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pK_a modulation of a bis(2-aminoimidazoline) DNA minor groove binder that targets the kinetoplast of *Trypanosoma brucei*

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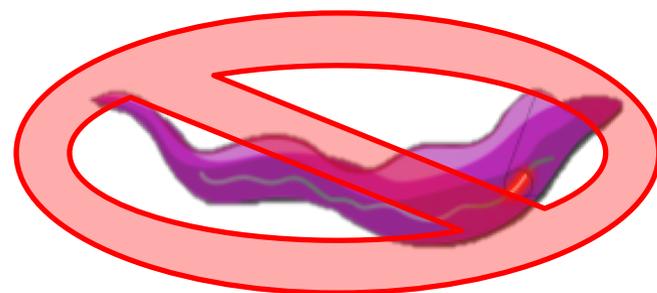
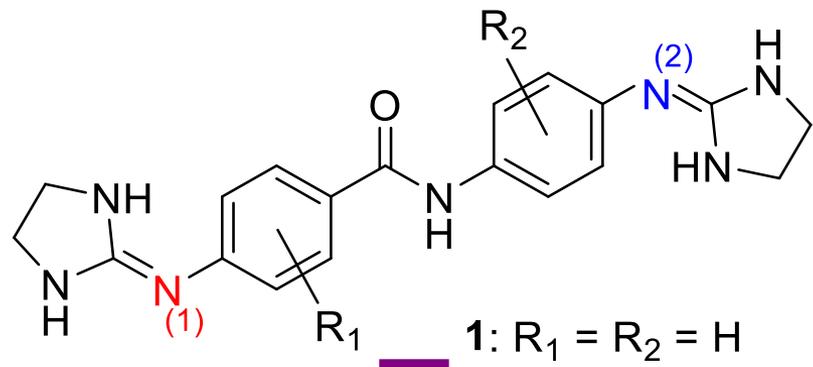
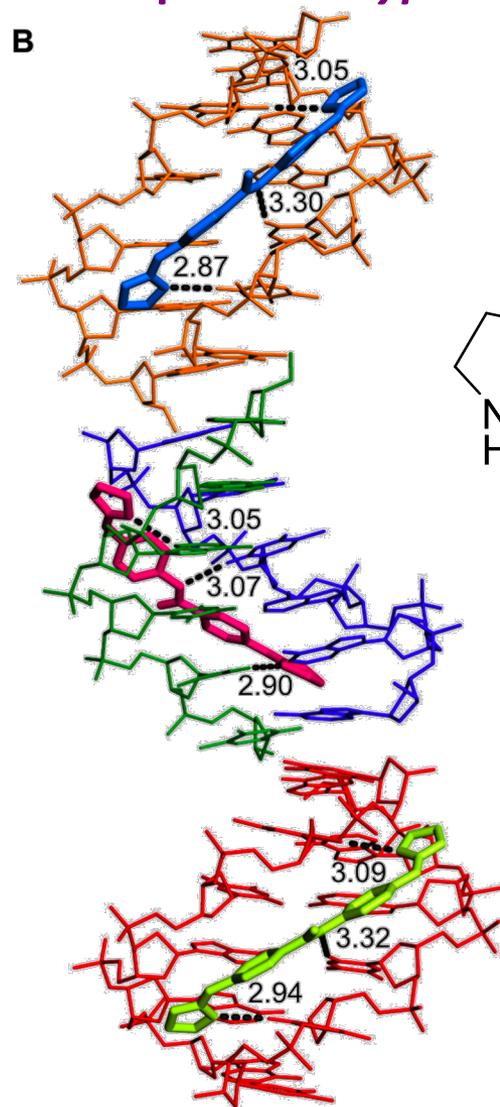
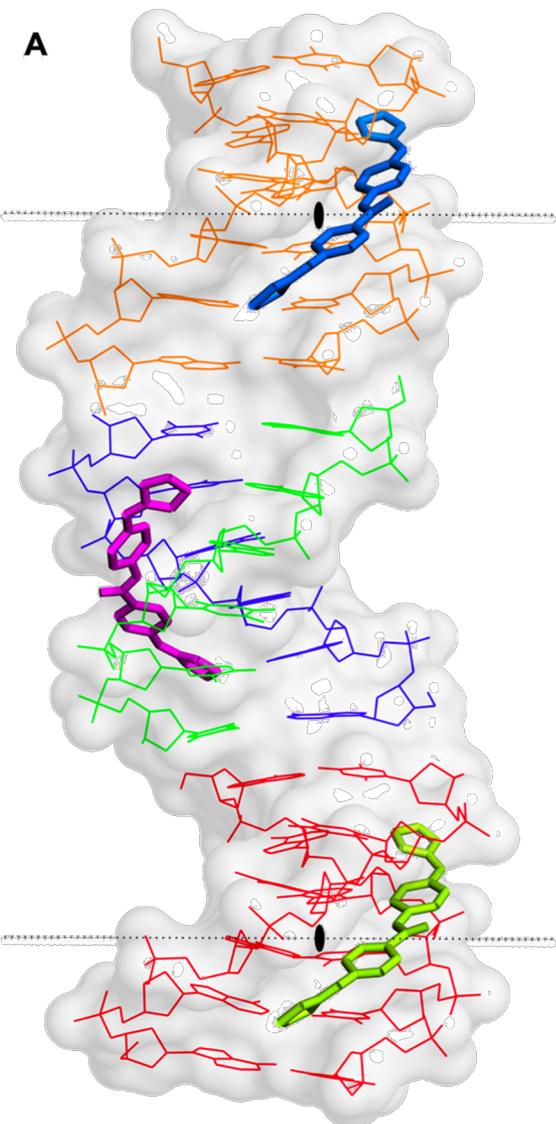
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pK_a modulation of a bis(2-aminoimidazoline) DNA minor groove binder that targets the kinetoplast of *Trypanosoma brucei*



100 % curative in a mouse model of first stage HAT.



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Abstract:

The parasite *Trypanosoma brucei*, etiologic agent of human African trypanosomiasis (i.e. sleeping sickness), contains a kinetoplast with the mitochondrial DNA (kDNA) comprising of >70 % AT base pairs. Hence, DNA minor groove binding molecules have been investigated as antitrypanosomal agents. Diphenyl-based bis(2-iminoimidazolidines) are promising DNA minor groove binders that are curative in mouse models of stage 1 trypanosomiasis but devoid of activity in the late(CNS)-stage disease, possibly due to poor brain penetration caused by their dicationic nature.

As a strategy to reduce the pK_a of the basic 2-iminoimidazolidine groups, halogen atoms ($R_1 = Cl, F$) were introduced in the structure of lead compound **1** [1] and the pK_a of the new compounds was determined. A reduction of 1–2 pK_a units for the imidazolidine group linked to the substituted phenyl ring was observed [1,2]. *In vitro* activities (EC_{50}) against wild type and resistant strains of *T. b. brucei* were in the submicromolar range with four compounds being more active and selective than **1** ($SI > 340$). The chloro-substituted derivative **5a**, which was curative *in vivo* in a mouse model of stage 1 infection by *T. b. rhodesiense*, appeared as a new promising lead compound.

Mechanistic studies were performed to identify the cellular target of these dicationic compounds. Altogether, our results show that **1** and **5a** share the same mechanism of action against *T. brucei*, acting specifically on the integrity of the kinetoplast by altering the structure and replication of kDNA [3].

Keywords: DNA minor groove binder, imidazolidine, dicationic compound, *Trypanosoma brucei*, kinetoplast



Neglected tropical diseases (NTDs)

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease that is almost invariably fatal if left untreated.

It is caused by subspecies of the protozoan parasite *Trypanosoma brucei* which is transmitted to humans by the tsetse fly vector.

Approximately 55 million people distributed over a surface of 340 000 km² in 33 sub-Saharan Africa countries are estimated to be at different levels of risk of contracting sleeping sickness¹.



DISEASE STAGES:

- **Haemolympathic stage**
(affects the blood)
- **Brain stage**
(affects the CNS)

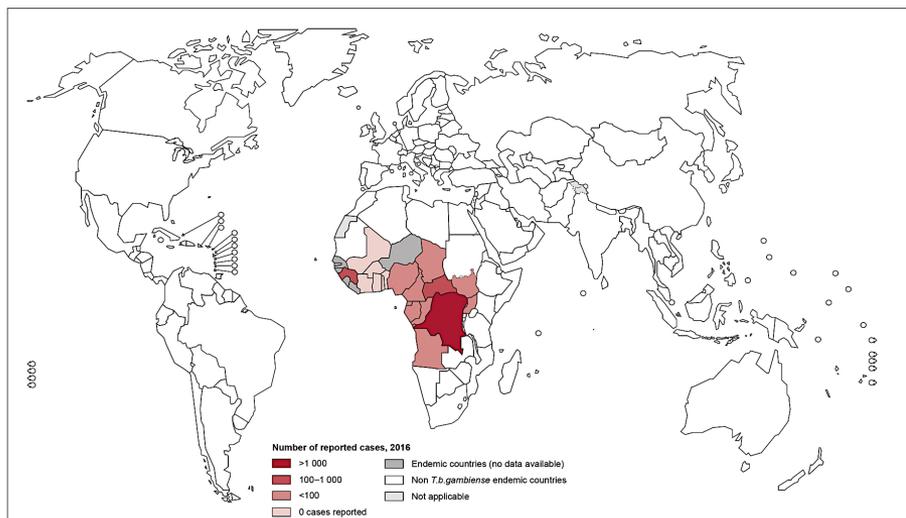


1. World Health Organization and Department of control of neglected tropical diseases. *Report of the second WHO stakeholders meeting on gambiense human African trypanosomiasis elimination*. **2016**; Geneva, 21–23.



Most of the affected populations live in remote rural areas with limited access to health services, which complicates the diagnosis and treatment of cases in Africa's poorest countries.

Distribution of human African trypanosomiasis (*T.b.gambiense*), worldwide, 2016



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2017. All rights reserved

Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization



Distribution of Human African Trypanosomiasis (*T.B. gambiense*), 2016.

Date : 27/Sep/2017

Source: National human African trypanosomiasis control programmes.

Annual country reports, 2016

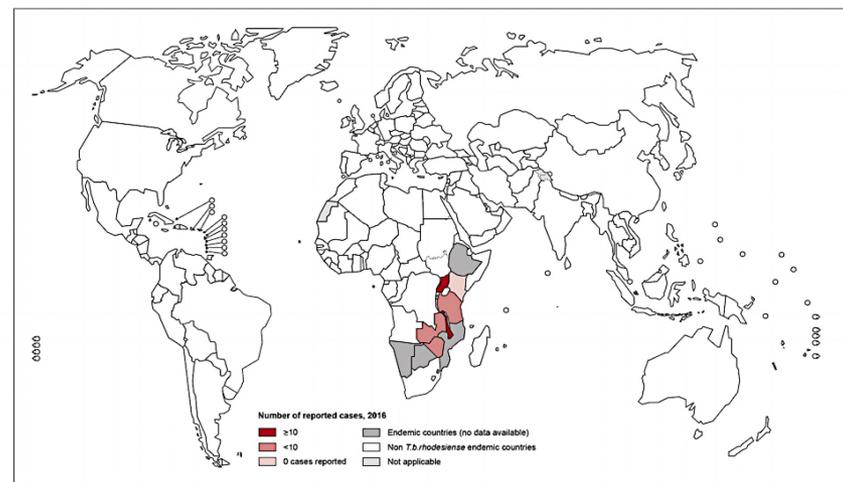
Distribution of Human African Trypanosomiasis (*T.B. rhodesiense*), 2016

Date : 27/Sep/2017

Source: National human African trypanosomiasis control programmes.

Annual country reports, 2016

Distribution of human African trypanosomiasis (*T.b.rhodesiense*), worldwide, 2016



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Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
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http://apps.who.int/neglected_diseases/ntddata/hat/hat.html Access: June 12th, 2018
<http://gamapserver.who.int/mapLibrary/app/searchResults.aspx> Access: June 12th, 2018



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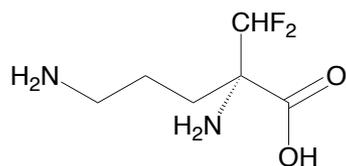
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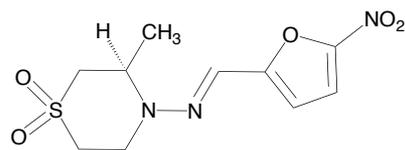
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Chemotherapy

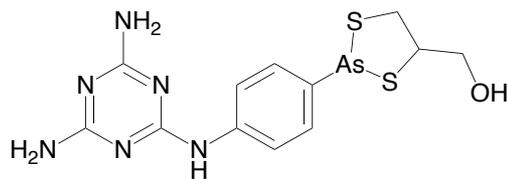
The current drugs used to treat HAT (suramin, pentamidine, melarsoprol and nifurtimox-eflornithine combination therapy, or NECT) are toxic and sometime ineffective due to the appearance of drug-resistant strains of *T. brucei*.¹ In the last decades, efforts have been made to discover improved drugs to treat HAT.



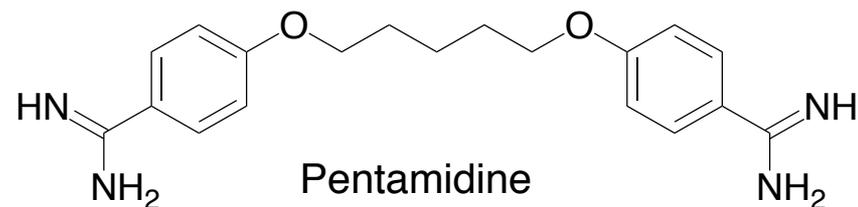
Eflornithine



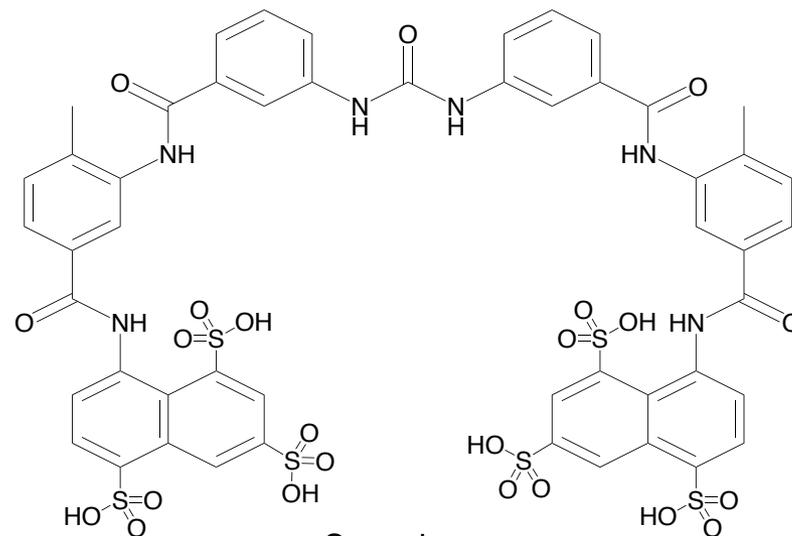
Nifurtimox



Melarsoprol



Pentamidine

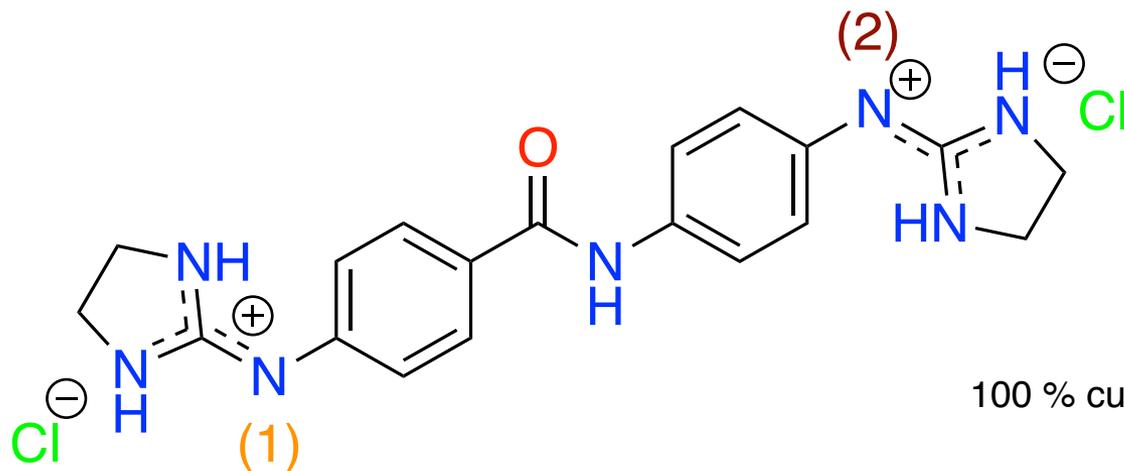


Suramin

1. Delespau V., de Koning H.P. Drugs and drug resistance in African trypanosomiasis. *Drug Resist. Updat.* **2007**; 10: 30–50.



Hit Identification



Hit compound I (FR60)

$IC_{50} = 0.025 \mu M^*$ $SI = >240$

$pK_{a(1)} = 9.04$ $pK_{a(2)} = 10.26$ ^b

% ionization at pH 7.4 = 98.7*

100 % curative in a mouse model of first stage HAT. *

In previous reports, we have shown that compound **1**, 4-((4,5-dihydro-1H-imidazol-2-yl)amino)-N-(4-((4,5-dihydro-1H-imidazol-2-yl)amino)phenyl)benzamide dihydrochloride, displayed excellent antitrypanosomal activity *in vitro* and were selective toward *Trypanosoma brucei*.

Compound **1** was curative by oral administration in a mouse model of acute *T. b. rhodesiense* infection demonstrating a great potential as chemotherapeutic agent.

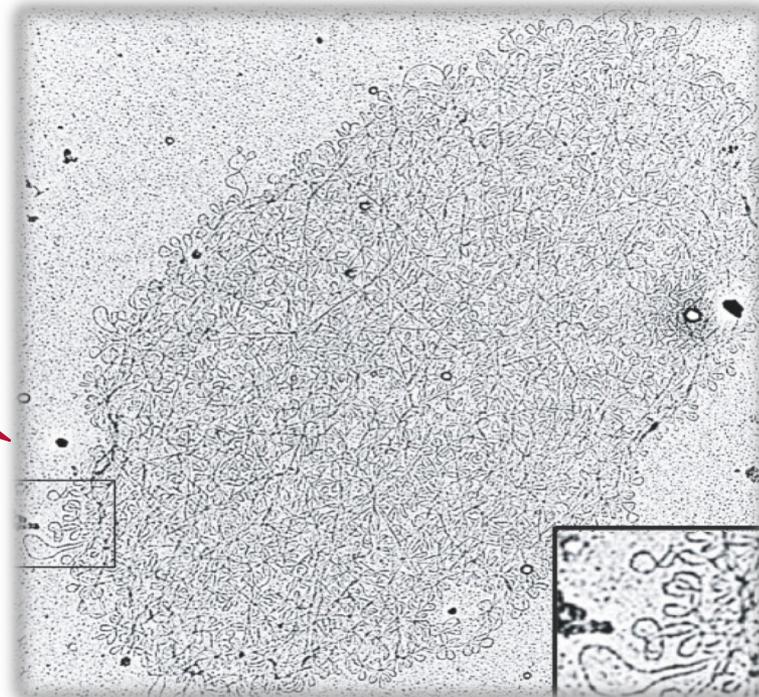
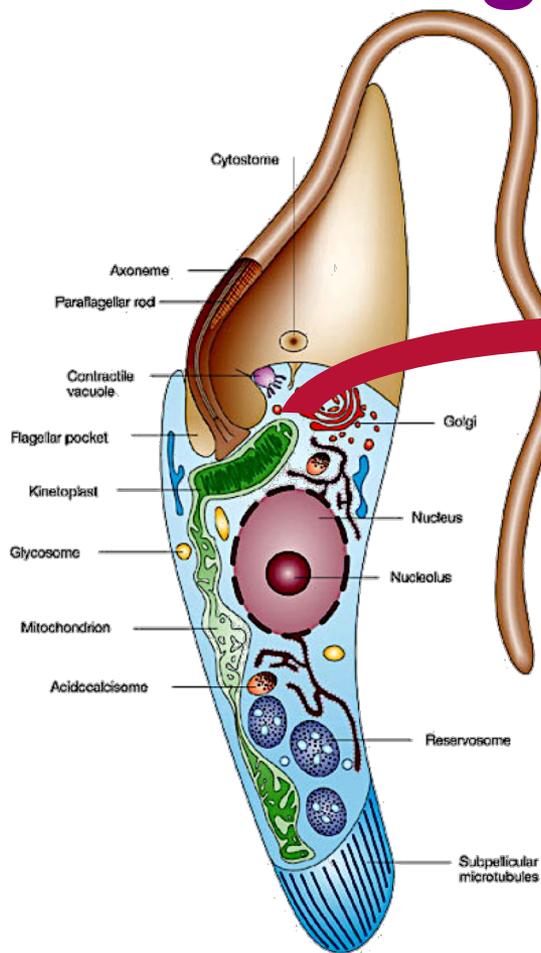
* Rodríguez, F.; Rozas, I.; Kaiser, M.; Brun, R.; Nguyen, B.; Wilson, W. D.; García, R. N.; Dardonville, C. New bis(2-aminoimidazoline) and bisguanidine DNA minor groove binders with potent *in vivo* antitrypanosomal and antiplasmodial activity. *J. Med. Chem.* 2008, 51, 909-923.

^b Beth A. Caine, Christophe Dardonville, and Paul L. A. Popelier (2018) Prediction of Aqueous pKa Values for Guanidine-Containing Compounds Using Ab Initio Gas-Phase Equilibrium Bond Lengths. *ACS Omega* 3 (4), 3835-3850



Parasite target

Electron micrograph of isolated kDNA networks²



Schematic representation of trypanosomatids¹

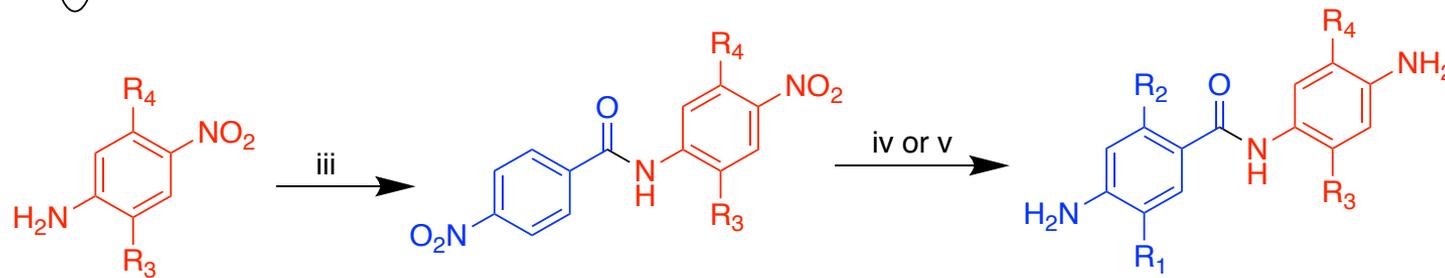
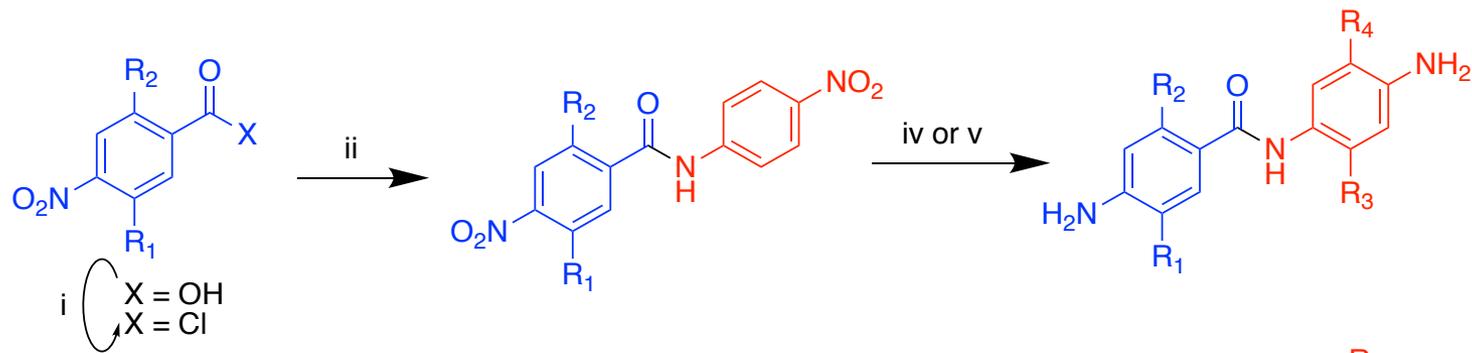
- The parasite *Trypanosoma brucei*, contains a kinetoplast with the **mitochondrial DNA (kDNA) comprising of >70 % AT base pairs**.
- Bisimidazole compounds are DNA minor groove binding molecules that specifically target AT base pairs, so the kinetoplast is a likely target of our compounds.
- We thus performed several experiments to understand the mode of action of these compounds.

¹ Vargas-Parada, L. Kinetoplastids and their Networks of Interlocked DNA. *Nature Education*, **2010**, 3(9):63

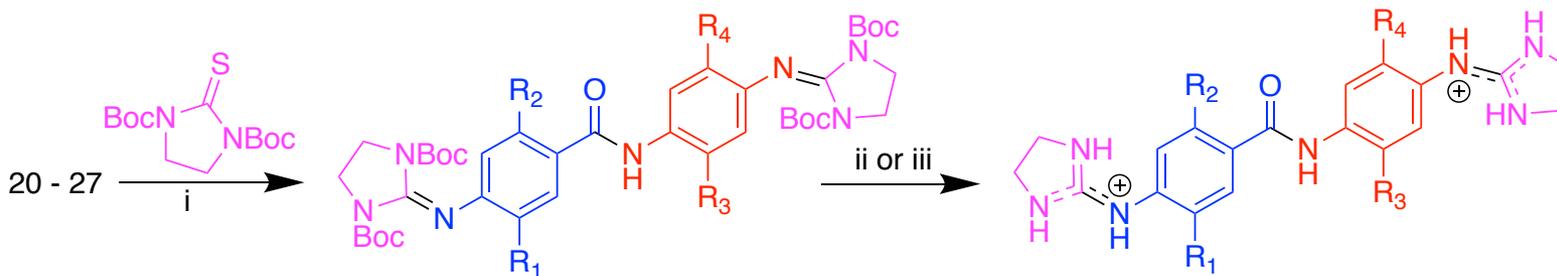
² Roy Chowdhury A, *et al.* The Killing of African Trypanosomes by Ethidium Bromide. *PLoS Pathogens*, **2010**, 6, e1001226



Synthetic strategy



- 2: $R^4 = \text{Cl}$, $R^1, R^2, R^3 = \text{H}$
- 3: $R^3 = \text{Cl}$, $R^1, R^2, R^4 = \text{H}$
- 4: $R^2 = \text{Cl}$, $R^1, R^3, R^4 = \text{H}$
- 5: $R^1 = \text{Cl}$, $R^2, R^3, R^4 = \text{H}$
- 6: $R^1 = \text{F}$, $R^2, R^3, R^4 = \text{H}$
- 7: $R^2 = \text{F}$, $R^1, R^3, R^4 = \text{H}$
- 8: $R^3 = \text{F}$, $R^1, R^2, R^4 = \text{H}$
- 9: $R^4 = \text{F}$, $R^1, R^2, R^3 = \text{H}$



Ríos Martínez, C. H.; Nué Martínez, J. J.; Ebiloma, G. U.; De Koning, H. P.; Alkorta, I.; Dardonville, C. Lowering the pK_a of a bisimidazolinium lead with halogen atoms results in improved activity and selectivity against *Trypanosoma brucei* in vitro. *Eur. J. Med. Chem.* **2015**, *101*, 806-817.



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Results and discussion

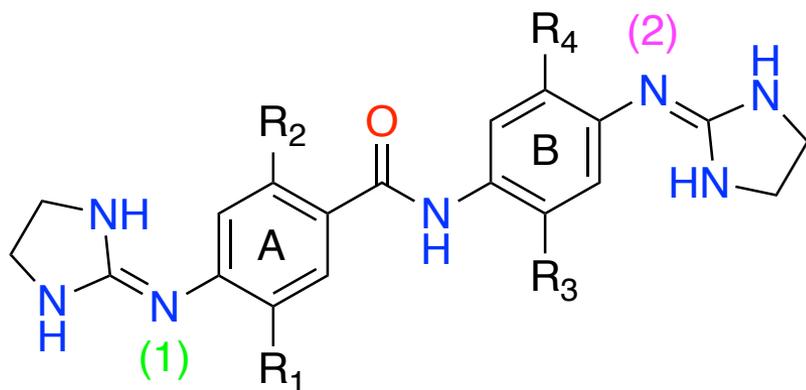
Physicochemical characterization: pK_a

index	R ¹	R ²	R ³	R ⁴	UV-metric		pH-metric	
					$pK_a^{(1)}$	$pK_a^{(2)}$	$pK_a^{(1)}$	$pK_a^{(2)}$
1					9.30	10.30	9.04	10.26
2				Cl	9.00	10.10	8.84	9.60
3				F	8.60	9.60		
4			Cl		8.50	10.10	8.97	9.62
5			F		8.50	9.70		
6		Cl			8.80	10.10	8.80	10.06
7		F			8.80	10.20	8.54	10.03
8	Cl				8.30	9.60	8.26	10.07
9	F				8.30	9.70	8.29	9.98

Influence of substituents on:

B ring: F reduces 0.6-0.7 pKa units (N1, N2)
Cl reduces 0,7-0,8 pKa units (N2 only)

A ring: F or Cl (R1) reduces 0,8 pKa units (N1)
F or Cl reduces 0,2 pKa units (N2 only)



Beth A. Caine, C. Dardonville, and P.L.A. Popelier *ACS Omega* 2018, 3 (4), 3835-3850



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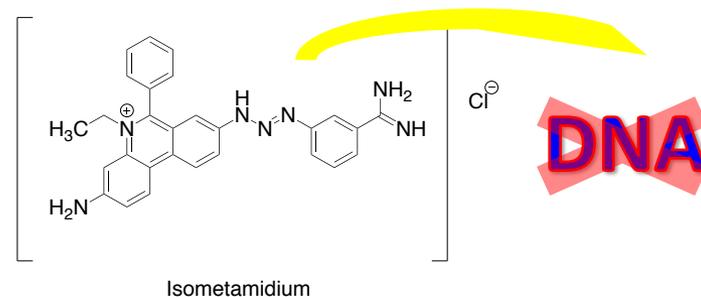
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Biological results: Resistance profile

Activity of compounds **1** and **2** against *T. brucei* 427WT and the isometamidium-resistant strain ISMR1 (dyskinetoplastic strain)

Compounds	<i>T. b. brucei</i> 427WT	<i>T. b. brucei</i> ISMR1	Resistance Factor vs. Tb247WT
	EC ₅₀ (μM) ± SEM	EC ₅₀ (μM) ± SEM	
1	0.83 ± 0.08 ^a	105.3 ± 3.2	127 ***
2	0.220 ± 0.002 ^a	29.0 ± 0.7	132 ***
Isometamidium	0.016 ± 0.001	1464 ± 94	92522 ***

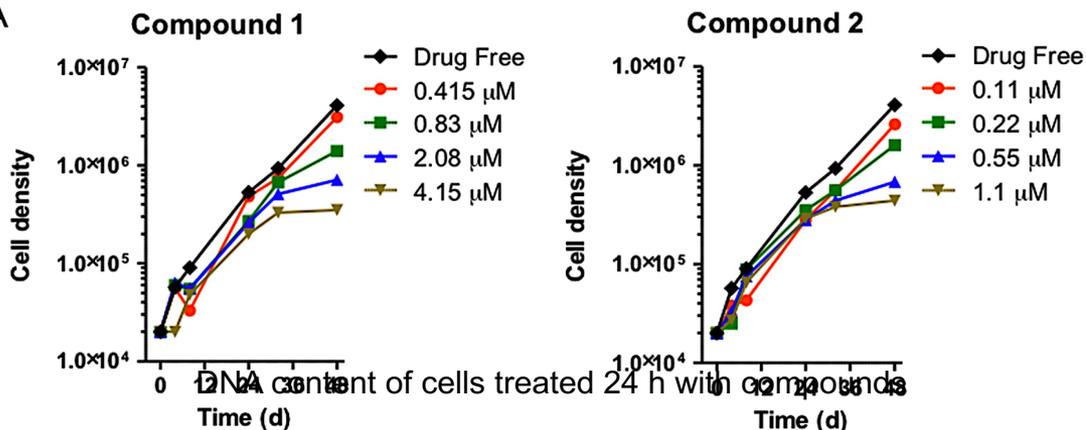
The compounds were significantly less effective against the dyskinetoplastic strain, with 127- and 132-fold increases in EC₅₀ values, respectively, indicating that the absence of kDNA has made the cells resistant to the test compounds.



Biological results: Alteration of the cell cycle of *T. brucei*

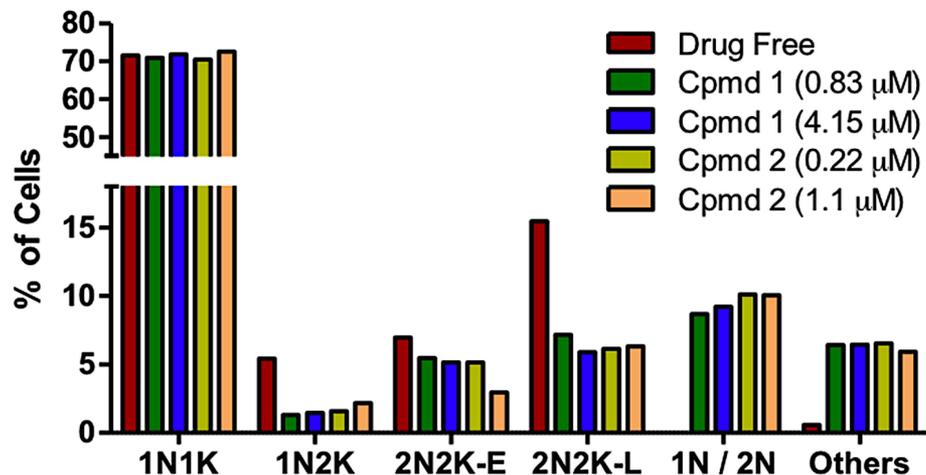
Growth curves

A



In the *T. brucei* cell cycle, replication and division of kDNA necessarily precedes nuclear division, and compounds that directly impact on kDNA are thus expected to interfere with cell division.

C



Indeed, both compounds dose-dependently reduced *T. brucei* growth rates and, at concentrations above EC_{50} , appeared to induce growth arrest after 24 h.

Functional and structural analysis of AT-specific minor groove binders that disrupt DNA-protein interactions and cause disintegration of the *Trypanosoma brucei* kinetoplast. *Nucleic Acids Res.* 2017, 45, 8378-8391.

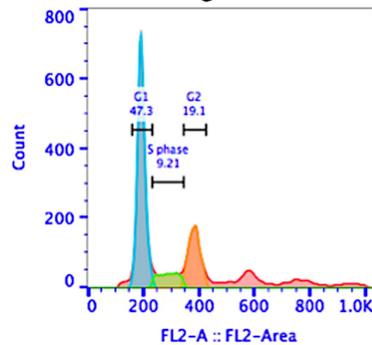


Biological results: Alteration of the cell cycle of *T. brucei*

Histograms of flow cytometric analysis

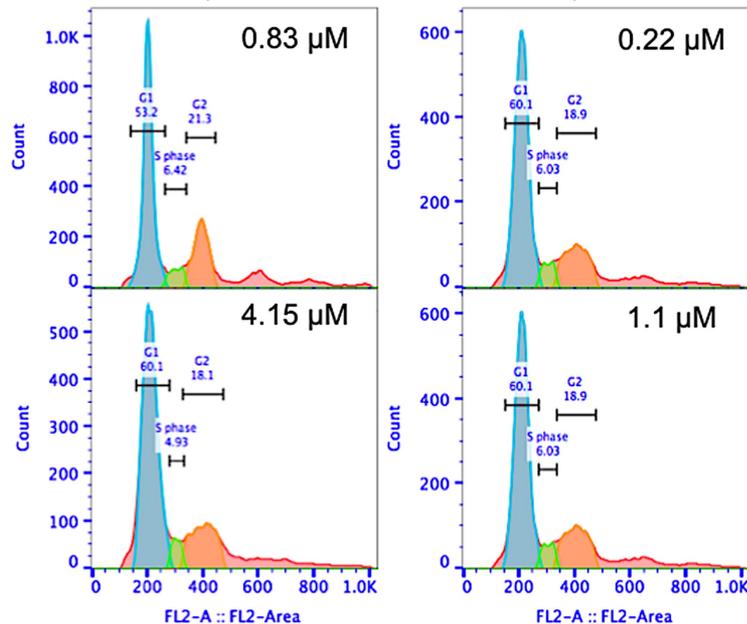
B

Drug Free



Compound 1

Compound 2



DNA content is clearly modified when treated with the compounds. Using flow cytometry we observed that our compounds act in the S-phase, when DNA synthesis is produced. So, compounds **1** and **2** impact on kDNA by interfering with cell division.

Functional and structural analysis of AT-specific minor groove binders that disrupt DNA–protein interactions and cause disintegration of the *Trypanosoma brucei* kinetoplast.

Nucleic Acids Res. **2017**, *45*, 8378-8391.



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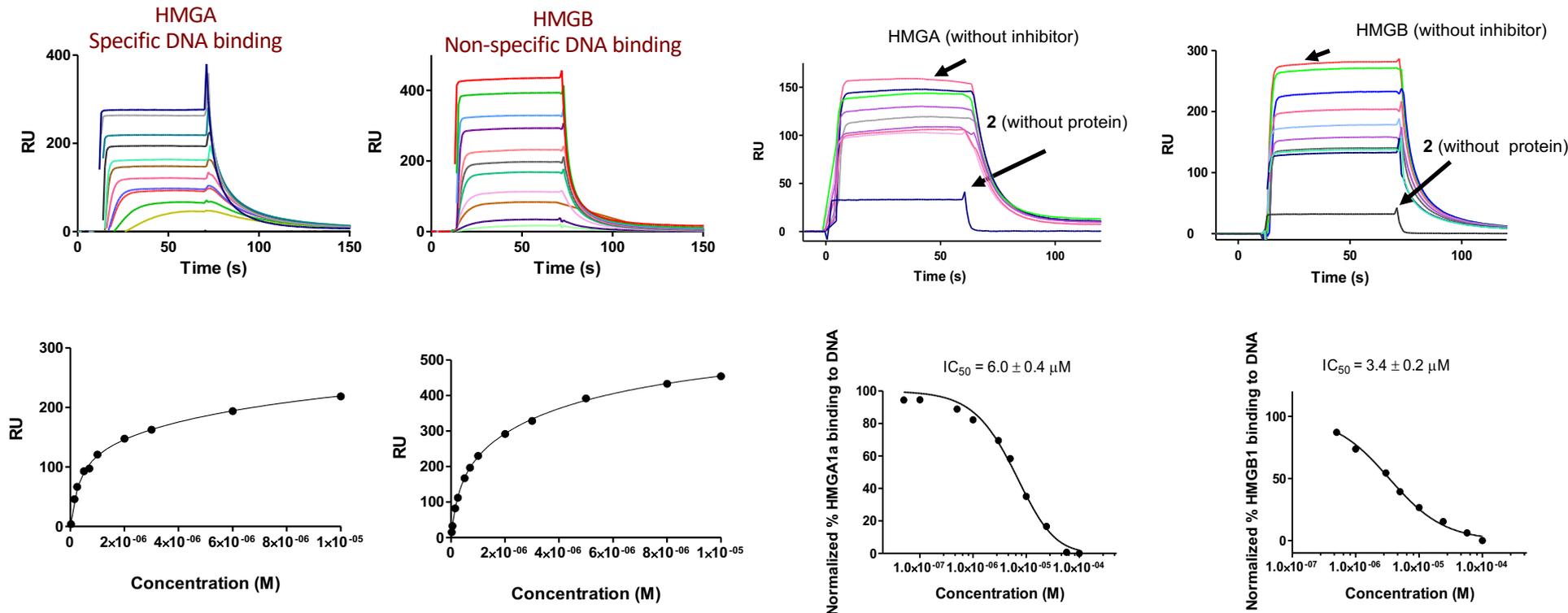


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Binding analysis: Compound 2 inhibits the binding of high mobility group proteins HMGA1a and HMGB1 to AT-rich DNA

Bingind assay

Competition assay

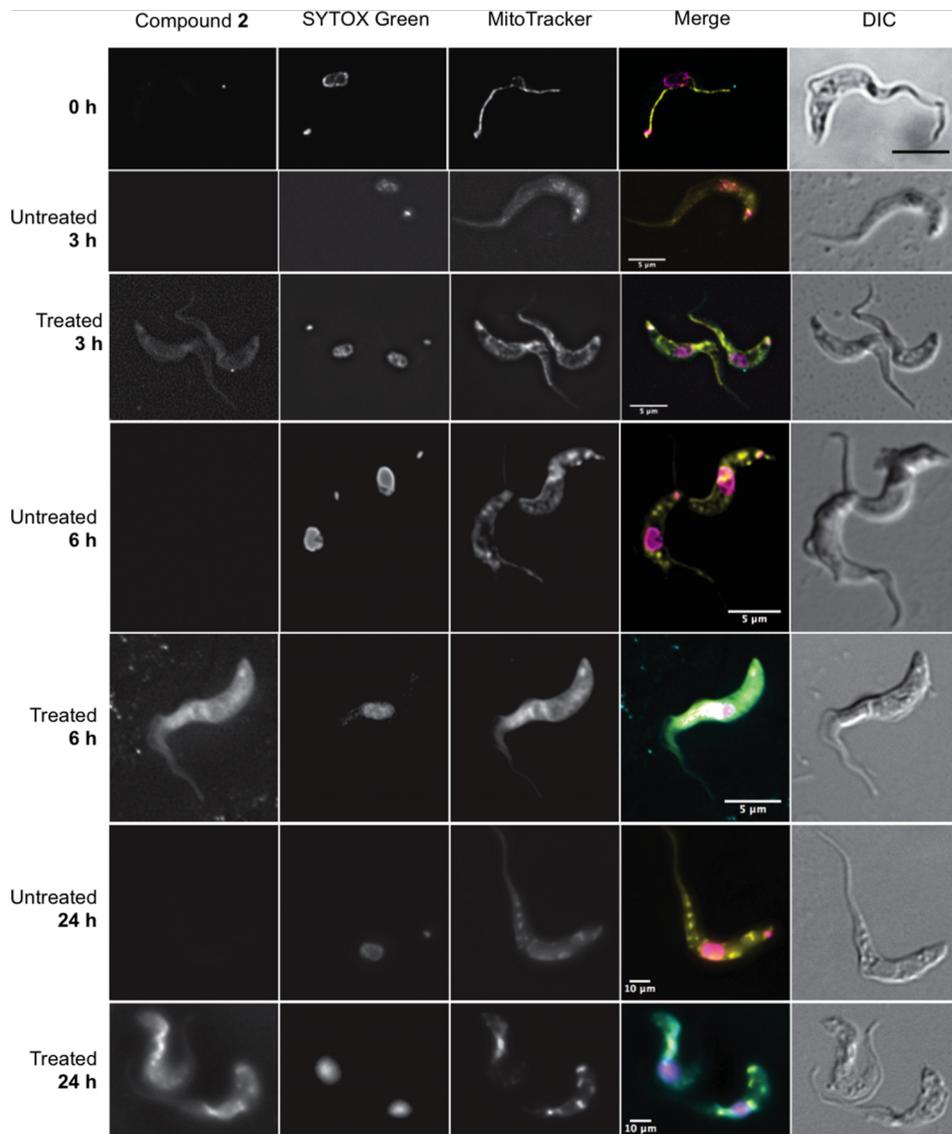


Using SPR technique for a binding assay, we observed how at crescent concentration of protein the detection of the DNA-protein complex exhibit a linear relation. Whereas in the competition assay we observe that introduction of the inhibitor reduce the formation of the DNA-protein complex. We showed that compound 2 can displace HMG-box containing proteins essential for kDNA function from their kDNA binding sites.

Millan et al. *Nucleic Acids Res.* 2017,45, 8378-8391.



Biological results: Fluorescent compound 2 accumulates in the mitochondrion of trypanosomes



Fluorescence technique shows compound 2 is accumulated inside the parasite in the mitochondrion of trypanosomes and after different times of interaction several damages in the cell are observed.

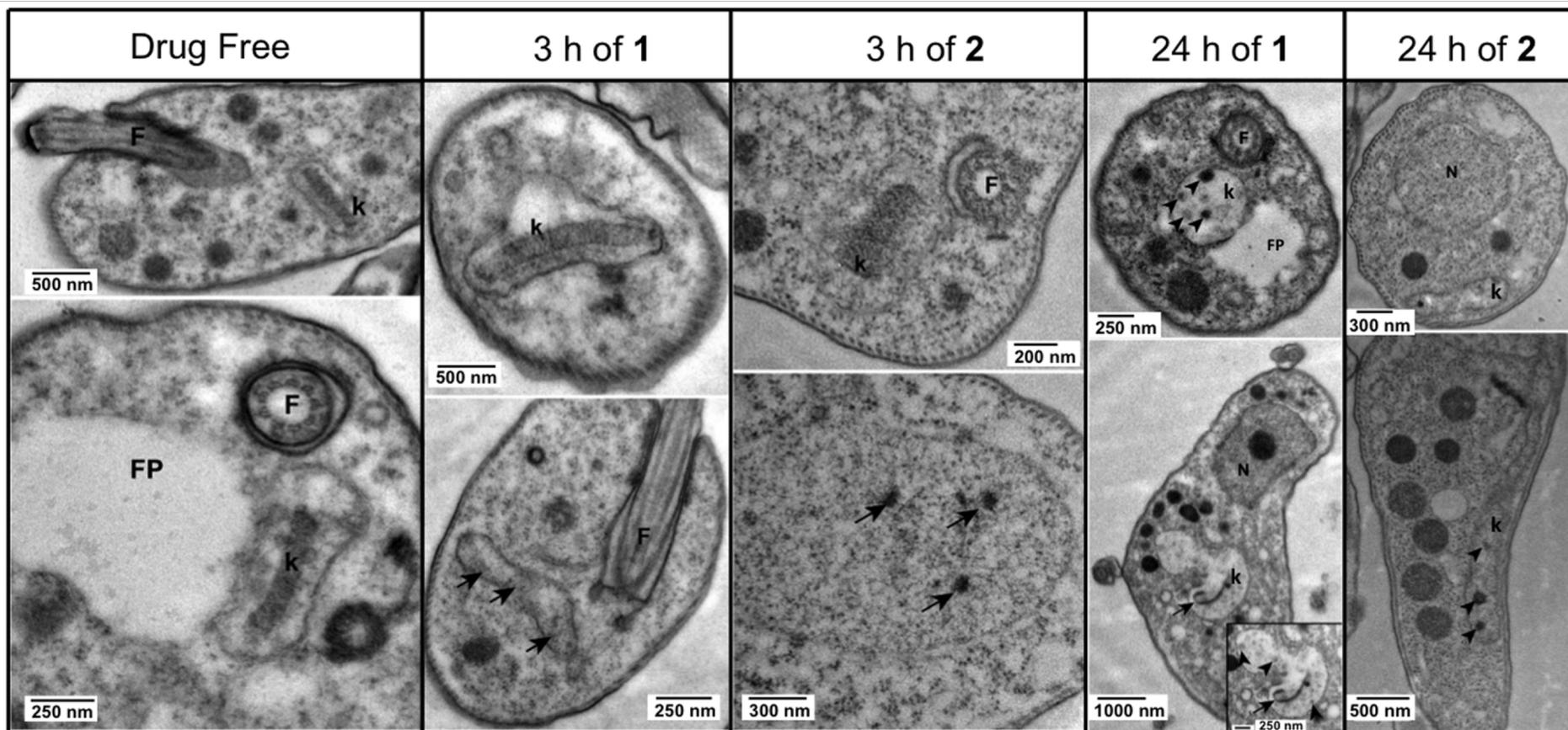
Millan et al. *Nucleic Acids Res.* 2017;45(14):8378-8391.



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Biological results: Compounds 1 and 2 cause destruction of the kinetoplast DNA network



TEM images show the compounds cause ultrastructural abnormalities in the kinetoplast, which were clearly far more severe at 24 h than at 3 h of incubation, when many cells appeared still undamaged.

Millan et al. *Nucleic Acids Res.* **2017**;45(14):8378-8391.



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Biological results: Compounds 1 and 2 show curative activity in a mouse model of first stage HAT

T. b. rhodesiense infection (mouse model of stage 1 HAT):

- Compound **1** is 100% curative by ip (4×5 mg/kg/day) and oral dosage (4×50 mg/kg/day)
- Chloro analogue **2 is 100% curative** at 4×20 mg/kg/day (ip)
- **At lower dosage** (4×4 mg/kg/day, ip), **2** increased the mean day of relapse of parasitemia at although **no cures were obtained**.



<https://www.freeimages.com/search/lab-rat>

Millan et al. *Nucleic Acids Res.* **2017**;45(14):8378-8391.



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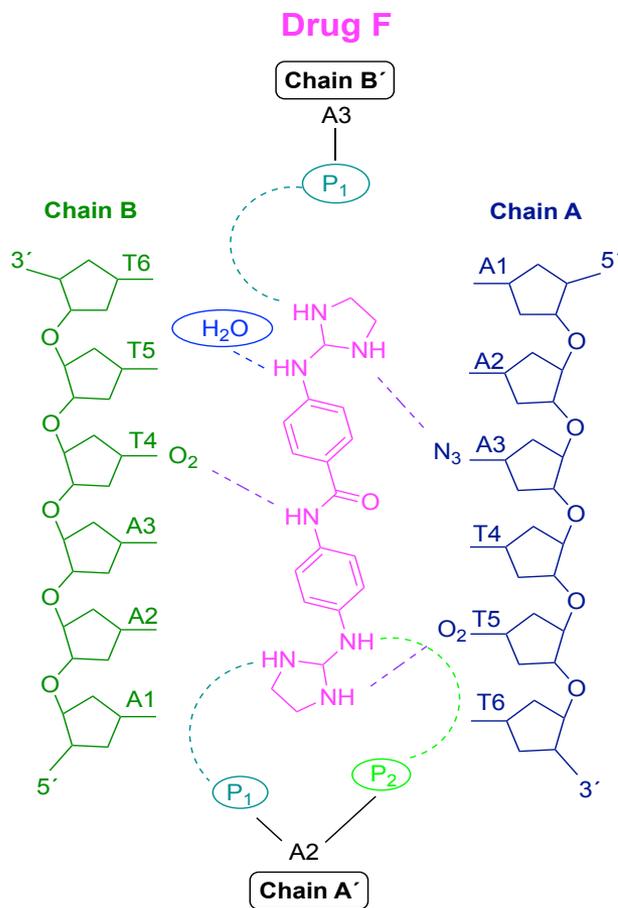
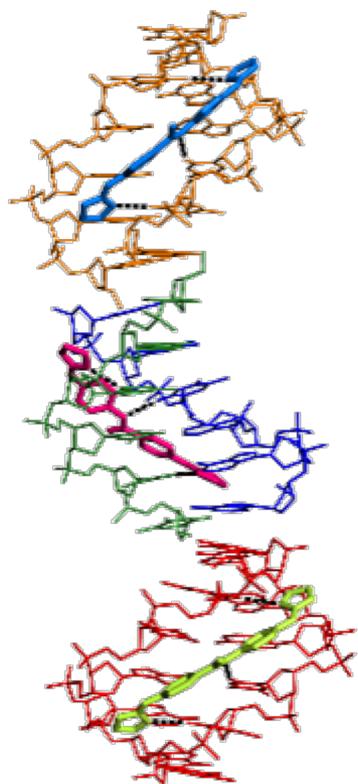


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Structural analysis: X-ray structure of the DNA–compound 1 complex at 1.25 Å

Drug conformation and interactions

DNA oligonucleotide [AAATTT]₂



The crystal structure of compound 1 bounded to an AT-rich DNA was solved at atomic resolution of 1.25 Å.

The drug molecules fill the central part of the minor groove of the duplexes.

The crystal is stabilized in part by the interaction of the central molecule, drug F (pink) with the DNA phosphates of neighboring molecules

Millan et al. *Nucleic Acids Res.* 2017;45(14):8378-8391.



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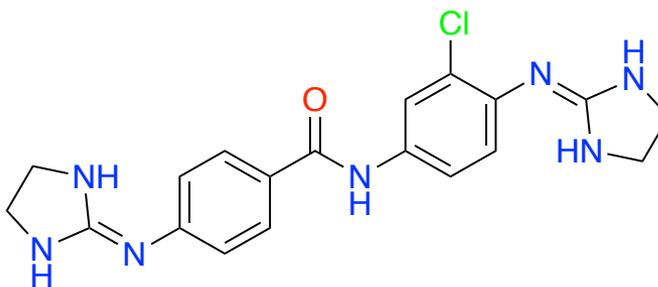
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Conclusions

- The introduction of halogens atoms in the structure of compound **1** led to a reduction of basicity of both imidazoline rings.
- Compound **2** with a chlorine atom in position R₄ was the most active against *T. brucei*.



- *N*-phenylbenzamide bis(2-aminoimidazolinium) compounds **1** and **2** share the same mechanism of action against *Trypanosoma brucei*, acting specifically on the integrity of the kinetoplast by altering the structure and replication of kDNA.



Acknowledgments

