Journal of Medicinal Chemistry

Article

Antiprotozoal Activity and DNA Binding of Dicationic Acridones

Sandra Montalvo-Quirós, Andrea Taladriz-Sender, Marcel Kaiser, and Christophe Dardonville

J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 02 Feb 2015

Just Accepted

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Antiprotozoal Activity and DNA Binding of Dicationic Acridones

Sandra Montalvo–Quirós[a], *Andrea Taladriz–Sender*[a§], *Marcel Kaiser*[b,c] *and Christophe Dardonville*[a*]

[a] Instituto de Química Médica, IQM–CSIC, Juan de la Cierva 3, E–28006 Madrid, Spain.

[b] Swiss Tropical and Public Health Institute, Socinstrasse, 57, CH-4002 Basel, Switzerland.

[c] University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland.

[*] Corresponding author. Tel.: +34 912587490; Fax.: +34 915644853; e-mail: dardonville@iqm.csic.es

§ Current address: Instituto de Química Orgánica General, IQOG–CSIC.

Abstract

Dicationic acridone derivatives were synthesized and their antiparasitic activity was evaluated. Acridones displayed in vitro nanomolar IC_{50}s against *Trypanosoma brucei rhodesiense* STIB900 with selectivity indices >1000. Compounds **1b**, **3a**, and **3b** were as potent as the reference drug melarsoprol in this assay. Submicromolar range activities were observed against wild type (NF54) and resistant (K1) strains of *Plasmodium falciparum* whereas no significant activity was detected against *Trypanosoma cruzi* or *Leishmania donovani*. Compounds **1a** and **1b** were curative in the STIB900 mouse model for human African trypanosomiasis. UV spectrophotometric titrations and circular dichroism (CD) experiments with fish sperm (FS)-DNA showed that these compounds form complexes with DNA with binding affinities in the 10^4 M^{-1} range. The biological and biophysical data show that antiparasitic activity, toxicity, and DNA binding of this series of acridones are dependent on the relative position of both imidazolinium cations on the heterocyclic scaffold.

Keywords: *Trypanosoma brucei*, *Trypanosoma cruzi, Plasmodium falciparum*, *Leishmania*, sleeping sickness, malaria, antiprotozoal chemotherapy, imidazoline, acridone, anthraquinone, UV titration, circular dichroism, DNA binder, intercalating agent.

Introduction

Available chemotherapy for parasitic tropical diseases such as African and American trypanosomiasis, leishmaniasis, and malaria is not satisfactory in many aspects; toxicity, inefficiency against resistant strains, a parenteral mode of administration and unaffordable price for low-income affected countries are all critical factors that seriously limit the use of these drugs.[1]

In the last years, our group has discovered very potent antitrypanosomal compounds that cured murine models of acute sleeping sickness.[2, 3] In addition, some of these compounds were also very active in vitro against *Plasmodium falciparum* even though this antimalarial activity was not always confirmed in vivo in the *P. berghei* mouse model.[3] These dicationic compounds (**I–VIII**, Figure 1), which contain a diphenyl scaffold and two 2-aminoimidazolinium moieties, bear a relatively short linker between both phenyl rings and share structural characteristics (e.g. diphenyl dicationic compound, short semi-rigid linker, crescent shape) with other related classes of antiprotozoal dicationic compounds such as the diamidines (Figure 1).[4, 5] In fact, similarly to diamidines the bis-2-aminoimidazolines are very effective and selective DNA minor groove binders.[2, 3, 6, 7] Binding to kinetoplast DNA (k-DNA) has been suggested to be in part responsible for the observed antitrypanosomal activity of the diamidine class of compounds either by inhibition of topoisomerase II or by direct inhibition of transcription.[8, 9]

Previous studies have shown that the *N*-phenylbenzamide scaffold (**I,** Figure 1) gives the most potent antitrypanosomal compounds in vivo[3, 10] as well as the best AT-selective DNA minor groove binders.[11, 12] In this work, we wanted to test whether conformational restriction of the scaffold of the bisimidazoline compounds would have an influence on their antiparasitic activity and/or DNA binding affinity. The acridone

heterocycle was chosen as surrogate of the *N*-phenylbenzamide linker between both cationic moieties as similar fused heterocyclic structures (e.g. acridone,[13, 14] anthraquinone,[15] dihydroanthracene,[3] 9*H*-fluorene,[3] 9*H*-fluoren-9-one[16]) have demonstrated antiprotozoal activities against *trichomonads*, *trypanosomes* and *Plasmodium* species. The effect on antiparasitic activity and DNA binding of N-10 methylation and NH ↔ CO replacement was also studied (**1a–c**, **2b** and **3a–c**, Figure 1).

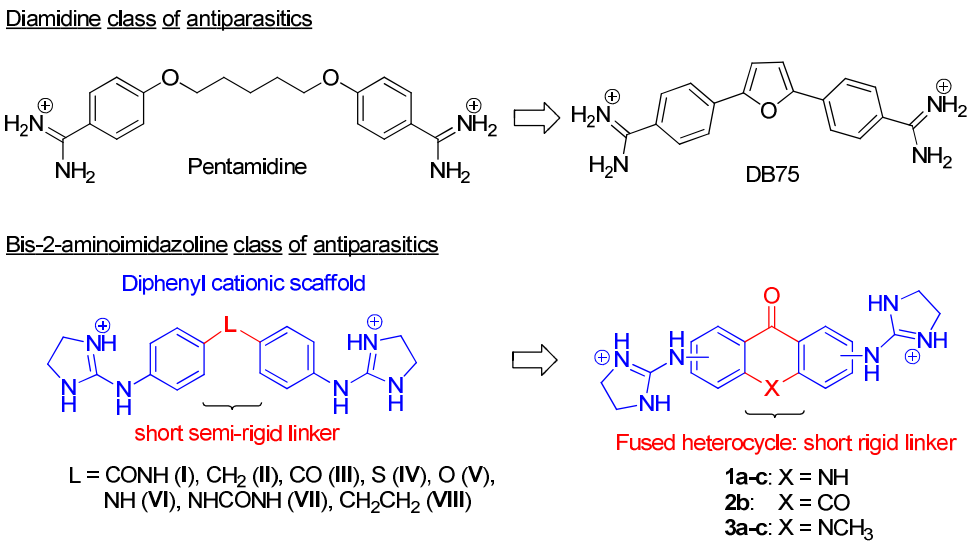


Figure 1. Dicationic Diphenyl Compounds of the Diamidine and Bis-2-aminoimidazoline Classes. Example of Conformational Restriction Leading to New Active Antiparasitic Compounds (compounds **1, 2,** and **3**).

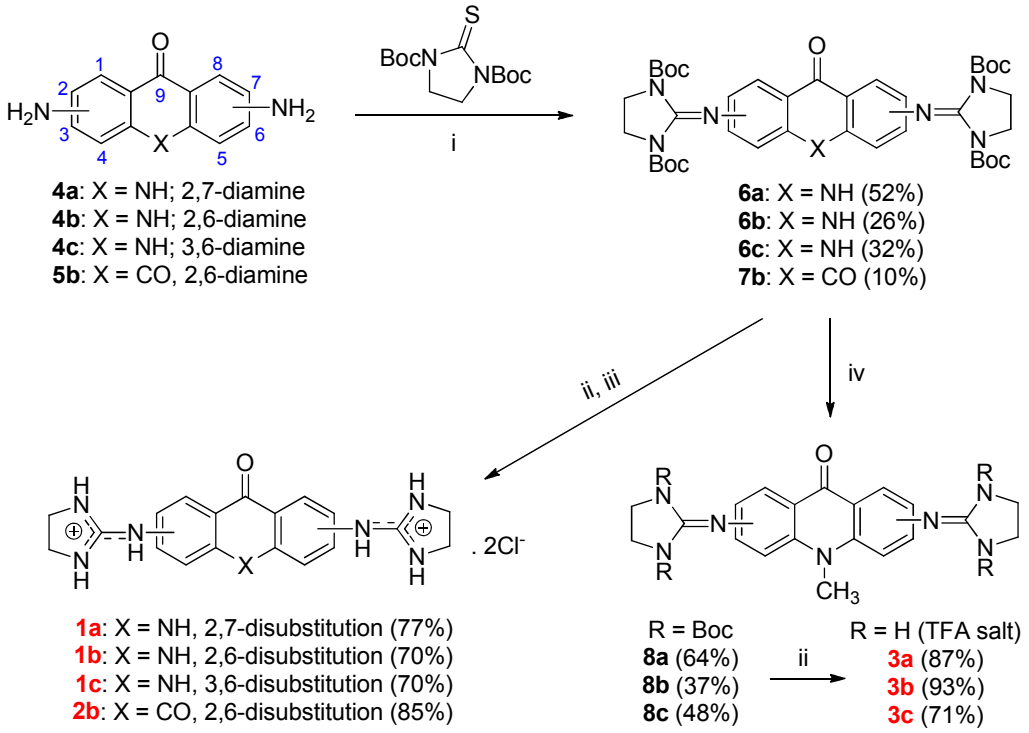
In this study, the compounds were screened in vitro against a panel of parasites (*Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*). Their DNA binding affinity and binding mode were studied by UV spectroscopy and circular dichroism (CD) experiments with fish sperm (FS) DNA. Preliminary in vivo studies in mouse models of human African trypanosomiasis and malaria.were also carried out with four molecules. The biological and biophysical data

gathered in this work confirmed the high antiprotozoal potential of this series of dicationic acridones and the importance of the substitution pattern on the observed antiprotozoal activity and DNA binding properties of the compounds.

Results

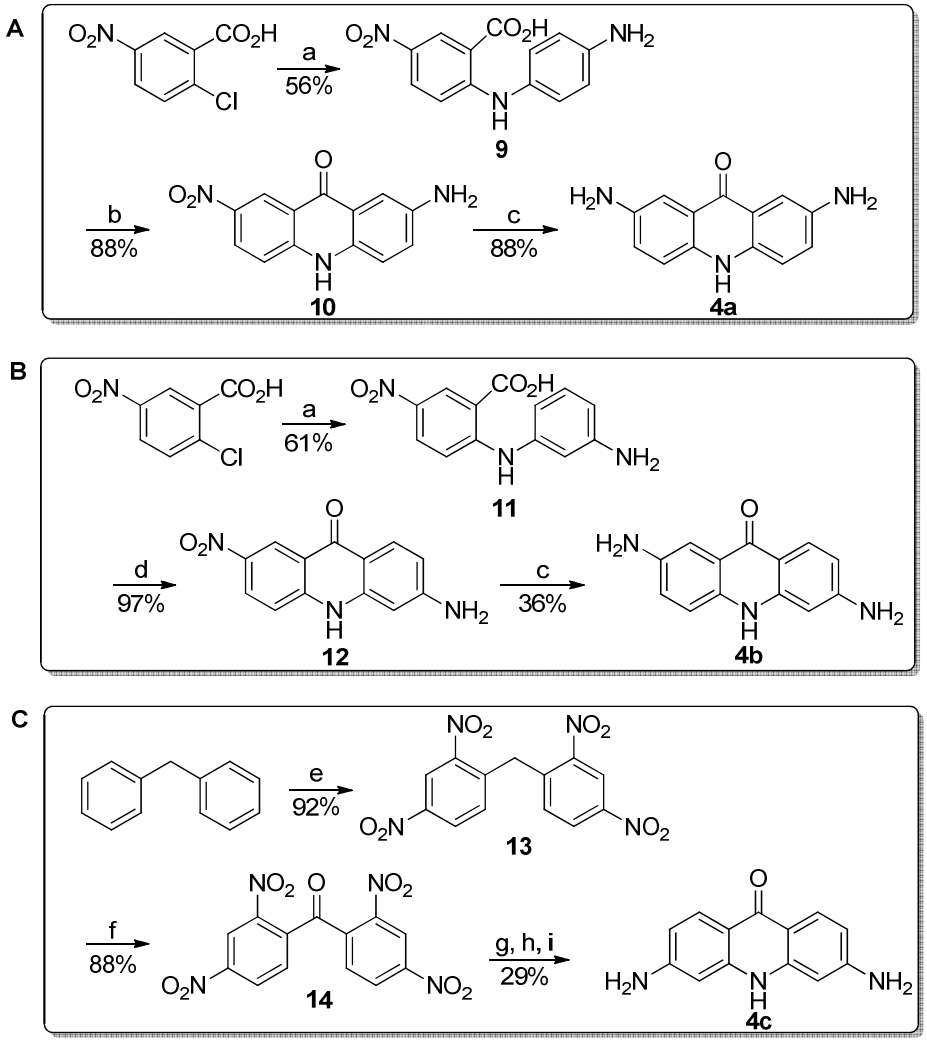
Chemistry. The dicationic target compounds **1a**–**c,** and **2b** were synthesized in two steps from the diamines **4a**–**c** and **5b**, respectively (Scheme 1). Diamines **4a** and **4b** were synthesized in three steps from 1-chloro-4-nitrobenzoic acid whereas **4c** was prepared in four steps from diphenylmethane (Scheme 2).[17] 2,6-Diaminoanthraquinone (**5b**) was commercially available. An excess of the classical *N,N′*-di(*tert*-butoxycarbonyl)imidazolidine-2-thione reagent was used to introduce the Boc-protected imidazoline ring as previously reported.[18] The isolated Boc-protected 2-aminoimidazoline compounds **6a**–**c** and **7b** were treated with TFA to yield the trifluoroacetate salts of the target compounds. For in vivo assays, the pharmacologically acceptable dihydrochloride salts of **1a**–**c** and **2b** were prepared in good yield with strongly basic anion exchange resin (Cl^- form). *N*-methylation of acridones **6a**–**c** with dimethylsulfate followed by acidic hydrolysis of the Boc-protecting groups yielded the *N*-methyl acridones **3a**–**c** as trifluoroacetate salts.

Scheme 1.[a] Synthesis of the Target Compounds



[a] Reagents and conditions: (i) $HgCl_2$, Et_3N, DMF, 0 ºC→ rt; (ii) TFA, CH_2Cl_2; (iii) strongly basic anion exchange resin (Cl^- form), H_2O, THF; (iv) Me_2SO_4, K_2CO_3, THF, Δ

Scheme 2.[a] Synthesis of the Starting Material Diamines



[a] Reagents and conditions. (a) 1,4-phenylenediamine or 1,3-phenylenediamine, K_2CO_3, Cu, $CuSO_4$ cat., H_2O, reflux; (b) Polyphosphoric acid, 100 ºC; (c) Na_2S•9 H_2O, NaOH, EtOH, H_2O, reflux; (d) H_2SO_4 conc., 100 ºC; (e) KNO_3, H_2SO_4, 15 ºC→70 ºC; (f) CrO_3, AcOH, reflux; (g) HCl conc., $SnCl_2$, 90–100 ºC; (h) EtOH, HCl conc., 100 ºC; (i) 20 % aqueous NaOH, reflux.

In Vitro Antiprotozoal Activity. The target compounds as well as seven synthetic intermediates were screened in vitro against *T. brucei*, *T. cruzi*, *L. donovani*, and *P. falciparum*. Cytotoxicity against rat L6-cells was also evaluated in order to calculate the selectivity index of the compounds (Table 1). None of the compound tested had significant activity against *T. cruzi* or *L. donovani* amastigotes. The target acridones **1a**–**1c** displayed low nanomolar activities against bloodstream trypanomastigotes of *T. b. rhodesiense* and excellent selectivities for the parasite (SI > 2000). Among these, the most active was the 2,6-disubstituted compound **1b** (IC_{50} = 7 nM) which was equipotent to the reference drug melarsoprol and approximately 3-times more potent than the *N*-phenylbenzamide linked compound **I** (Figure 1, IC_{50} = 25 nM).[3] In contrast, the 2,6-disubstituted anthraquinone analogue **2b** was 92- and 328-fold less active than **I** and **1b**, respectively, indicating that NH ↔ CO replacement is not tolerated. Interestingly, methylation at N-10 did not affect the activity for the 2,6- (**3b**) and 3,6- (**3c**) disubstituted acridones whereas it did increase by 10-fold the anti-*T. brucei* effect of the 2,7-disubstituted compound (**3a**). Hence, in this series the in vitro antitrypanosomal activity profile was dependent on the substitution pattern of the acridone scaffold, with 2,6-disubstitution >> 3,6 > 2,7 for N-10(H) acridone derivatives **1a–c**, and 2,7 ≈ 2,6 > 3,6 for *N*-methylated analogues **3a–c**.

In general, all of these compounds were less active against *P. falciparum* with submicromolar IC_{50}s except the anthraquinone derivative **2b** that displayed a reversed activity profile with nanomolar IC_{50} against *P. falciparum* (61 nM) and micromolar IC_{50} against *T. brucei* (2.29 μM). The aromatic amines **4a**, **4b**, **10** and **12** lacking the imidazolinium moieties were mostly inactive against the parasites tested which confirms that the presence of two cations is necessary to get nanomolar antiparasitic activities.

Table 1. In Vitro Antiprotozoal Activity of the Target Compounds and Synthetic Intermediates[o]

Compound	*T. b. rhod.*[a]	*T. cruzi*[b]	*L. don.*[c]	*P. falc.*		Cytotox.[f]
				K1[d]	NF54[e]	
	IC_{50} (Selectivity index)[g] μM					CC_{50} (μM)
4a	2.9	149	321	2.5	nd[h]	75
4b	19.2	269	288	4.8	nd	23
6a	2.2	50	>127	33	nd	73
1a	**0.069** (>3333)	>230	177	**0.34** (>666)	nd	>230
1b	**0.007** (15257)	>230	73	nd	**0.83** (128)	107
1c	**0.062** (2210)	>230	113	nd	**0.25** (546)	137
2b[i]	2.3	104	145	**0.06** (1229)	nd	75
3a[i]	**0.007** (14843)	nd	nd	nd	**0.22** (468)	104
3b[i]	**0.008** (12287)	nd	nd	nd	**0.56** (175)	98
3c[i]	**0.060** (1647)	nd	nd	nd	**0.09** (1149)	99
9	55	292	23	52	nd	>365
10	19.5	287	77	49	nd	186
11	124	271	61	70	nd	266
12	67	279	291	34	nd	147
melarsoprol	0.005					
benznidazole		1.7				
miltefosine			0.45			
chloroquine				0.13	0.012	
podophyllotoxin						0.012

[a] *T. b. rhodesiense* STIB900 trypomastigotes. [b] *T. cruzi* Tulahuen C4 intracellular amastigotes. [c] *L. donovani* MHOM-ET-67/L82 axenic amastigotes. [d] *P. falciparum* intraerythrocytic stage: the K1 strain is resistant to chloroquine and pyrimethamine. [e] The NF54 strain is sensitive to all known antimalarials.[f] Rat skeletal myoblast L6-cells. [g] SI = [CC_{50} /IC_{50} (parasite)]. [h] Not determined. [i] Trifluoroacetate salt.

In Vivo Antitrypanosomal and Antimalarial Activity. A preliminary evaluation of the in vivo potential of these dicationic acridones in the *T. b. rhodesiense* STIB900 mouse model was carried out with the three non-methylated regioisomers **1a**–**1c**. This model which reproduces the acute rhodesiense infection is quite difficult to cure and represents a stringent model of first stage sleeping sickness. In this assay, mice were treated intraperitoneally with the compounds for 4 consecutive days and parasitemia

was monitored over 60 days. Mice that survived 60 days free of parasites were considered as cured. The intraperitoneal administration of 20 mg/kg of the compounds was curative for **1a** (2/4 mice) and **1b** (3/4 mice) whereas **1c** showed acute toxicity at this dose. At lower non-toxic dosage (5 mg/kg ip) **1c** was not curative and increased only moderately the mean time of relapse of parasitemia. Compound **1a** was also tested by oral dosage (50 mg/kg) showing a moderate effect although no cures were obtained (Table 2). This indicates that the oral bioavailability of this class of dicationic acridones is modest even though there is room for improvement.

Compound **2b** which was the best antimalarial compound from the in vitro screening was tested in the *P. berghei* mouse model by intraperitoneal administration.[19, 20] Unfortunately, this compound was inactive on a four days treatment at 20 mg/kg/day while higher dosage (50 mg/kg) was not tolerated.

Table 2. In Vivo Activity in the STIB900 Mouse Model of Stage 1 Sleeping Sickness.

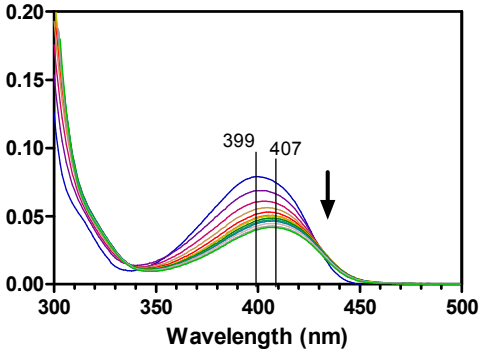
Compound	Dosage route[a]	Dosage (mg/kg)	Cured[b]/Infected	Mean day of relapse
Control	-	-	0/4	7[c]
1a	ip	4 × 20	2/4	> 45.5
	po	4 × 50	0/4	22
1b	ip	4 × 20	**3/4**	**> 50**
1c	ip	4 × 20	T[d]	T[d]
		4 × 5	0/4	14

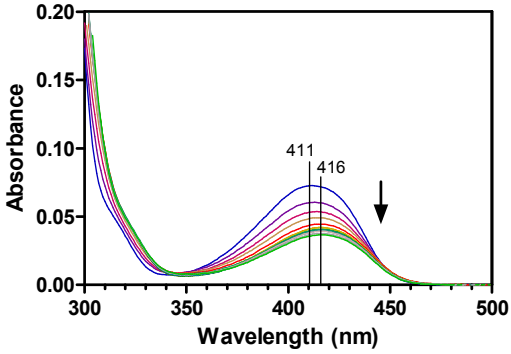
[a] ip = intraperitoneal, po = per os (oral); [b] Number of mice that are alive and parasite free after 60 days; [c] All mice were positive and euthanized at day 7. [d] Toxic after second administration.

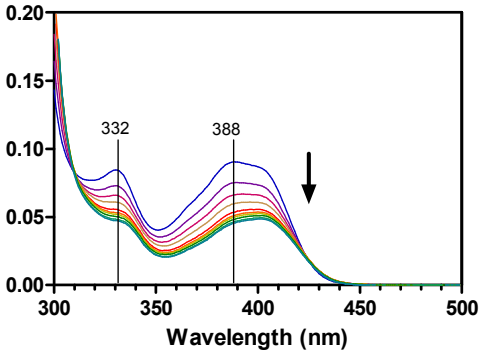
DNA Binding Studies. Since diphenyl bis-2-aminoimidazolines are DNA minor groove binders[2, 3, 6, 11] and acridone and anthraquinone derivatives generally bind by

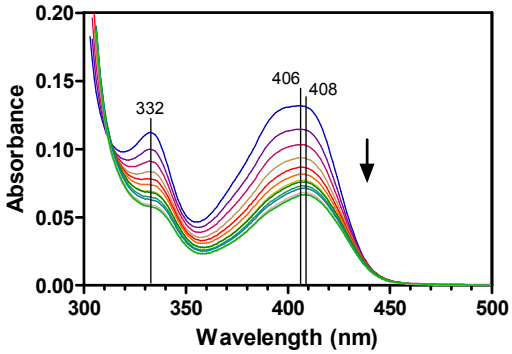
intercalation, we were interested in evaluating the binding affinity of the new compounds with DNA quantitatively.

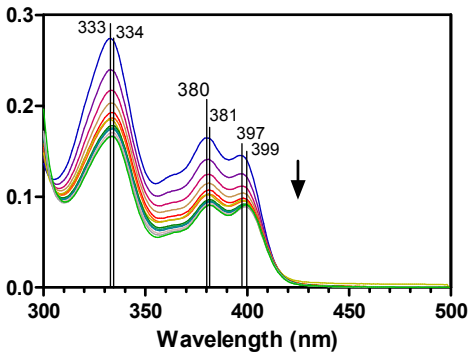
Spectrophotometric Titrations. All of these acridone compounds have strong absorption bands in the 250–300 nm range and weaker absorption bands above 300 nm. The bands over 300 nm, which do not overlap with the DNA absorbance at 260 nm, were useful for the spectrophotometric titrations. UV-vis spectra of compounds **1a-c**, **2b**, and **3a-c** were recorded in the 250–500 nm wavelength range in the presence of increasing amounts of FS-DNA. Addition of FS-DNA induced strong hypochromicity (approximately 46–49% decrease in extinction coefficient at the compound peaks wavelengths) and large bathochromic shifts for **1a**, **2b**, and **3a** indicating the formation of a complex with DNA (Figure 2 and Table 3). It is known that these effects are generally more pronounced for intercalators than for groove binders.[21-24] Hence, the results may indicate a prevailing intercalative binding mode in FS-DNA for **1a**, **2b**, and **3a**. In contrast, addition of FS-DNA to **1b**, **1c, 3b**, and **3c** led to high hypochromicities and weak bathochromic and hypsochromic shifts, respectively, indicating that binding to DNA occurred, albeit in a different way. These results may indicate a mixed intercalative and groove binding mode in FS-DNA. Apparently, these dicationic compounds have different binding behaviours which appear to be dependent on the respective position of the imidazoline rings on the acridone scaffold and the presence of a methyl substituent at N-10 of the acridone ring. The binding constants calculated from Scatchard plot analysis of the titration experiments using the (non–co–operative) neighbour exclusion model of McGhee and von Hippel[25] were in the range $9.6 – 31.4 \times 10^4$ M^{-1} (Table 3) which is consistent with binding affinities found for other intercalators.

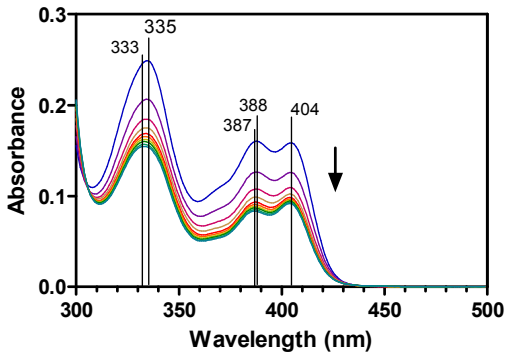

1a
399
407
Wavelength (nm)


3a
411
416
Absorbance
Wavelength (nm)


1b
332
388
Wavelength (nm)


3b
332
406
408
Absorbance
Wavelength (nm)


1c
333
334
380
381
397
399
Wavelength (nm)


3c
333
335
387
388
404
Absorbance
Wavelength (nm)

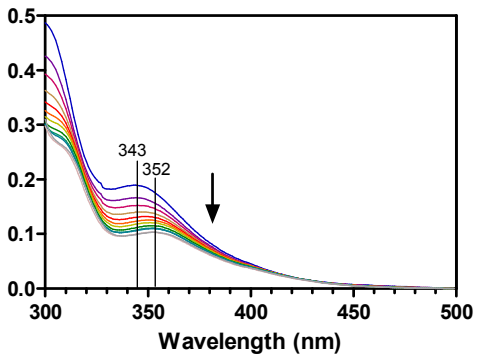

2b
343
352
Wavelength (nm)

Figure 2. Spectrophotometric titration of compounds **1a-c**, **2b**, and **3a-c** (20 μM) with FS-DNA in 10 mM phosphate buffer (pH 7) at 25 ºC. FS-DNA concentrations ranged from 0 to 2.08×10^{-4} M (**1a**), 0 to 1.08×10^{-4} M (**1b**), 0 to 1.2×10^{-4} M (**1c**), 0 to 1.85×10^{-4} M (**2b**), 0 to 3.47×10^{-4} M (**3a**), 0 to 2.28×10^{-4} M (**3b**), and 0 to 1.58×10^{-4} M (**3c**). The arrows indicate increasing concentration of DNA.

Table 3. Binding Constants and UV-vis Absorption Data Determined by UV-Spectrophotometric Titrations with FS-DNA

Cmpd	λ_{max} free (nm)	λ_{max} bound (nm)	$\Delta\lambda$ (nm)	Hypochromicity[a] (%)	$K \times 10^4$ (M^{-1}) (n)[b]
1a	399	407	+8	49	13.1 ± 1.0 (0.6–0.7)
1b	332	332	0	47	31.4 ± 0.1 (1.2–1.7)
	388	nd[c]	nd	nd	
1c	333	334	+1	41	26.5 ± 3.2 (0.5–1.1)
	380	381	+1	47	
	397	399	+2	40	
2b	343	352	+9	46	9.6 ± 0.5 (1.9–2.3)
3a	411	416	+5	48	25.0 ± 4.9 (0.5–1.0)
3b	406	408	+2	50	11.9 ± 0.4 (1.3–2.1)
3c	335	333	-2	35	17.5 ± 0.9 (2.3–2.4)
	388	387	-1	46	
	404	404	0	41	

[a] H = [(Absorbance $cmpd_{free}$ - Absorbance complex) / Abs. $cmpd_{free}$]×100. [b] Fitting with the McGhee & von Hippel neighbour exclusion model.[25] The neighbour exclusion parameter "n" (expressed in bp) is indicated between brackets. [c] Not determined due to overlapping bands.

Circular Dichroism (CD) Spectroscopy. CD spectroscopy can provide useful information on the mode of binding of achiral ligands to DNA. An induced CD (ICD) signal observed in the presence of DNA indicates ligand-DNA interaction, the intensity and sign of which depend on the binding mode. In general, minor groove B-DNA binders with a transition moment oriented along the groove show strong positive ICD. In contrast, intercalators display small ICD signals, the sign of which depends on the orientation of the ligand transition moment, either parallel (positive ICD) or perpendicular (negative ICD) to the dyad axis of DNA.[26]

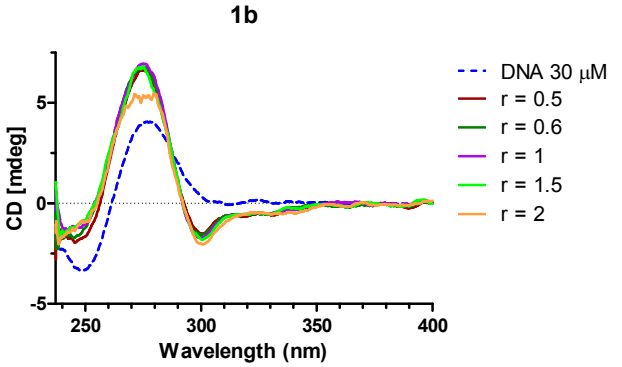
The binding mode of four representative compounds (**1b**, **1c**, **2b**, and **3c**) with FS-DNA was studied by CD spectroscopy in the wavelength range 230–500 nm. In these experiments, the concentration of DNA was kept constant (30 μM) and different ligand/DNA mixing ratios, r, were studied (Figure 3). These achiral compounds had no CD signal when free in solution but all of them induced an increase in intensity of the band at 275 nm when mixed with FS-DNA. However, it is difficult to say if this signal, which ovelaps with the DNA band (λ_{max} = 278 nm), was due to an ICD band of the ligand or to a conformational change of the DNA.

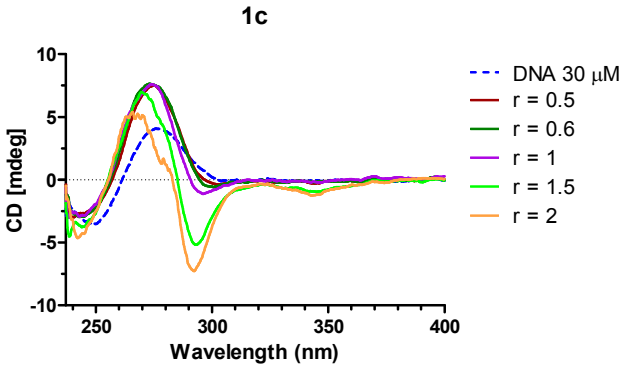
The CD spectrum of **1b** in presence of DNA showed a weak negative ICD band at 300 nm, the intensity of which augmented with increasing mixing ratio. A clear isoelliptic point was also observed at 292 nm in the whole range of drug/DNA ratios indicating a single dominant binding mode. This may be indicative of an intercalative-binding mode. CD spectra of **1c** showed variation depending on the binding ratio. At low ratio (0.5–0.6) a positive band of unvarying intensity overlapping with the DNA band was observed at 275 nm. At high drug/DNA ratio (1–2), the intensity of the positive band decreased whereas a negative ICD band at 297 nm appeared gradually upon increasing r. Red shift for $r \geq 1$ and deviation of the isoelliptic point for all different ratio were also observed possibly indicating heterogenous mode of binding (e.g. groove binding and

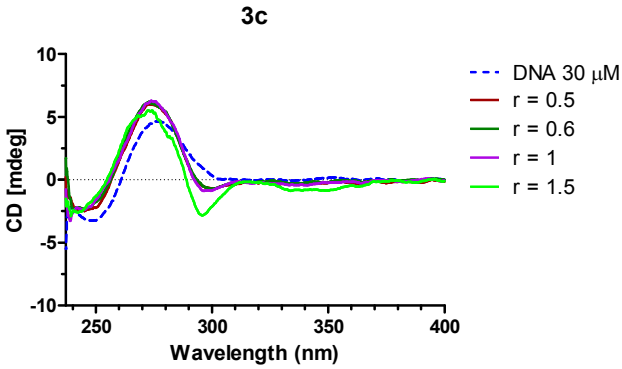
intercalation). Even though the CD spectras of **1c** and **3c** showed similar profiles some difference were apparent indicating similar but not identical modes of binding to FS-DNA. At drug/DNA ratio of 0.5–1, **3c** showed a weak negative band at 297 nm with an isoelliptic point at 286 nm, indicative of one dominant binding mode. At higher ratio (r = 1.5) the negative band at 297 nm was red shifted and more pronounced, albeit less intense than for **1c** at the same binding ratio. These results are consistent with the presence of mixed binding modes in DNA at higher r.

CD spectra of the DNA–anthraquinone **2b** complex were quite different from that observed with acridones **1b**, **1c**, and **3c**. No ICD signal was observed at r = 0.5–0.6 whereas a positive ICD signal at approximately 280 nm (overlapping with DNA band) and a weak negative ICD at 380 nm appeared upon increment of r to 1 and 1.5. This may indicate intercalation or external stacking at high compound to DNA concentration. Altogether, these results are consistent with a prevailing intercalation mode of binding even though other types of binding interaction (e.g. groove binding, electrostatic) cannot be ruled out at this point.

In summary, UV titrations and CD results showed that these compounds bind to DNA and suggest the existence of different binding modes (intercalation and/or minor groove) depending on the structure and the compound to DNA ratio. Similar results were described with the diamidine drug DAPI, an intercalator that also has very favorable interaction in the minor groove at AT sequences.[21, 23, 27, 28] Recent findings by Nagle et al.[29] with a series of rigid core (fluorene and dihydroanthracene) bis-2-aminoimidazolinium derivatives also support our observations that the relative position of both cations influences the binding mode of these dicationic acridones. Further work with specific oligonucleotides will be needed to elucidate the precise mode of binding to DNA and sequence specificity of these dicationic acridones.


1b
CD [mdeg]
Wavelength (nm)
DNA 30 μM
r = 0.5
r = 0.6
r = 1
r = 1.5
r = 2


1c
CD [mdeg]
Wavelength (nm)
DNA 30 μM
r = 0.5
r = 0.6
r = 1
r = 1.5
r = 2


3c
CD [mdeg]
Wavelength (nm)
DNA 30 μM
r = 0.5
r = 0.6
r = 1
r = 1.5

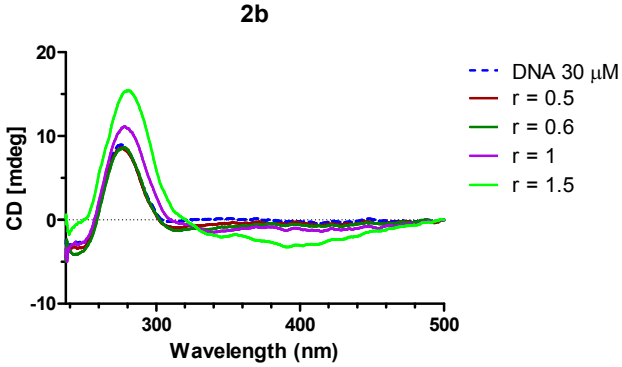

2b
CD [mdeg]
Wavelength (nm)
DNA 30 μM
r = 0.5
r = 0.6
r = 1
r = 1.5

Figure 3. CD spectra of **1b**, **1c**, **3c**, and **2b** with FS-DNA (30 μM) at different mixing ratio (r = 0.5, 0.6, 1.0, 1.5, 2.0). The spectra were recorded at 20 ºC in PBS containing 6% DMSO (v/v).

Discussion

Dicationic bisimidazoline derivatives of the *N*-phenylbenzamide scaffold have shown great promise as antitrypanosomal agents and DNA minor groove binders.[3] In this study, we have tested the effect on antiprotozoal activity and DNA binding affinity of conformational restriction of the *N*-phenylbenzamide scaffold. The influence of the relative position of both imidazolinium cations on the heterocyclic scaffold was also studied. On the one hand, the conformational restriction produced by the introduction of the acridone scaffold led to an increase in trypanocidal activity (about 3-fold compared to the *N*-phenylbenzamide derivative **I**) for three compounds (**1b**, **3a**, and **3b**). In fact, these acridones derivatives which have low nanomolar in vitro activity (< 10 nM) against *T. b. rhodesiense* STIB900 and outstanding selectivity (> 12000) represent, with the fluorene derivative reported before,[3] the most potent class of bis(2-aminoimidazolines) synthesized up to now. These IC_{50} values are comparable to that of the reference drugs melarsoprol and pentamidine in this assay.[30] On the other hand, the substitution of the acridone by an anthraquinone heterocycle led to a drastic loss of activity against *T. brucei* (100-fold) but hardly affected the antiplasmodial activity (IC_{50} = 61 nM vs 28 nM for **I**). This CO ↔ NH replacement also had a dramatic effect on the acute toxicity in vivo (e.g. the 2,6-anthraquinone derivative **2b** was toxic after the third application at 50 mg/kg ip). Hence, the presence of nitrogen (N-10) in the scaffold structure was favorable for high level anti-*T. brucei* action. Noteworthy, methylation at N-10 did not modify significantly the anti-*T. brucei* activity and cytotoxicity except for **3a** the activity and cytotoxicity of which was increased 10- and 2-times, respectively.

Remarkably, the relative disposition of both imidazoline cations with respect to the heterocyclic scaffold was critical to the observed in vitro and in vivo activities. Clearly, the acridone scaffold with a 2,6-disubstitution pattern gave the best results against *T. brucei* in vitro (**1b** and **3b**) and in vivo (**1b**). In contrast, the 2,6-disubstituted anthraquinone derivative **2b** moderately affected *T. brucei* growth whereas it showed a pronounced in vitro activity against the *P. falciparum* chloroquine-resistant strain K1. This result is consistent with a previous report by Winkelmann and Raether[15] which showed that 2,6-diguanidine anthraquinones have no effect on *T. brucei*. In this series, the 3,6-disubstitution pattern was clearly the worst in vivo as it provoked acute toxicity at low dosage (20 mg/kg). As preliminary screen, one compound, **1a**, was tested by the oral route in the STIB900 mouse model. Even though no cures were obtained, a significant increase in the mean day of relapse of parasitemia was observed (22 vs 7 days for control mice) indicating weak oral bioavailability. Further work will be necessary to determine whether other compounds of this series have better oral bioavailability or if this property can be improved by structure modification.

The bis(2-aminoimidazoline) class of molecules binds strongly to the minor groove of AT-rich DNA.[2, 3, 6, 7, 11, 31, 32] Since dicationic compounds can accumulate to high levels within the mitochondrion of *T. brucei* driven electrostatically by the negatively charged environment of the inner mitochondrial matrix,[33] this interaction with DNA could well be the basis for their antitrypanosomal activity as AT-content of *T. brucei* kDNA is approximately 72%.[34] Based on the planar tricyclic structure of these derivatives, an intercalation mode of binding was expected for the dicationic acridones and anthraquinone analogues reported here. This mode of binding has been observed previously with related 3,6-bis(3-alkylguanidino)acridines.[35] In general, the effect of nitrogen methylation at *N*–10 on DNA binding affinity was small indicating that N(10)H does not contribute much to DNA binding. This is consistent with a prevalent

intercalative mode of binding as the *N*-methyl group is co-planar to the flat acridone ring and, as such, should not have major influence on the intercalation between adjacent base pairs. Our data support the view that the 3,6-disubstituted acridones **1c** and **3c** have a different (e.g. minor groove) mode of binding to DNA compared to 2,6- and 2,7-disubstituted acridones. In fact, 3,6-disubstituted acridones reported here have a crescent shape similar to other dicationic minor groove binders such as 3,6- and 2,7-bisimidazoline carbazoles,[36] furamidine[37], or **VI**[7] (Figure 1) that match the groove curvature and can fit into the minor groove at AT-rich sequences.

Conclusions

We have shown here that the conformational restriction of the *N*-phenylbenzamide scaffold by transformation into an acridone heterocycle led to dicationic bisimidazoline compounds with potent in vitro activity against *T. brucei* and *P. falciparum*. Compound **1b** in particular was as active as melarsoprol in vitro and also curative in the STIB900 mouse model of stage 1 HAT. Clearly, the antiprotozoal activity depends on the relative position of both imidazolinium cations on the fused-heterocyclic scaffold, the 2,6-disubtitution pattern giving the best results against *T. brucei*. Finally, we have shown that these compounds bind to DNA with binding constants in the 10^4 M^{-1} range. The substituents' (cations) relative position also influenced the DNA binding mode even though intercalation seemed to be the prevailing one. Since no apparent correlation was observed between DNA binding affinity and in vitro activity against *T. brucei*, we conclude that additional mechanisms of action may be involved in the antitrypanosomal action of these dicationic acridones.

Experimental section

Chemistry. All dry solvents and reagents were purchased from Aldrich or Fluka in Sure/Seal bottles. All reactions requiring anhydrous conditions or an inert atmosphere were performed under a positive pressure of N_2. All reactions were monitored by HPLC–MS or Thin Layer Chromatography (TLC) using silica gel 60 F_{254} plates (Merck). Chromatography was performed with Isolute SI prepacked columns. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 300 or Varian Inova 500 spectrometer. Chemical shifts of the ^{1}H NMR spectra were internally referenced to TMS (δ 0 ppm) for $CDCl_3$ or the residual proton resonance of the deuterated solvents: D_2O (δ 4.79 ppm), CD_3OD (δ 3.31 ppm) and DMSO-d_6 (δ 2.50 ppm). Signal splitting patterns are described as: singlet (s), broad singlet (br s), doublet (d), triplet (t), quadruplet (q), multiplet (m), or combination thereof. *J* values are given in Hz. Melting points were determined in open capillary tubes with a SMP3–Stuart Scientific apparatus or Mettler Toledo MP70 melting point system, and are uncorrected. All compounds are >95% pure by HPLC or combustion analysis otherwise noted. Elemental analysis was performed on a Heraeus CHN–O Rapid analyser. Analytical results were within ± 0.4 % of the theoretical values unless otherwise noted. Analytical HPLC–MS was run with an Xbridge C18–3.5 μm (2.1×100 mm) column on a Waters 2695 separation module coupled with a Waters Micromass ZQ spectrometer using electrospray ionization (ESI^+). The following HPLC conditions were used: column temperature = 30 ºC, gradient time = 5 min, H_2O/CH_3CN (10:90 → 90:10) (HCO_2H 0.1 %), flow rate = 1 mL/min, UV detection: photodiode array (λ = 190−400 nm). Accurate mass were measured with an Agilent Technologies Q–TOF 6520 spectrometer using electrospray ionization.

The starting material diamines (**4a–c**) were synthesized according to the procedures described in a patent by Neidle et al.[17] The synthesis and characterization of **4a–c** and

the Boc-protected imidazoline intermediates (**6a**–**c**, **7b**, and **8a–c)** is given as Supporting Information.

General Procedure for the Removal of the Boc-protecting Groups and Regeneration of the Hydrochloride Salts. The Boc-protected compound (**6a**, **6b**, **6c**, or **7b**) dissolved in CH_2Cl_2 (2 mL) was treated with TFA (2 mL). The clear solution was stirred at room temperature for 5 h. The volatiles were removed under vacuum and Et_2O was added to the flask. The crude residue was triturated with a spatula to yield the TFA salt of the product. The etheral phase was discarded and the solid dissolved in 30 mL of water (for **1a**, **1c**, **2b**) was stirred overnight with Amberlyte IRA400 strongly basic anion exchange resin (Cl^- form) [Note that for **1b**, 1 mL of THF was added as co-solvent to dissolve the TFA salt before adding the resin]. The resin was filtered off and washed with water. The aqueous filtrate was extracted successively with CH_2Cl_2 (10 mL) and EtOAc (10 mL), and filtered through a flutted paper. Rotatory evaporation of the water yielded the dihydrochloride salt of the compound as a solid.

Dihydrochloride salt of 2,7-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)acridin-9(10H)-one (1a). Starting from **6a** (111.5 mg) and following the general procedure, the dihydrochloride salt of **1a** was obtained as a yellow solid (49 mg, 77%). HPLC > 95%; mp > 250 ºC (decomp.). ^{1}H NMR (300 MHz, D_2O) δ 8.19 (br s, 2H, Ar*H*), 7.73 (br s, 4H, Ar*H*), 3.88 (s, 8H, *CH_2CH_2*). ^{13}C NMR (75 MHz, D_2O + CD_3OD) δ 178.6, 159.5, 139.6, 131.2, 130.5, 130.4, 120.3, 120.2, 43.8. LRMS (ES^+) *m/z* 362 $(M+H)^+$, 182 $[(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 362.1745 $[M+H]^+$ ($C_{19}H_{20}N_7O$ requires 362.1724).

Dihydrochloride salt of 2,6-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)acridin-9(10H)-one (1b). Starting from **6b** (244 mg) and following the general procedure, the dihydrochloride salt of **1b** was obtained as a greenish solid (97 mg, 70%). HPLC > 95%; mp > 350 ºC. ^{1}H NMR (300 MHz, DMSO) δ 12.37 (s, 1H, *NH*), 11.31 (s, 1H,

NH), 10.64 (s, 1H, *NH*), 8.70 (s, 2H, *NH*), 8.37 (s, 2H, *NH*), 8.25 (d, *J* = 8.7, 1H, Ar*H*), 8.03 (d, *J* = 2.4, 1H, Ar*H*), 7.66 (m, 2H, Ar*H*), 7.45 (s, 1H, Ar*H*), 7.16 (dd, *J* = 1.9, 8.8, 1H, Ar*H*), 3.73 (s, 4H, CH_2CH_2), 3.68 (s, 4H, CH_2CH_2). ^{1}H NMR (300 MHz, D_2O) δ 7.49 (d, *J* = 8.4, 1H, Ar*H*), 7.27 (s, 1H, Ar*H*), 7.19 (d, *J* = 8.4, 1H, Ar*H*), 6.92 (d, *J* = 8.9, 1H, Ar*H*), 6.70 (d, *J* = 8.9, 1H, Ar*H*), 6.55 (s, 1H, Ar*H*), 3.86 (s, 4H, CH_2CH_2), 3.82 (s, 4H, CH_2CH_2). ^{13}C NMR (75 MHz, D_2O) δ 176.9, 158.2, 157.3, 140.8, 140.7, 138.4, 129.6, 129.5, 127.9, 119.5, 119.2, 118.3, 116.4, 115.6, 107.2, 43.2. LRMS (ES^+) *m/z* 362.4 $(M+H)^+$, 181.5 $[(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 362.1737 $[M+H]^+$ ($C_{19}H_{20}N_7O$ requires 362.1724).

Dihydrochloride salt of 3,6-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)acridin-9(10H)-one (1c). Starting from **6c** (102 mg) and following the general procedure, the dihydrochloride salt of **1c** was obtained as a yellow solid (40 mg, 70%). HPLC > 95%; mp > 250 ºC (decomp.). ^{1}H NMR (300 MHz, D_2O) δ 7.66 (d, *J* = 8.8, 2H, Ar*H*), 6.80 (dd, *J* = 2.1, 8.8, 2H, Ar*H*), 6.67 (d, *J* = 2.1, 2H, Ar*H*), 3.90 (s, 8H, CH_2CH_2). ^{13}C NMR (75 MHz, D_2O) δ 177.0, 157.5, 141.1, 140.8, 128.0, 117.1, 115.7, 107.5, 43.2. LRMS (ES^+) *m/z* 362.5 $(M+H)^+$, 181.6 $[(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 362.1739 $[M+H]^+$ ($C_{19}H_{20}N_7O$ requires 362.1724).

Dihydrochloride salt of 2,6-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)anthraquinone (2b). Starting from **7b** (94 mg) and following the general procedure, the dihydrochloride salt of **2b** was obtained as an orangish solid (46 mg, 85%). HPLC > 95%; mp > 250 ºC (decomp.). ^{1}H NMR (300 MHz, D_2O) δ 8.09 (d, *J* = 8.5, 2H, Ar*H*), 7.80 (d, *J* = 2.3, 2H, Ar*H*), 7.61 (dd, *J* = 8.5, 2.3, 2H, Ar*H*), 3.90 (s, 8H, CH_2CH_2). ^{13}C NMR (75 MHz, D_2O) δ 182.8, 157.9, 142.3, 134.7, 130.2, 129.9, 127.3, 119.2, 43.3. LRMS (ES^+) *m/z* 375 $[(M+H)^+$, 100%]. ESI–HRMS *m/z* 375.1555 $[M+H]^+$ ($C_{20}H_{19}N_6O_2$ requires 375.1564).

Ditrifluoroacetate salt of 2,7-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)-10-methylacridin-9(10H)-one (3a). A solution of **8a** (13 mg, 0.167 mmol) dissolved in CH_2Cl_2 (0.5 mL) and trifluoroacetic acid (0.2 mL) was stirred at room temperature for 66 h. The solvents were removed under vacuum and the crude residue dissolved in a little MeOH was filtered through a plug of cotton to remove insoluble matter. The clear solution was concentrated to ca. 0.2 mL and diethyl ether was added to precipitate the product. The flask was allowed to stand for a few hours in the refrigerator, the supernatant was removed, and the solid precipitate was rinsed with Et_2O. Compound **3a** was obtained as a yellow amorphous solid (8.8 mg, 87%). HPLC > 95%; mp 234 ºC. ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (d, *J* = 2.7, 2H, Ar*H*), 7.98 (d, *J* = 9.2, 2H, Ar*H*), 7.78 (dd, *J* = 2.7, 9.2, 2H, Ar*H*), 4.06 (s, 3H, N*CH*$_3$), 3.83 (s, 8H, *CH*$_2$*CH*$_2$). ^{13}C NMR (126 MHz, CD_3OD) δ 178.56, 160.53, 142.75, 132.12, 131.17, 123.44, 122.90, 119.39, 44.26, 34.91. LRMS (ES^+) *m/z* 376.5 [$(M+H)^+$], 188.7 [$(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 376.1896 $[M+H]^+$ ($C_{20}H_{22}N_7O$ requires 376.1880).

Ditrifluoroacetate salt of 2,6-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)-10-methylacridin-9(10H)-one (3b). Using the same procedure as above starting from **8b** (8 mg, 0.103 mmol) yielded **3b** as a yellowish amorphous solid (5.8 mg, 93%). HPLC = 93%; mp 166–170 ºC. ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (d, *J* = 8.6, 1H, Ar*H*), 8.31 (d, *J* = 2.7, 1H, Ar*H*), 7.96 (d, *J* = 9.2, 1H, Ar*H*), 7.76 (dd, *J* = 2.7, 9.2, 1H, Ar*H*), 7.70 (d, *J* = 1.9, 1H, Ar*H*), 7.28 (dd, *J* = 1.9, 8.7, 1H, Ar*H*), 4.03 (s, 3H, N*CH*$_3$), 3.88 (s, 4H, *CH*$_2$*CH*$_2$), 3.82 (s, 4H, *CH*$_2$*CH*$_2$). ^{13}C NMR (126 MHz, CD_3OD) δ 178.38, 160.51, 159.72, 145.12, 143.10, 143.04, 131.94, 131.15, 130.44, 123.86, 122.98, 120.94, 119.26, 117.62, 110.29, 44.27, 44.26, 34.83. LRMS (ES^+) *m/z* 376.6 [$(M+H)^+$], 188.8 [$(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 376.1892 $[M+H]^+$ ($C_{20}H_{22}N_7O$ requires 376.1880).

Ditrifluoroacetate salt of 3,6-bis((4,5-dihydro-1*H*-imidazol-2-yl)amino)-10-methylacridin-9(10H)-one (3c). Using the same procedure as above starting from **8c**

(10 mg, 0.129 mmol) yielded **3c** as a brownish amorphous solid (5.5 mg, 71%). HPLC = 93%; mp 180–181 ºC. ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (d, *J* = 8.6, 2H, Ar*H*), 7.67 (d, *J* = 1.9, 2H, Ar*H*), 7.27 (dd, *J* = 1.9, 8.6, 2H, Ar*H*), 3.97 (s, 3H, NCH_3), 3.87 (s, 8H, CH_2CH_2). ^{13}C NMR (126 MHz, CD_3OD) δ 178.20, 159.72, 145.34, 142.92, 130.40, 121.30, 117.57, 110.34, 44.27, 34.77. LRMS (ES^+) *m/z* 376.5 $[(M+H)^+]$, 188.8 $[(M+2H)^{2+}$, 100%]. ESI–HRMS *m/z* 376.1899 $[M+H]^+$ ($C_{20}H_{22}N_7O$ requires 376.1880).

Biology

In vitro antiprotozoal activity. An Alamar blue-based assay was used to determine the in vitro activity against bloodstream forms *T. b. rhodesiense*[38] and axenically grown amastigotes of *L. donovani*,[39] and the cytoxicity to rat myoblast L6-cells as previously reported.[20] Activity against intracellular amastigotes of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (LacZ) gene[40] was determined with the colorimetric assay using chlorophenyl red β-D-galactopyranoside (CPRG)-Nonidet as reported.[20] Antimalarial activity against erithrocytic stage of *P. falciparum* (NF54 chloroquine sensitive strain and K1 chloroquine/pyrimethamine resistant strain) was determined with a [^{3}H]hypoxanthine incorporation assay.[20, 41]

In vivo activity against *T. b. rhodesiense*. Four female NMRI mice were used per experimental group. Each mouse was inoculated i.p. with 10^4 bloodstream forms of STIB900, respectively. Heparinized blood from a donor mouse with approximately 5 × 10^6 /mL parasitaemia was suspended in PSG to obtain a trypanosome suspension of 1 × 10^5 /mL. Each mouse was injected with 0.25 mL. Compounds were formulated in 100% DMSO and diluted 10-fold in distilled water. Compound treatment was initiated 3 days post-infection on four consecutive days for all administration routes (i.p., p.o.) in a volume of 0.1 mL/10 g. Three mice served as infected-untreated controls. They were

not injected with the vehicle alone since we have established in our laboratory (over many years) that these vehicles do not affect parasitaemia nor the mice. Parasitaemia was monitored using smears of tail-snip blood twice a week after treatment for two weeks followed by once a week until 60 days post-infection. Mice were considered cured when there was no parasitaemia relapse detected in the tail blood over the 60-day observation period. Mean relapse days (MRD) were determined as day of relapse post-infection of mice.

All the in vivo efficacy studies in mice were conducted at the Swiss Tropical and Public Health Institute (Basel) according to the rules and regulations for the protection of animal rights ("Tierschutzverordnung") of the Swiss "Bundesamt für Veterinärwesen". They were approved by the veterinary office of Canton Basel-Stadt, Switzerland.

DNA binding

Spectrophotometric titrations. UV-vis spectra were measured on a Perkin–Elmer Lambda 35 UV–vis spectrophotometer in a 1.5 mL quartz cuvette (1 cm pathlength) in 10 mM phosphate buffer (pH 7) previously degassed by sonication. A stock solution of FS-DNA (23 mg in 5 mL of phosphate buffer) was prepared and shaken gently for 1 h. The solution was diluted 100× and the UV spectrum at 260 nm was recorded (Abs_{260} = 1.1016). The concentration of FS-DNA stock solution (8.6 mM) was worked out from the following equation: $Abs_{260} = 100 \times C \times d \times \varepsilon_{260}$ using the extinction coefficient 12800 $M(bp)^{-1}.cm^{-1}$ at 260 nm for FS-DNA.[42] Stock solutions of the compounds **1a-c**, **2b**, and **3a-c** (1 mL, C = 20 μM in phosphate buffer) were prepared from 1 mM stock solutions in DMSO by dilution with 10 mM phosphate buffer. The final amount of DMSO in the stock solution was 2%.

Spectrophotometric titrations were performed by sequential addition of aliquots of DNA solution (C = 860 μM) to 800 μL of the compound (20 μM) until saturation was observed. A 1 min incubation time was allowed between each addition. The experiments were performed at 25 ºC. The data was processed with Excel and the percentage of hypochromicity (peak to peak) and bathochromic shift was calculated. *K* values were determined using Scatchard plots and non linear curve fitting with the (non–co–operative) McGhee and von Hippel neighbour exclusion model.[25]

CD experiments. Circular dichroism studies were recorded using a Jasco J-815 spectropolarimeter from 220 to 500 nm with 1 cm quartz cuvettes at 20 ºC. The spectra were averaged over three scans with a scan speed of 200 nm/min and a buffer blank correction. A 30 μM FS-DNA solution in 10 mM phosphate buffer (pH 7) with 6% DMSO was first scanned; the compounds at increasing concentration ratios were then titrated into the same cuvette, and the complexes were scanned under the same conditions. For each compound (**1b**, **1c**, **2b**, and **3c**), six dilutions in phosphate buffer with 6% DMSO (15, 20, 30, 45, and 60 μM) were prepared starting from 1 mM stock solutions in DMSO. The desired ratios of compound to DNA (r = 0.5, 0.6, 1.0, 1.5, and 2.0) were obtained by adding compound to the cell containing a constant amount of DNA. FS-DNA was purchased from ACROS (CAS 68938-01-2).

Acknowledgements. This work was supported by grants from the Spanish Ministerio de Ciencia e Innovación (Grant SAF2009-10399). SMQ was recipient of a JAE-Intro fellowship from the CSIC.

Supporting information available. Synthesis and characterization of starting material diamines (**4a**, **4b**, and **4c**) and Boc-protected intermediates **6a–c, 7b**, and **8a–c**.

Corresponding Author

*Phone: +34 912587490. Fax. +34 915644853. E-mail: dardonville@iqm.csic.es

Abbreviations Used

FS–DNA, fish sperm–DNA; ICD signal, induced circular dichroism signal; k–DNA, kinetoplast–DNA; r, ligand/DNA ratio.

References

1. WHO. *Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases*; World Health Organization: Geneva, 2010.

2. Dardonville, C.; Barrett, M. P.; Brun, R.; Kaiser, M.; Tanious, F.; Wilson, W. D. DNA binding affinity of bisguanidine and bis(2-aminoimidazoline) derivatives with in vivo antitrypanosomal activity. *J. Med. Chem.* **2006,** 49, 3748-3752.

3. Rodríguez, F.; Rozas, I.; Kaiser, M.; Brun, R.; Nguyen, B.; Wilson, W. D.; García, R. N.; Dardonville, C. New bis(2-aminoimidazoline) and bisguanidine DNA minor groove binders with potent in vivo antitrypanosomal and antiplasmodial activity. *J. Med. Chem.* **2008,** 51, 909-923.

4. Werbovetz, K. Diamidines as antitrypanosomal, antileishmanial and antimalarial agents. *Curr. Opin. Invest. Drugs* **2006,** 7, 147-157.

5. Soeiro, M. N. C.; De Souza, E. M.; Stephens, C. E.; Boykin, D. W. Aromatic diamidines as antiparasitic agents. *Expert Opin. Invest. Drugs* **2005,** 14, 957-972.

6. Glass, L. S.; Nguyen, B.; Goodwin, K. D.; Dardonville, C.; Wilson, W. D.; Long, E. C.; Georgiadis, M. M. Crystal structure of a trypanocidal 4,4'-

bis(imidazolinylamino)diphenylamine bound to DNA. *Biochemistry* **2009,** 48, 5943-5952.

7. Acosta-Reyes, F. J.; Dardonville, C.; de Koning, H. P.; Natto, M.; Subirana, J. A.; Campos, J. L. In and out of the minor groove: interaction of an AT-rich DNA with the drug CD27. *Acta Cryst.* **2014,** D70, 1614-1621.

8. Wilson, W. D.; Tanious, F. A.; Mathis, A.; Tevis, D.; Hall, J. E.; Boykin, D. W. Antiparasitic compounds that target DNA. *Biochimie* **2008,** 90, 999-1014.

9. Wilson, W. D.; Nguyen, B.; Tanious, F. A.; Mathis, A.; Hall, J. E.; Stephens, C. E.; Boykin, D. W. Dications that target the DNA minor groove: compound design and preparation, DNA interactions, cellular distribution and biological activity. *Curr. Med. Chem. - Anticancer Agents* **2005,** 5, 389-408.

10. Ríos Martínez, C.; Miller, F.; Ganeshamoorthy, K.; Glacial, F.; Kaiser, M.; de Koning, H.; Eze, A.; Lagartera, L.; Herraiz, T.; Dardonville, C. A new non-polar *N*-hydroxy imidazoline lead compound with improved activity in a murine model of late stage *T. b. brucei* infection is not cross-resistant with diamidines. *Antimicrob. Agents Chemother.* **2015,** 59, 890-904.

11. Nagle, P. S.; Rodriguez, F.; Nguyen, B.; Wilson, W. D.; Rozas, I. High DNA affinity of a series of peptide linked diaromatic guanidinium-like derivatives. *J. Med. Chem.* **2012,** 55, 4397-4406.

12. Ríos Martínez, C. H.; Lagartera, L.; Kaiser, M.; Dardonville, C. Antiprotozoal activity and DNA binding of *N*-substituted *N*-phenylbenzamide and 1,3-diphenylurea bisguanidines. *Eur. J. Med. Chem.* **2014,** 81, 481-491.

13. Kelly, J. X.; Smilkstein, M. J.; Brun, R.; Wittlin, S.; Cooper, R. A.; Lane, K. D.; Janowsky, A.; Johnson, R. A.; Dodean, R. A.; Winter, R.; Hinrichs, D. J.; Riscoe, M. K. Discovery of dual function acridones as a new antimalarial chemotype. *Nature* **2009,** 459, 270-273.

14. Fernández-Calienes Valdés, A. Acridine and acridinones: old and new structures with antimalarial activity. *Open Med. Chem. J.* **2011,** 5, 11-20.

15. Winkelmann, E.; Raether, W. Chemotherapeutically active anthraquinones. I. Aminoanthraquinones. *Arznei-forschung* **1979,** 29, 1504-1509.

16. Arafa, R. K.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Stephens, C. E.; Boykin, D. W. Synthesis, DNA affinity, and antiprotozoal activity of fused ring dicationic compounds and their prodrugs. *J. Med. Chem.* **2005,** 48, 5480-5488.

17. Neidle, S.; Harrison, R. J.; Kelland, L. R.; Gowan, S. M.; Martin, R.; Reszka, T. Therapeutic acridones and acridine compounds. PCT/GB01/03046, 31-01-2002, 2002.

18. Dardonville, C.; Goya, P.; Rozas, I.; Alsasua, A.; Martin, M. I.; Borrego, M. J. New aromatic iminoimidazolidine derivatives as alpha1-adrenoceptor antagonists: a novel synthetic approach and pharmacological activity. *Bioorg. Med. Chem.* **2000,** 8, 1567-1577.

19. Peters, W. *Chemotherapy and Drug Resistance in Malaria*. Academic Press: London, 1987; Vol. 1.

20. Dardonville, C.; Fernandez-Fernandez, C.; Gibbons, S. L.; Jagerovic, N.; Nieto, L.; Ryan, G.; Kaiser, M.; Brun, R. Antiprotozoal activity of 1-phenethyl-4-aminopiperidine derivatives. *Antimicrob. Agents Chemother.* **2009,** 53, 3815-3821.

21. Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jones, R. L.; Fox, K.; Wydra, R. L.; Strekowski, L. DNA sequence dependent binding modes of 4',6-diamidino-2-phenylindole (DAPI). *Biochemistry* **1990,** 29, 8452-8461.

22. Wilson, W. D.; Tanious, F. A.; Watson, R. A.; Barton, H. J.; Strekowska, A.; Harden, D. B.; Strekowski, L. Interaction of unfused tricyclic aromatic cations with DNA: a new class of intercalators. *Biochemistry* **1989,** 28, 1984-1992.

23. Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jones, R. L.; Strekowski, L.; Boykin, D. W. Binding of 4',6-diamidino-2-phenylindole (DAPI) to GC and mixed

sequences in DNA: intercalation of a classical groove-binding molecule. *J. Am. Chem. Soc.* **1989,** 111, 5008-5010.

24. Breslin, D. T.; Yu, C.; Ly, D.; Schuster, G. B. Structural modification changes the DNA binding mode of cation-substituted anthraquinone photonucleases: association by intercalation or minor groove binding determines the DNA cleavage efficiency. *Biochemistry* **1997,** 36, 10463-10473.

25. McGhee, J. D.; von Hippel, P. H. Theoretical aspects of DNA-protein interactions: co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. *J. Mol. Biol.* **1974,** 86, 469-489.

26. Garbett, N. C.; Ragazzon, P. A.; Chaires, J. B. Circular dichroism to determine binding mode and affinity of ligand-DNA interactions. *Nat. Protoc.* **2007,** 2, 3166-3172.

27. Portugal, J.; Waring, M. J. Assignment of DNA binding sites for 4',6-diamidine-2-phenylindole and bisbenzimide (Hoechst 33258). A comparative footprinting study. *Biochim. Biophys. Acta* **1988,** 949, 158-168.

28. Kapuscinski, J.; Szer, W. Interactions of 4',6-diamidine-2-phenylindole with synthetic polynucleotides. *Nucleic Acids Res.* **1979,** 6, 3519-3534.

29. Nagle, P. S.; McKeever, C.; Rodriguez, F.; Nguyen, B.; Wilson, W. D.; Rozas, I. Unexpected DNA Affinity and Sequence Selectivity through Core Rigidity in Guanidinium-Based Minor Groove Binders. *J. Med. Chem.* **2014,** 57, 7663-7672.

30. Hu, L.; Patel, A.; Bondada, L.; Yang, S.; Wang, M. Z.; Munde, M.; Wilson, W. D.; Wenzler, T.; Brun, R.; Boykin, D. W. Synthesis and antiprotozoal activity of dicationic 2,6-diphenylpyrazines and aza-analogues. *Bioorg. Med. Chem.* **2013,** 21, 6732-6741.

31. Nagle, P. S.; Quinn, S. J.; Kelly, J. M.; O'Donovan, D. H.; Khan, A. R.; Rodriguez, F.; Nguyen, B.; Wilson, W. D.; Rozas, I. Understanding the DNA binding of

novel non-symmetrical guanidinium/2-aminoimidazolinium derivatives. *Org. Biomol. Chem.* **2010,** 8, 5558-5567.

32. Nagle, P. S.; Rodriguez, F.; Kahvedzic, A.; Quinn, S. J.; Rozas, I. Asymmetrical diaromatic guanidinium/2-aminoimidazolinium derivatives: synthesis and DNA affinity. *J. Med. Chem.* **2009,** 52, 7113-7121.

33. Lanteri, C. A.; Tidwell, R. R.; Meshnick, S. R. The mitochondrion is a site of trypanocidal action of the aromatic diamidine DB75 in bloodstream forms of *Trypanosoma brucei*. *Antimicrob. Agents Chemother.* **2008,** 52, 875-882.

34. Chen, K. K.; Donelson, J. E. Sequences of two kinetoplast DNA minicircles of *Tryptanosoma brucei*. *Proc. Natl Acad. Sci. U S A* **1980,** 77, 2445-2449.

35. Plsikova, J.; Janovec, L.; Koval, J.; Ungvarsky, J.; Mikes, J.; Jendzelovsky, R.; Fedorocko, P.; Imrich, J.; Kristian, P.; Kasparkova, J.; Brabec, V.; Kozurkova, M. 3,6-Bis(3-alkylguanidino)acridines as DNA-intercalating antitumor agents. *Eur. J. Med. Chem.* **2012,** 57, 283-295.

36. Tanious, F. A.; Ding, D.; Patrick, D. A.; Tidwell, R. R.; Wilson, W. D. A new type of DNA minor-groove complex: carbazole dication-DNA interactions *Biochemistry* **1997,** 36, 15315-15325.

37. Nguyen, B.; Tardy, C.; Bailly, C.; Colson, P.; Houssier, C.; Kumar, A.; Boykin, D. W.; Wilson, W. D. Influence of compound structure on affinity, sequence selectivity, and mode of binding to DNA for unfused aromatic dications related to furamidine. *Biopolymers* **2002,** 63, 281-297.

38. Räz, B.; Iten, M.; Grether-Bühler, Y.; Kaminsky, R.; Brun, R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) in vitro. *Acta Trop.* **1997,** 68, 139-147.

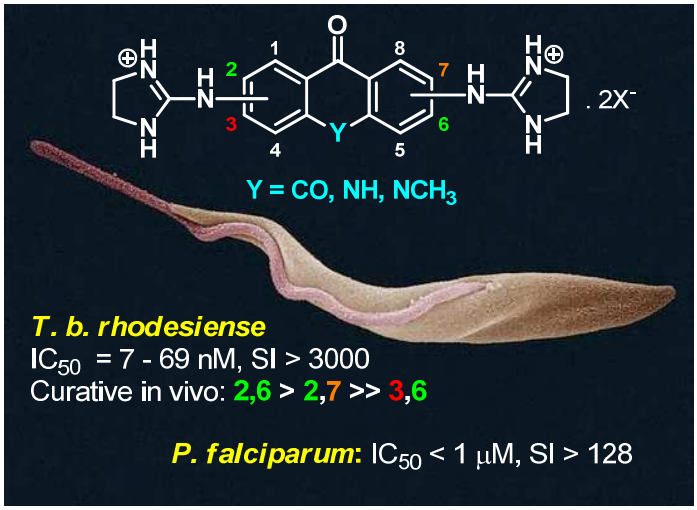
39. Mikus, J.; Steverding, D. A simple colorimetric method to screen drug cytotoxicity against *Leishmania* using the dye Alamar Blue®. *Parasitol. Int.* **2000,** 48, 265-269.

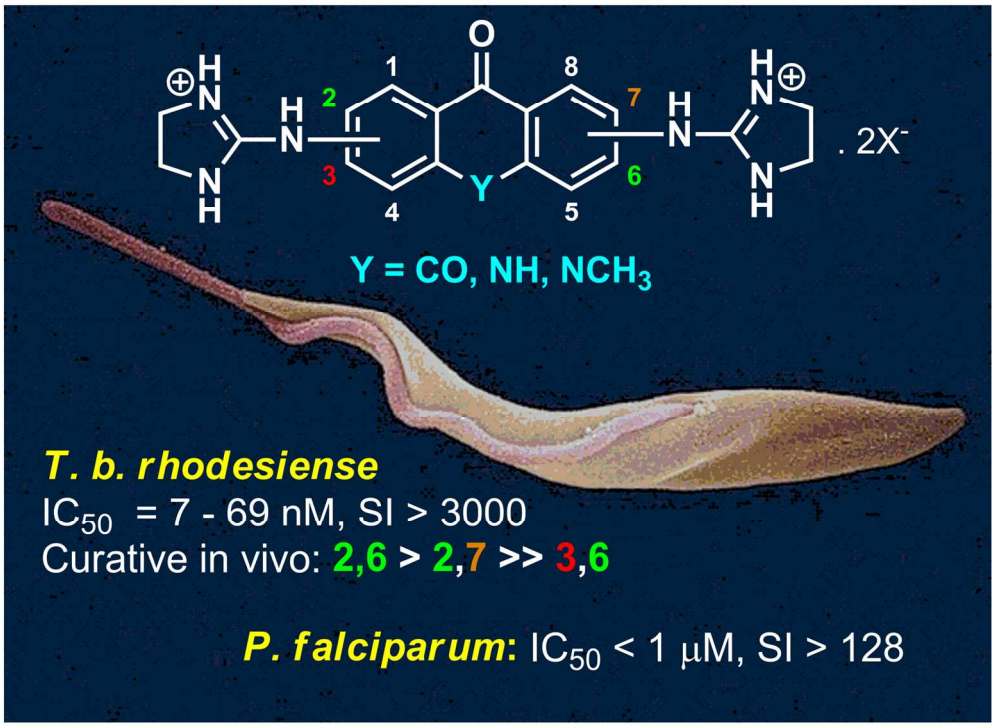
40. Buckner, F. S.; Verlinde, C. L.; La Flamme, A. C.; Van Voorhis, W. C. Efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing beta-galactosidase. *Antimicrob. Agents Chemother.* **1996,** 40, 2592-2597.

41. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979,** 16, 710-718.

42. Mullice, L. A.; Laye, R. H.; Harding, L. P.; Buurma, N. J.; Pope, S. J. A. Rhenium complexes of chromophore-appended dipicolylamine ligands: syntheses, spectroscopic properties, DNA binding and X-ray crystal structure. *New J. Chem.* **2008,** 32, 2140-2149.

Table of Contents graphic





71x52mm (600 x 600 DPI)