quinine	100	48	300[e]
artemisinin	12	13	5[e]

[a] Data is concentration (nM) for 50% inhibition. [b–d] Data from the Walter Reed Army Institute of Research, Washington, DC, using methods outlined in ref 45. Derivation of the W2 and D6 strains is outlined in ref 46 and the K1 in ref 47. [e] Values are MIC (equivalent to IC_{99}). The authors thank Ms. Sodsri Thaithong for these data.

Table 3 compares the activities of the 1′-NH_2, 1′-NMe_2, and 1′-CH_2NMe_2 substituted 9-anilino-3,6-diaminoacridines **7a,c,d** with four other drugs currently used as antimalarial agents (chloroquine, mefloquine, quinine, and artemisinin) against three different strains of *P. falciparum*, using the infected erythrocyte assay. It is clear from these results that there are major differences in effectiveness, even between different 9-anilinoacridines, against different strains of the parasites, suggesting that the drug targets have subtle structural differences in the different strains. Other studies have shown that different DNA-intercalating drugs which inhibit topoisomerase II cause quite different patterns of DNA cleavage,[38–40] raising the possibility that different cleavage sites in different strains could contribute to the preferential effects of the drugs on different strains of malarial parasites. If this is the case, further studies of structure–activity relationships among 9-anilinoacridines may provide new derivatives with increased effectiveness against individual strains of malaria or against evolving resistant parasites.

The 1′-SO_2NH_2 derivative **7n** was evaluated against *P. berghei* in mice, using a standard protocol,[41] but no *in vivo* antimalarial activity was observed at a dose of 30 mg/kg.[42] Despite the low mammalian cell toxicity observed in the present study, the drug proved toxic at 100 mg/kg. One possible reason for this is that *P. falciparum* and *P. berghei* have significant differences in the topo II enzyme. Alternatively, the drug may undergo rapid metabolic inactivation *in vivo*, possibly by N-acetylation (a proven route of metabolism of ethidium bromide).[43] To evaluate this, further studies on potential prodrugs of **7n** are in progress.

Conclusions

These results confirm that 3,6-diamino substitution on 9-anilinoacridines confers high potency against *P. falciparum* parasites in erythrocyte cell cultures. In contrast, the nature of substituents on the aniline is much less important, allowing the use of electron-withdrawing groups

which improve the *in vitro* therapeutic index (IVTI) by lowering mammalian cell toxicity and which possibly contribute to lower rates of oxidative metabolism. The need for 3,6-diamino substitution cannot be understood solely in terms of high basicity, despite literature precedents, and may be more related to overall drug hydrophobicity and its effects on drug uptake (previous work[15] suggested a requirement for high hydrophobicity). Several analogues in the series (e.g., **7d,n**) have IVTIs (ratios of IC_{50}s in mammalian cells and against parasite cultures) exceeding 500. There appears to be a positive relationship between ability to inhibit parasite growth in culture and ability to inhibit parasite topoisomerase II activity in an isolated enzyme assay. The 1′-SO_2NH_2 derivative **7n** showed a high IVTI (1000) and was a potent inhibitor of both *P. falciparum in vitro* (IC_{50}[P] 20 nM) and *P. falciparum*-derived topoisomerase II. The inactivity of **7n** against *P. berghei* in mice was disappointing; the reasons for this may include poor drug distribution, rapid metabolism, and/or rapid cellular sequestration of the highly basic drug, possibly into lysosomes.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical and were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were measured on Bruker AM-400 or AM-200 spectrometers (Me_4Si). For clarity, aniline protons are designated with primes (e.g., H-2′ and H-3′). Mass spectra were recorded on a VG 7070 spectrometer at nominal 5000 resolution.

3,6-Diamino-9-[(4-methylphenyl)amino]acridine (7k): Example of general method A of Scheme 1. A solution of 4-aminotoluene (0.33 g, 1.5 mmol) was added to a solution of 3,6-diacetamido-9-chloroacridine (10) (0.59 g, 1.5 mmol) in *N*-methyl-2-pyrrolidinone (20 mL). The mixture was stirred at 20 °C for 3 h and then diluted with EtOAc. Most of the MeOH was evaporated under reduced pressure, and the resulting precipitate of crude diacetamide (11, R = 4-Me) was collected by filtration and washed with EtOAc. This was then dissolved in 2 N ethanolic HCl (20 mL) and heated under reflux for 1 h. The cooled mixture was diluted with EtOAc, and the resulting precipitate was filtered, washed with EtOAc, and recrystallized from MeOH/EtOAc to give 3,6-diamino-9-[(4-methylphenyl)amino]acridine (**7k**) as the hydrated dihydrochloride salt (0.11 g, 21% yield: mp (MeOH/EtOAc) 227 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 2.31 (s, 3 H, CH_3), 6.59–6.67 (m, 4 H, H-2, H-7, H-5, H-6), 7.06 (d, J = 8.3 Hz, 2 H, H-2′, H-6′), 7.19 (d, J = 8.3 Hz, 2 H, H-3′, H-5′), 7.74 (d, J = 9.2 Hz, 2 H, H-1, H-8), 9.87 (s, 1 H, NH), 12.80 (s, 1 H, NH). Anal. ($C_{20}H_{18}N_4$·2HCl·1.5H_2O) C,H,N,Cl.

Similar reactions gave the following compounds.

3,6-Diamino-9-[(4-aminophenyl)amino]acridine (7a): (from hydrolysis of the crude triacetate) as the trihydrochloride salt (30% yield); mp (MeOH/EtOAc) 315–317 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 6.48 (d, J = 2.1 Hz, 2 H, H-4, H-5), 6.73 (dd, J = 9.4, 2.2 Hz, 2 H, H-2, H-7), 7.19 (d, J = 8.8 Hz, 2 H, H-3′, H-5′), 7.40 (d, J = 8.8 Hz, 2 H, H-2′, H-6′), 7.52 (d, J = 9.3 Hz, 2 H, H-1, H-8). Anal. ($C_{19}H_{19}N_5$·3HCl·0.5H_2O) C,H,Cl; N: calcd, 17.2; found, 16.6.

***N*-[3-Methoxy-4-[(3,6-diamino-9-acridinyl)amino]phenyl]methanesulfonamide (7f)**: as the dihydrochloride salt (45% yield); mp (MeOH/EtOAc) >250 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 3.06 (s, 3 H, SCH_3), 3.59 (s, 3 H, OCH_3), 6.56 (dd, J = 9.4, 2.0 Hz, 2 H, H-2, H-7), 6.65 (d, J = 2.1 Hz, 2 H, H-4, H-5), 6.90 (dd, J = 8.5, 2.3 Hz, 1 H, H-6′), 7.00 (d, J = 2.2 Hz, 1 H, H-2′), 7.23 (d, J = 8.45 Hz, 1 H, H-5′), 7.70 (d, J = 9.4 Hz, 2 H, H-1, H-8), 9.64 (s, 1 H, NH), 9.95 (s, 1 H, NH), 12.63 (s, 1 H, NH). Anal. ($C_{21}H_{21}N_5O_3S$·2HCl·0.5H_2O) C,H,N,Cl.

3,6-Diamino-9-(phenylamino)acridine (7h): as the dihydrochloride salt (45% yield); mp (MeOH/EtOAc) 230–232 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 6.64 (dd, J = 9.3, 2.1 Hz, 2 H, H-2, H-7), 6.69 (d, J = 2.1 Hz, 2 H, H-4, H-5), 7.15 (m, 3 H, ArH), 7.36 (t, J = 7.4 Hz, 2 H, ArH), 7.75 (d, J = 9.3 Hz, 2 H, H-1, H-8), 9.92 (s, 1 H, NH), 12.95 (s, 1 H, NH). Anal. ($C_{19}H_{17}N_4$·2HCl·H_2O) C,H,N,Cl.

3,6-Diamino-9-[(4-hydroxyphenyl)amino]acridine (7i): as the dihydrochloride salt; mp (MeOH/EtOAc) >350 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 6.56 (dd, J = 9.3, 2.0 Hz, 2 H, H-2, H-7), 6.61 (d, J = 2.0 Hz, 2 H, H-4, H-5), 6.82 (d, J = 8.6 Hz, 2 H, H-2′, H-6′), 7.05 (d, J = 8.7 Hz, 2 H, H-3′, H-5′), 7.72 (d, J = 9.4 Hz, 2 H, H-1, H-8), 9.85 (s, 1 H, NH), 12.52 (s, 1 H, NH). Anal. ($C_{19}H_{16}N_4O$·2HCl·0.5H_2O) C,H,N,Cl.

3,6-Diamino-9-[(4-methoxyphenyl)amino]acridine (7j): as the hydrochloride salt (22% yield); mp (MeOH/EtOAc) >200 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 3.79 (s, 3 H, OCH_3), 6.99 (dd, J = 9.3, 2.1 Hz, 2 H, H-2, H-7), 6.62 (d, J = 2.0 Hz, 2 H, H-4, H-5), 6.97 (d, J = 9.0 Hz, 2 H, H-2′, H-6′), 7.16 (d, J = 7.0 Hz, 2 H, H-3′, H-5′), 7.69 (d, J = 9.3 Hz, 2 H, H-1, H-8), 9.87 (s, 1 H, NH), 12.58 (s, 1 H, NH). Anal. ($C_{21}H_{19}N_3O_4S$·HCl·0.5H_2O) C,H,N,Cl.

***N,N*-Dimethyl-4-[(3,6-diamino-9-acridinyl)amino]benzenesulfonamide (7p)**: as the hydrochloride salt (11% yield); mp (MeOH/EtOAc) 295 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 2.58 (s, 6 H, 2 × CH_3), 6.73–6.81 (m, 8 H, H-2, H-7, H-4, H-5, 2 × NH_2), 7.14 (d, J = 8.7 Hz, 2 H, H-3′, H-5′), 7.62 (d, J = 8.7 Hz, 2 H, H-2′, H-6′), 7.75 (d, J = 9.2 Hz, 2 H, H-1, H-8), 10.10 (s, 1 H, NH), 13.35 (s, 1 H, NH). Anal. ($C_{21}H_{21}N_5O_2S$·HCl·H_2O) C,H,N.

***N,N*-Pentamethylene-4-[(3,6-diamino-9-acridinyl)amino]benzenesulfonamide (7q)**: as the hydrochloride salt (8% yield); mp (MeOH/EtOAc) 235 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 1.66 (t, J = 6.5 Hz, 4 H, 2 × CH_2), 3.12 (t, J = 6.3 Hz, 4 H, 2 × CH_2), 6.74–6.80 (m, 4 H, H-2, H-7, H-4, H-5), 7.19 (d, J = 8.7 Hz, 2 H, H-3′, H-5′), 7.68 (d, J = 8.7 Hz, 2 H, H-2′, H-6′), 7.74 (d, J = 9.1 Hz, 2 H, H-1, H-8), 10.13 (s, 1 H, NH), 13.29 (s, 1 H, NH). Anal. ($C_{23}H_{23}N_5O_2S$·HCl·1.5H_2O) C,H,N.

A similar reaction of the amine from *N,N*-dimethyl-4-nitrobenzylamine and 3-acetamido-9-chloroacridine followed by hydrolysis gave 3-amino-9-[[4-[(*N,N*-dimethylamino)methyl]phenyl]amino]acridine (**6d**) as the trihydrochloride salt (12% yield): mp 228 °C; ^{1}H NMR (D_2O) δ 3.04 (s, 6 H, $N(CH_3)_2$), 4.45 (s, 2 H, CH_2), 6.24 (s, 1 H, H-5), 6.55 (d, J = 9.5 Hz, 1 H, H-2), 7.05 (d, J = 7.8 Hz, 2 H, H-2′, H-6′), 7.19 (d, J = 9.4 Hz, 1 H, ArH), 7.26 (t, J = 7.7 Hz, 1 H, ArH), 7.41 (d, J = 8.4 Hz, 1 H, ArH), 7.54 (d, J = 8.0 Hz, 2 H, H-3′, H-5′), 7.63 (d, J = 8.6 Hz, 1 H, ArH), 7.78 (t, J = 7.8 Hz, 1 H, ArH). Anal. ($C_{22}H_{24}N_4$·3HCl·2H_2O) C,H.

***N*-Methyl-4-[(3,6-diamino-9-acridinyl)amino]benzenesulfonamide (7o): Example of general method B of Scheme 1.** 3,6-Diamino-9-oxoacridan (**8**) (1 g, 4.4 mmol) was heated in a mixture of propionic acid (15 mL) and propanoic anhydride (10 mL) at 80 °C for 1 h. The cooled mixture was poured into water (500 mL) and stirred for 1 h, and the solid was then collected and washed well with water to give 3,6-dipropionamido-9-oxoacridan (**12**) (1.3 g, 92%): mp >320 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.13 (t, J = 7.5 Hz, 6 H, 2 × CH_3), 2.41 (q, J = 7.5 Hz, 4 H, 2 × CH_2), 7.20 (dd, J = 8.8, 1.8 Hz, 2 H, H-2, H-7), 8.09 (d, J = 8.8 Hz, 2 H, H-1, H-8), 8.14 (d, J = 1.7 Hz, 2 H, H-4, H-5), 10.25 (s, 2 H, 2 × NH), 11.63 (s, 1 H, NH). The crude acridone **12** (1 g, 3.11 mmol) was heated under reflux in $POCl_3$ (25 mL) in the presence of a few drops of nitrobenzene for 20 min. The homogeneous mixture was then cooled, and excess reagent was removed under reduced pressure. The residue was treated with ice and NH_4OH, and the resulting precipitate was filtered, washed well with water, and dried. Recrystallization from MeOH in the presence of Et_3N gave 3,6-dipropionamido-9-chloroacridine (**13**) (1.0 g, 90% yield): mp 267 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 1.15 (t, J = 7.45 Hz, 6 H, 2 × CH_3), 2.45 (q, J = 7.6 Hz, 4 H, 2 × CH_2), 7.77 (dd, J = 9.35, 1.6 Hz, 2 H, H-2, H-7), 8.29 (d, J = 9.3 Hz, 2 H, H-1, H-8), 8.56 (br s, 2 H, H-4, H-5), 10.39 (s, 2 H, 2 × NH). Anal. ($C_{19}H_{18}N_3O_2Cl$·CH_3OH) C,H,N. (MeOH detected by NMR.)

The above chloroacridine **13** (0.50 g, 1.40 mmol) was reacted with *N*-methyl-4-(phenylamino)benzenesulfonamide (0.2 g, 1.54 mmol) in *N*-methyl-2-pyrrolidinone (30 mL) as for method A. Dilution with EtOAc after 3 h at 20 °C gave *N*-methyl-4-[(3,6-dipropionamido-9-acridinyl)amino]benzenesulfonamide (14, R = SO_2NHMe) (0.24 g, 32% yield): mp (MeOH/EtOAc) 276–280 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.14 (t, J = 7.45 Hz, 6 H, 2 × CH_3),

2.43–2.50 (m, 7 H, 2 × CH_2, CH_3), 3.17 (s, 1 H, NH), 7.42–7.50 (m, 4 H, H-3′, H-5′, H-2, H-7), 7.76 (d, J = 8.65 Hz, 2 H, H-2′, H-6′), 8.15 (d, J = 9.4 Hz, 2 H, H-1, H-8), 8.67 (br s, 2 H, H-4, H-5), 10.84 (s, 2 H, 2 × NH), 11.12 (s, 1 H, NH), 13.39 (s, 1 H, NH). Anal. ($C_{26}H_{27}N_5O_4S{\cdot}HCl{\cdot}0.5H_2O$) C,H,N,Cl.

This product (0.24 g, 0.44 mmol) was heated under reflux in 2 N ethanolic HCl for 1 h and then diluted with EtOAc. The resulting precipitate of impure **7o** was collected, recrystallized from MeOH/EtOAc, and then converted to the free base with NH_4OH. This was chromatographed on alumina, eluting with CH_2Cl_2/MeOH mixtures to remove major impurities, and then reconverted to the hydrochloride salt and recrystallized to give *N*-methyl-4-[(3,6-diamino-9-acridinyl)amino]benzenesulfonamide (**7o**) as the hydrochloride salt (59% yield): mp (MeOH/EtOAc) 297 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 2.08 (s, 3 H, CH_3), 6.77–6.78 (m, 4 H, H-4, H-5, H-3′, H-5′), 7.13 (d, J = 9.4 Hz, 2 H, H-2, H-7), 7.64 (d, J = 7.5 Hz, 2 H, H-2′, H-6′), 7.77 (d, J = 9.7 Hz, 2 H, H-1, H-8), 10.11 (s, 1 H, NH), 13.50 (s, 1 H, NH). Anal. ($C_{20}H_{19}N_5O_2S{\cdot}HCl{\cdot}H_2O$) C,H,N.

4-[(3,6-Diamino-9-acridinyl)amino]benzenecarboxamide (7r): Example of general method C of Scheme 1. A solution of 3,6-diazido-9-chloroacridine[25] (**16**) (0.2 g, 0.68 mmol) and 4-aminobenzenecarboxamide (0.10 g, 0.74 mmol) in *N*-methyl-2-pyrrolidinone (10 mL) was treated with a few drops of concentrated HCl and then stirred at 20 °C for 1 h. The mixture was diluted with EtOAc, and the resulting precipitate was collected and washed with EtOAc to give 4-[(3,6-diazido-9-acridinyl)amino]benzenecarboxamide (**17r**) (0.25 g, 85% yield): mp (MeOH/EtOAc) >370 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.27 (d, J = 8.6 Hz, 2 H, H-2, H-7), 7.40 (d, J = 7.45 Hz, 2 H, H-3′, H-5′), 7.44 (s, 1 H, NH), 7.51 (s, 2 H, H-4, H-5), 7.97 (d, J = 8.6 Hz, 2 H, H-2′, H-6′), 8.04 (s, 1 H, NH), 8.19 (d, J = 9.4 Hz, H-1, H-8). Anal. ($C_{20}H_{13}N_9O{\cdot}HCl$) C,H,N,Cl.

A solution of **17r** (82 mg, 0.19 mmol) in MeOH was hydrogenated over Pt/C catalyst for 0.5 h. The solvent was evaporated, and the residue was washed with EtOAc, filtered, and dried to give 4-[(3,6-diamino-9-acridinyl)amino]benzenecarboxamide (**7r**) (55 mg, 76% yield). Crystallization from MeOH/EtOAc/HCl gave the dihydrochloride salt: mp 368 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 6.73 (d, J = 9.4 Hz, 2 H, H-2, H-7), 6.77 (br s, 2 H, H-4, H-5), 7.08 (d, J = 8.1 Hz, 2 H, H-3′, H-5′), 7.78 (d, J = 9.4 Hz, 2 H, H-1, H-8), 7.82 (d, J = 8.05 Hz, 2 H, H-2′, H-6′), 10.09 (s, 1 H, NH), 13.35 (s, 1 H, NH). Anal. ($C_{20}H_{17}N_5O{\cdot}2HCl$) C,H,N.

Similar reactions gave the following compounds.

3,6-Diazido-9-[(4-acetamidophenyl)amino]acridine (17a): as the hydrochloride salt (65% yield); mp (MeOH/EtOAc) 205 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 2.09 (s, 3 H, CH_3), 7.24 (dd, J = 9.3, 2.1 Hz, 2 H, H-2, H-7), 7.37 (d, J = 8.8 Hz, 2 H, H-2′, H-6′), 7.50 (d, J = 2.1 Hz, 2 H, H-4, H-5), 7.75 (d, J = 8.1 Hz, 2 H, H-3′, H-5′), 8.19 (d, J = 9.3 Hz, 2 H, H-1, H-8), 10.28 (s, 1 H, NH), 11.41 (br s, 1 H, NH), 14.25 (br s, 1 H, NH). Anal. ($C_{21}H_{15}N_9O{\cdot}HCl$) C,H,N. Hydrogenation of **17a** (Pt/C/H_2/MeOH) followed by hydrolysis (2 N HCl in EtOH) gave 3,6-diamino-9-[(4-aminophenyl)amino]acridine (**7a**) (85% yield): mp and mixed mp 315–317 °C.

3,6-Diazido-9-[[4-(*N*-methylamino)phenyl]amino]acridine (17b): as the dihydrochloride salt (66% yield); mp (MeOH/EtOAc) 172 °C dec; ^{1}H NMR (CD_3OD) δ 3.03 (s, 3 H, CH_3), 7.17 (dd, J = 9.4, 2.2 Hz, 2 H, H-2, H-7), 7.29 (d, J = 8.8 Hz, 2 H, H-3′, H-5′), 7.41 (d, J = 2.1 Hz, 2 H, H-4, H-5), 7.43 (d, J = 8.8 Hz, 2 H, H-2′, H-6′), 8.20 (d, J = 9.35 Hz, 2 H, H-1, H-8). Anal. ($C_{20}H_{15}N_9{\cdot}2HCl{\cdot}0.5H_2O$) C,H,N. Hydrogenation of **17b** (Pt/C/H_2/MeOH) gave 3,6-diamino-9-[[4-(*N*-methylamino)phenyl]amino]acridine (**7b**) as the dihydrochloride salt (96% yield): mp (MeOH/EtOAc) 268 °C; ^{1}H NMR (D_2O) δ 3.25 (s, 3 H, NCH_3), 6.42 (s, 2 H, H-4, H-5), 6.69 (d, J = 9.3 Hz, 2 H, H-2, H-7), 7.13 (d, J = 8.0 Hz, 2 H, H-2′, H-6′), 7.41 (d, J = 9.1 Hz, 2 H, H-1, H-8), 7.53 (d, J = 8.35 Hz, 2 H, H-3′, H-5′). Anal. ($C_{20}H_{19}N_5{\cdot}2HCl{\cdot}2H_2O$) C,H,N.

3,6-Diazido-9-[[4-(*N,N*-dimethylamino)phenyl]amino]acridine (17c): as the dihydrochloride salt (53% yield); mp (MeOH/EtOAc) 180 °C dec; ^{1}H NMR (CD_3OD) δ 3.24 (s, 6 H, 2 × CH_3), 7.18 (dd, J = 9.35, 2.3 Hz, 2 H, H-2, H-7), 7.41 (d, J = 2.2 Hz, 2 H, H-4, H-5), 7.46 (m, 4 H, H-2′, H-3′, H-5′, H-6′), 8.19 (d, J = 9.35 Hz, 2 H, H-1, H-8). Anal. ($C_{21}H_{17}N_9{\cdot}2HCl$) C,H,Cl; N: calcd, 26.9; found, 27.4. Hydrogenation of **17c** (Pt/C/H_2/MeOH) gave 3,6-diamino-9-[[4-(*N,N*-dimethylamino)phenyl]amino]acridine (**7c**) (95% as a hygroscopic trihydrochloride salt): mp (MeOH/EtOAc) >300 °C; ^{1}H NMR (D_2O) δ 3.38 (s, 6 H, 2 × CH_3), 6.27 (br s, 2 H, H-4, H-5), 6.52 (d, J = 8.75 Hz, 2 H, H-2, H-7), 6.88 (d, J = 5.7 Hz, 2 H, H-2′, H-6′), 7.17 (d, J = 8.5 Hz, 2 H, H-1, H-8), 7.54 (d, J = 7.5 Hz, 2 H, H-3′, H-5′). Anal. ($C_{21}H_{23}N_5{\cdot}3HCl{\cdot}4H_2O$) C,H,N,Cl.

3,6-Diazido-9-[[4-[(*N,N*-dimethylamino)methyl]phenyl]amino]acridine (17d): as the dihydrochloride salt (49% yield); mp (MeOH/EtOAc) 190 °C dec; ^{1}H NMR (CD_3OD) δ 2.91 (s, 6 H, 2 × CH_3), 4.39 (s, 2 H, CH_2), 7.15 (dd, J = 9.3, 2.2 Hz, 2 H, H-2, H-7), 7.42 (d, J = 2.1 Hz, 2 H, H-4, H-5), 7.64 (d, J = 8.4 Hz, 2 H, H-2′, H-6′), 7.64 (d, J = 8.45 Hz, 2 H, H-3′, H-5′), 8.18 (d, J = 9.3 Hz, 2 H, H-1, H-8). Anal. ($C_{22}H_{19}N_9{\cdot}2HCl$) C,H,N. Hydrogenation of **17d** (Pt/C/H_2/MeOH) gave 3,6-diamino-9-[[4-[(*N,N*-dimethylamino)methyl]phenyl]amino]acridine (**7d**) (99% yield as the hydrated dihydrochloride salt): mp (MeOH/EtOAc) 244 °C dec; ^{1}H NMR (D_2O) δ 2.95 (s, 6 H, 2 × CH_3), 4.36 (s, 2 H, CH_2), 6.34 (d, J = 1.74 Hz, 2 H, H-4, H-5), 6.58 (dd, J = 9.3, 1.9 Hz, 2 H, H-2, H-7), 6.98 (d, J = 8.2 Hz, 2 H, H-2′, H-6′), 7.30 (d, J = 9.3 Hz, 2 H, H-1, H-8), 7.44 (d, J = 8.3 Hz, 2 H, H-3′, H-5′). Anal. ($C_{22}H_{23}N_5{\cdot}2HCl{\cdot}4H_2O$) C,H,N.

***N*-[2-Methoxy-4-[*N*-(3,6-diazido-9-acridinyl)amino]phenyl]methanesulfonamide (17g)**: as the hydrochloride salt (46% yield); mp (MeOH/EtOAc) 202 °C dec; ^{1}H NMR (CD_3OD) δ 3.29 (s, 3 H, SCH_3), 3.83 (s, 3 H, OCH_3), 6.93 (dd, J = 8.5, 2.3 Hz, 1 H, H-5′), 7.07 (d, J = 2.2 Hz, 1 H, H-3′), 7.14 (dd, J = 9.3, 2.3 Hz, 2 H, H-2, H-7), 7.36 (d, J = 2.2 Hz, 2 H, H-4, H-5), 7.51 (d, J = 8.5 Hz, 1 H, H-6′), 8.16 (d, J = 9.3 Hz, 2 H, H-1, H-8). Anal. ($C_{21}H_{17}N_9O_3S{\cdot}HCl$) C,H,N. Hydrogenation of **17g** (Pt/C/H_2/MeOH) gave *N*-[2-methoxy-4-[*N*-(3,6-diamino-9-acridinyl)amino]phenyl]methanesulfonamide (**7g**) (71% yield), which was crystallized from MeOH/EtOAc as the hydrochloride salt: mp 232 °C dec; ^{1}H NMR (CD_3OD) δ 2.94 (s, 3 H, SCH_3), 3.80 (s, 3 H, OCH_3), 6.62 (d, J = 2.8 Hz, 2 H, H-4, H-5), 6.71 (dd, J = 9.4, 2.3 Hz, 2 H, H-2, H-7), 6.74 (dd, J = 8.6, 2.3 Hz, 1 H, H-5′), 6.87 (d, J = 2.3 Hz, 1 H, H-3′), 7.36 (d, J = 8.4 Hz, 1 H, H-6′), 7.76 (d, J = 9.4 Hz, 2 H, H-1, H-8). Anal. ($C_{21}H_{21}N_5O_3S{\cdot}HCl{\cdot}2H_2O$) C,H,N,S.

3,6-Diazido-9-[[4-[(*N*-pentylamino)methyl]phenyl]amino]acridine (17l): as the dihydrochloride salt (51% yield); mp (MeOH/EtOAc) 210 °C dec; ^{1}H NMR (CD_3OD) δ 0.96 (t, J = 7.0 Hz, 3 H, CH_3), 1.41 (m, 4 H, 2 × CH_2), 1.75 (quintet, J = 7.7 Hz, 4 H, 2 × CH_2), 3.07 (t, J = 8.1 Hz, 2 H, CH_2), 4.26 (s, 2 H, CH_2), 7.05 (d, J = 8.8 Hz, 2 H, H-2, H-7), 7.33 (d, J = 8.2 Hz, 2 H, H-2′, H-6′), 7.37 (s, 2 H, H-4, H-5), 7.58 (d, J = 8.4 Hz, 2 H, H-3′, H-5′), 8.12 (d, J = 9.1 Hz, 2 H, H-1, H-8). Anal. ($C_{25}H_{25}N_9{\cdot}2HCl$) C,H,N,Cl. Hydrogenation of **17l** (Pt/C/H_2/MeOH) gave 3,6-diamino-9-[[4-[(*N*-pentylamino)methyl]phenyl]amino]acridine (**7l**) as the trihydrochloride salt (67% yield): mp (MeOH/EtOAc) 200 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 0.88 (t, J = 6.7 Hz, 3H, CH_3), 1.28–1.30 (m, 4 H, 2 × CH_2), 1.66 (m, 2 H, CH_2), 2.85 (t, J = 7.5 Hz, 2 H, CH_2), 4.07 (s, 2 H, CH_2), 6.68 (dd, J = 9.3, 2.0 Hz, 2 H, H-2, H-7), 6.71 (d, J = 2.0 Hz, 2 H, H-4, H-5), 6.95 (s, 4 H, 2 × NH_2), 7.14 (d, J = 6.50 Hz, 2 H, H-2′, H-6′), 7.49 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 7.76 (d, J = 9.3 Hz, 2 H, H-1, H-8), 9.22 (br s, 2 H, N^+H_2), 9.97 (s, 1 H, NH), 13.09 (br s, 1 H, NH). Anal. ($C_{25}H_{29}N_5{\cdot}3HCl{\cdot}0.5H_2O$) C,H,N.

3,6-Diazido-9-[[4-(piperidinylmethyl)phenyl]amino]acridine (17m): as the hydrochloride salt (51% yield); mp (MeOH/EtOAc) 230 °C dec; ^{1}H NMR (CD_3OD) δ 2.08 (m, 2 H, CH_2), 2.21 (m, 2 H, CH_2), 3.25 (m, 2 H, CH_2), 3.56 (m, 2 H, CH_2), 4.47 (s, 2 H, CH_2N), 7.18 (dd, J = 9.3, 2.2 Hz, 2 H, H-2, H-7), 7.44 (d, J = 2.2 Hz, 2 H, H-4, H-5), 7.50 (d, J = 8.5 Hz, 2 H, H-2′, H-6′), 7.69 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 8.19 (d, J = 9.3 Hz, 2 H, H-1, H-8). Anal. ($C_{21}H_{17}N_9O_3S{\cdot}HCl$) C,H; N: calcd, 24.3; found, 23.8. Hydrogenation of **17m** (Pt/C/H_2/MeOH) gave 3,6-diamino-9-[[4-(piperidinylmethyl)phenyl]amino]acridine (**7m**) (67% yield), which was crystallized from MeOH/EtOAc as the dihydrochloride salt: mp >320 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.91 (m, 4 H, 4 × CH_2), 3.02 (m, 4 H, 4 × CH_2), 4.76 (s, 2 H, CH_2N), 6.69 (m, 4 H, H-2, H-7, H-4, H-5), 6.94 (s, 4 H, 2 × NH_2), 7.13 (d, J = 8.4 Hz, 2 H, H-2′, H-6′), 7.53 (d, J = 8.1 Hz, 2 H, H-3′, H-5′), 7.76 (d, J = 9.2 Hz, 2 H, H-1, H-8), 9.97 (s, 1 H, NH), 11.00 (s, 1 H, NH), 13.07 (s, 1 H, NH). Anal. ($C_{24}H_{25}N_5{\cdot}2HCl{\cdot}2H_2O$) C,H,N.

4-[(3,6-Diazido-9-acridinyl)amino]benzenesulfonamide (17n): as the hydrochloride salt (77% yield): mp (MeOH/EtOAc) 210 °C dec; ^{1}H NMR ((CD$_3$)$_2$SO) δ 7.28 (d, J = 9.35 Hz, 2 H, H-2, H-7), 7.41 (s, 2 H, SO$_2$NH$_2$), 7.46 (d, J = 8.4 Hz, 2 H, H-3′, H-5′), 7.58 (s, 2 H, H-4, H-5), 7.85 (d, J = 8.3 Hz, 2 H, H-2′, H-6′), 8.23 (d, J = 9.3 Hz, H-1, H-8). Anal. ($C_{19}H_{13}N_9O_2S{\cdot}HCl{\cdot}MeOH$) C,H,N. (MeOH detected by NMR.) Hydrogenation of **17n** (Pt/C/H$_2$/MeOH) gave 4-[(3,6-diamino-9-acridinyl)amino]benzenesulfonamide (**7n**) (69% yield), which was crystallized from MeOH/EtOAc as the hydrochloride salt: mp 245 °C (lit.[23] mp 243–246 °C).

***N*-Methyl-4-[(3,6-diazido-9-acridinyl)amino]benzenecarboxamide (17s)**: as the hydrochloride salt (43% yield); mp (MeOH/EtOAc) >300 °C; ^{1}H NMR ((CD$_3$)$_2$SO) δ 2.80 (d, J = 4.2 Hz, 3 H, CH$_3$), 7.25 (dd, J = 10.2, 1.7 Hz, 2 H, H-2, H-7), 7.44 (d, J = 8.3 Hz, 2 H, H-3′, H-5′), 7.56 (s, 2 H, H-4, H-5), 7.94 (d, J = 8.4 Hz, 2 H, H-2′, H-6′), 8.20 (d, J = 9.3 Hz, 2 H, H-1, H-8), 8.53 (q, J = 4.55 Hz, 1 H, NH), 11.45 (br s, 1 H, NH). Anal. ($C_{21}H_{15}N_9O{\cdot}HCl$) C,H,N,Cl. Hydrogenation of **17s** (Pt/C/H$_2$/MeOH) gave *N*-methyl-4-[(3,6-diamino-9-acridinyl)amino]benzenecarboxamide (**7s**) (73% yield), which was crystallized from MeOH/EtOAc as the dihydrochloride salt: mp 264–271 °C; ^{1}H NMR ((CD$_3$)$_2$SO) δ 2.77 (d, J = 4.5 Hz, 3 H, CH$_3$), 6.70–6.72 (m, 4 H, H-2, H-7, H-4, H-5), 6.97 (s, 4 H, 2 × NH$_2$), 7.08 (d, J = 8.7 Hz, 2 H, H-3′, H-5′), 7.74–7.81 (m, 4 H, H-1, H-8, H-2′, H-6′), 8.36 (q, J = 4.4 Hz, 2 H, CONH$_2$), 9.98 (s, 1 H, NH), 13.11 (s, 1 H, NH). Anal. ($C_{21}H_{15}N_9O{\cdot}2HCl{\cdot}0.5H_2O$) C,H,N,Cl.

***N,N*-Dimethyl-4-[(3,6-diazido-9-acridinyl)amino]benzenecarboxamide (17t)**: as the hydrochloride salt (85% yield); mp (MeOH/EtOAc) 200 °C dec; ^{1}H NMR (CD$_3$OD) δ 3.08 (s, 3 H, CH$_3$), 3.13 (s, 3 H, CH$_3$), 7.16 (dd, J = 9.3, 2.4 Hz, 2 H, H-2, H-7), 7.41 (m, 4 H, H-4, H-5, H-3′, H-5′), 7.56 (d, J = 8.6 Hz, 2 H, H-2′, H-6′), 8.17 (d, J = 9.3 Hz, 2 H, H-1, H-8). Anal. ($C_{22}H_{18}N_9O{\cdot}HCl$) C,H,N,Cl. Hydrogenation of 17t (Pt/C/H$_2$/MeOH) gave *N,N*-dimethyl-4-[(3,6-diamino-9-acridinyl)amino]benzenecarboxamide (7t) (89% yield), which was recrystallized from MeOH/EtOAc as the hydrochloride salt: mp 264–271 °C; ^{1}H NMR ((CD$_3$)$_2$SO) δ 2.95 (s, 6 H, 2 × CH$_3$), 6.71 (m, 4 H, H-2, H-7, H-4, H-5), 6.96 (s, 4 H, 2 × NH$_2$), 7.09 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 7.37 (d, J = 8.5 Hz, 2 H, H-2′, H-6′), 7.78 (d, J = 9.8 Hz, 2 H, H-1, H-8), 9.96 (s, 1 H, NH), 13.11 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_5O{\cdot}HCl{\cdot}3H_2O$) C,H,N.

3,6-Bis(*N,N*-dimethylamino)-9-anilinoacridine (20h): Example of method D of Scheme 2. A mixture of 3,6-bis(dimethylamino)-9-(methylthio)acridine (19) (prepared from 3,6-bis(dimethylamino)acridine (18)[26]) (1.00 g, 3.2 mmol) and excess phenol (4.0 g) was heated in an oil bath at 100 °C for 15 min. Aniline (0.60 g, 6.4 mmol) was added, and the mixture was stirred for 15 h at 100 °C and then cooled to 20 °C. A mixture of concentrated HCl (4 mL) in Me$_2$CO (37.5 mL) was added, and the mixture was stirred at 20 °C for 2 h. The resulting orange precipitate was filtered and recrystallized twice from MeOH/EtOAc to give 3,6-bis(*N,N*-dimethylamino)-9-anilinoacridine (**20h**) as the hydrochloride salt (0.63 g, 73%): mp (MeOH/EtOAc) >330 °C; ^{1}H NMR (CD$_3$OD) δ 3.17 (s, 12 H, 2 × N(CH$_3$)$_2$), 6.56 (d, J = 2.5 Hz, 2 H, H-5, H-6), 6.89 (dd, J = 9.7, 2.5 Hz, 2 H, H-2, H-7), 7.20–7.39 (m, 3 H, ArH), 7.40 (d, J = 7.45 Hz, 2 H, ArH), 7.83 (d, J = 9.7 Hz, 2 H, H-1, H-8). Anal. ($C_{23}H_{24}N_4{\cdot}HCl$) C,H,N,Cl.

4-[(3,6-Dimethoxy-9-acridinyl)amino]benzenesulfonamide (21n). A mixture of 3,6-dimethoxy-9-chloroacridine (from 3,6-dimethoxy-9-oxoacridan[19,24] and POCl$_3$) (0.22 g, 0.70 mmol) and sulfanilamide (0.24 g, 1.4 mmol) was dissolved in *N*-methyl-2-pyrrolidinone (13 mL). Two drops of concentrated HCl was added, and the mixture was stirred at 40 °C for 30 min. Dilution with EtOAc gave a precipitate, which was collected by filtration and recrystallized from MeOH/EtOAc to give 4-[(3,6-dimethoxy-9-acridinyl)amino]benzenesulfonamide (**21n**) as the hydrochloride salt (0.28 g, 95% yield): mp (MeOH/EtOAc) >265 °C dec; ^{1}H NMR ((CD$_3$)$_2$SO) δ 4.01 (s, 6 H, 2 × OCH$_3$), 7.18 (dd, J = 9.5, 2.2 Hz, 2 H, H-2, H-7), 7.35–7.41 (m, 6 H, H-4, H-5, H-3′, H-5′, NH$_2$), 7.81 (d, J = 8.6 Hz, 2 H, H-2′, H-6′), 8.15 (d, J = 9.5 Hz, 2 H, H-1, H-8), 11.00 (br s, 1 H, NH). Anal. ($C_{21}H_{19}N_3O_4S{\cdot}HCl{\cdot}0.5H_2O$) C,H,N,Cl.

4-[(3,6-Dichloro-9-acridinyl)amino]benzenesulfonamide (22n). A mixture of 3,6,9-trichloroacridine (from 3,6-dichloro-9-oxoacridan[19,27] and POCl$_3$) (0.15 g, 0.54 mmol) and sulfanilamide (0.10 g, 0.59 mmol) in *N*-methyl-2-pyrrolidinone (10 mL) was treated with a drop of concentrated HCl and stirred at room temperature for 3 h followed by dilution with EtOAc to give 4-[(3,6-dichloro-9-acridinyl)amino]benzenesulfonamide (**22n**) as the hydrochloride salt (0.20 g, 82% yield): mp (MeOH/EtOAc) 295–300 °C; ^{1}H NMR ((CD$_3$)$_2$SO) δ 3.16 (s, 2 H, NH$_2$), 7.38–7.46 (m, 4 H, ArH), 7.84 (d, J = 8.5 Hz, 2 H, ArH), 7.97 (br s, 2 H, ArH), 8.15 (d, J = 8.5 Hz, 2 H, ArH). Anal. ($C_{19}H_{13}Cl_2N_3O_2S{\cdot}HCl{\cdot}H_2O$) C,H,N,Cl.

Similarly was prepared 3,6-dichloro-9-[[4-[(*N,N*-dimethylamino)methyl]phenyl]amino]acridine (**22d**) as the dihydrochloride salt (60% yield): mp (MeOH/EtOAc) 308 °C dec; ^{1}H NMR (D$_2$O) δ 2.95 (s, 6 H, 2 × CH$_3$), 4.43 (s, 2 H, CH$_2$), 7.37–7.39 (m, 4 H, ArH), 7.64 (d, J = 7.90 Hz, 2 H, ArH), 7.70 (s, 2 H, ArH), 7.81 (d, J = 9.3 Hz, 2 H, ArH). Anal. ($C_{22}H_{19}Cl_2N_3{\cdot}2HCl$) C,H,N,Cl.

Mammalian Cell Inhibition Assay. These were carried out using the human Jurkat leukemia cell line, as described previously.[15] The IC$_{50}$ values (μM) recorded in Table 1 are the concentration of drug which reduced cell growth to 50% of that of untreated controls following a 72-h exposure. Cells were counted with an improved Neubauer hemocytometer.

Parasite Inhibition Assay. The activities of the compounds against a chloroquine- and pyrimethamine-resistant K1 strain of *P. falciparum* were determined as described previously.[15] Briefly, suspensions of *P. falciparum*-infected erythrocytes (0.5% initial parasitemia) were incubated with drugs for 24 h at 37 °C, and drug activity was measured as the concentration required to inhibit the incorporation of [^{3}H]hypoxanthine into parasites by 50%, compared with untreated controls.

Topoisomerase II Assay. Extracts containing human topoisomerase II activity were obtained from Jurkat leukemia cells, which were grown in flasks to midlog phase (approximately 10^9 cells, 2 L) and recovered by centrifugation. Following resuspension and one wash in Tris-buffered saline (Tris-HCl, 25 mM (pH 7.4), NaCl, 130 mM, KCl, 50 mM), cells were resuspended in buffer C (2 mL: Tris-HCl, 200 mM (pH 8), KCl, 200 mM, MgCl$_2$, 5 mM, EGTA, 1 mM) containing protease inhibitors (NP40, 0.1% (v/v), 2-mercapthoethanol, 20 mM, aprotinin, 1% (v/v), leupeptin, 100 μg/mL, α-macroglobulin, 10 μg/mL, toluenesulfonyl fluoride, 1 mM). Following a 30-min incubation at 4 °C, the cells were sonicated (MSE Soniprep ultrasonic disintegrator) for 2 × 15-s bursts at a medium power setting. A one-third volume of polyethyleneglycol (PEG)/salt solution (PEG, 24% (w/v), KCl, 2 M, Na$_2$S$_2$O$_5$, 10 mM) was added, and the suspension was incubated for 30 min at 4 °C. The resulting precipitate was removed by centrifugation in a microcentrifuge at approximately 5000*g* for 10 min at 4 °C, and the supernatant containing the topoisomerase II activity was recovered. Topoisomerase II extracts were immediately stored in aliquots at −70 °C. Prior to inclusion in drug inhibition assays, extracts were evaluated for topoisomerase II activity and diluted appropriately to give the required amount of P4 DNA unknotting activity. The assays were carried out as described,[35,44] by measuring the degree of unknotting and linearization of bacteriophage P4 DNA. The resulting DNA species were separated by 0.7% agarose gel electrophoresis, stained in ethidium bromide (10 μg/mL), visualized under UV illumination, and photographed using Polaroid 667 film. Preparation of *P. falciparum* topoisomerase II extracts and their use in the kinetoplast decatenation assay followed published procedures.[15]

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