Ciclopirox olamine, novel activity against *Trypanosoma cruzi*: *in vitro* mechanism of action and combination studies

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INTRODUCTION

Chagas disease, caused by *Trypanosoma cruzi,* is mainly endemic to South America. There are ~7-8 million people infected with *T. cruzi* worldwide[1] with heart disease the primary cause of morbidity[2]. We identified ciclopirox olamine (CPX), an anti-fungal agent, with activity against both life cycle stages of *T. cruzi in vitro* (Figure 1). Supplementation with iron demonstrates that the mode of action of CPX against *T. cruzi* is largely mediated by iron chelation. Combination studies have identified CPX to have an additive effect *in vitro* with the drugs used to treat Chagas disease.

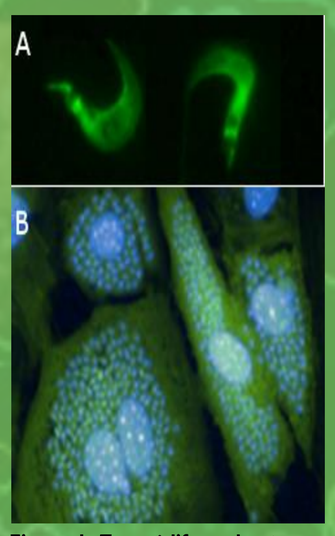

Figure 1. *T. cruzi* life cycle stages. (A) *T. cruzi* trypomastigotes identified with CellTracker green. (B) *T. cruzi* amastigotes within 3T3 fibroblasts, stained with Hoechst and HCS CellMask Green. Hoechst (blue) stains both the nucleus of the host cell and the parasite.

MATERIALS AND METHODS

IRON CHELATION ASSAY (384 well)

Ferric sulphate (FC), in two-fold dilutions from a final concentration of 100 µM to 0.781 µM was added to *T. cruzi* infected 3T3 cells. After 2 hrs, doses of CPX or the iron chelator desferrioxamine (DFO) were added and plates incubated for 48 hrs, then fixed and stained with Hoechst and HCS CellMask Green (Life Technologies). Imaging and analysis were performed using the PerkinElmer Opera QEHS (Figure 2). The same process was applied to host cell free trypomastigotes, however PrestoBlue (Life Technologies) was added to live cells, incubated for 6 hrs and fluorescence read on an Envision plate reader (PerkinElmer).

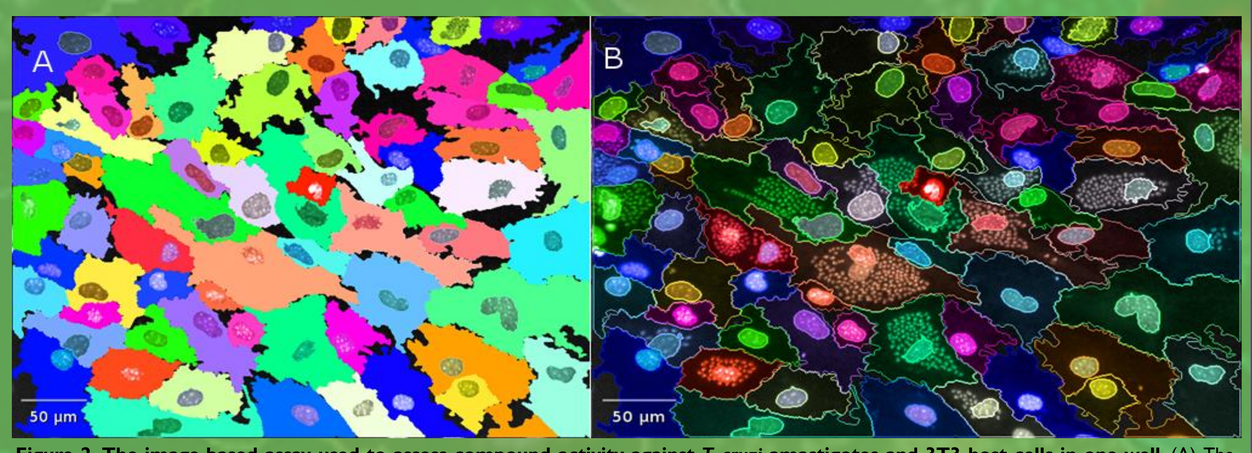

Figure 2. The image-based assay used to assess compound activity against *T. cruzi* amastigotes and 3T3 host cells in one well. (A) The script, applied on the Opera confocal imaging system, identified the nucleus and the cytoplasm (coloured) of each individual host cell, (B) and identifies spots within the host cell cytoplasm.

COMBINATION STUDIES (384 well)

Separate dose response plates were prepared of CPX, and the drugs used to treat Chagas, benznidazole (BZ) and nifurtimox (NFX). Concentrations ranged from16 x the IC_{50} value (3 µM CPX; 4 µM BZ or 1 µM NFX) to 0.004 x the IC_{50} value, in two-fold dilutions. CPX and BZ; and CPX and NFX plates were combined, to give doses from 0.004:16 to 16:0.004 CPX: drug. IC_{50} values were calculated in the *T. cruzi* assays from the activity of two-fold dilution series. CPX:BZ doses are shown in Figure 3.

BENZNIDAZOLE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
B	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
C	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
D	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
E	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
F	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
G	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
H	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
I	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
J	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
K	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
L	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
M	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
N	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	
O	0	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	

KEY
1:0 CPX / BZ
16:1 CPX: BZ
1:16 CPX: BZ

CICLOPIROX OLAMINE

	1	3	4	5	6	7	8	9	10	11	12	13	14	16
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
E	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
F	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
G	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
H	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
I	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
J	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
K	3	3	3	3	3	3	3	3	3	3	3	3	3	3
L	6	6	6	6	6	6	6	6	6	6	6	6	6	6
M	12	12	12	12	12	12	12	12	12	12	12	12	12	12
N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
O	48	48	48	48	48	48	48	48	48	48	48	48	48	48

Figure 3. Plate layout and concentrations for combination studies of CPX and BZ. Concentrations are shown in µM (final in the assay) for each compound. Plates are combined and dose combinations range from 0.004:16 CPX: BZ (0.01 µM CPX: 64 µM BZ) to 16:0.004 CPX:BZ (48 µM CPX: 0.02 µM BZ), in two- fold dilution series. Controls in plate were each compound with no combination (16 CPX: 0 BZ; 16 BZ: 0 CPX).

RESULTS

IRON CHELATION ASSAY

In the presence of 6.25 µM FC, the IC_{50} value of CPX was reduced 9.5 fold in the amastigote assay. Supplementing with 3.12 µM FC reduced the CPX IC_{50} value 11.9 fold in the trypomastigote assay (Figure 4). The maximum change in the IC_{50} value for DFO was 3.3 fold displayed in the amastigote assay, with the addition of 3.12 µM FC.

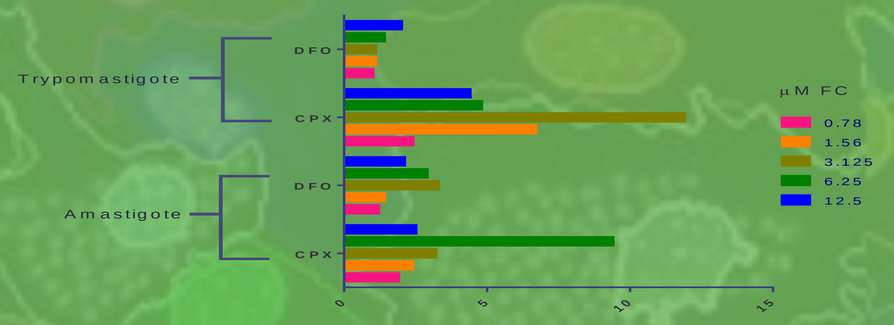

Figure 3. The effect of supplementation of ferric citrate (FC) on the IC_{50} value of desferrioxamine (DFO) and ciclopirox olamine (CPX) *in vitro* against *T. cruzi life* cycle stages. Image- and fluorescence-based assays were used to determine the activity of combinations of CPX and FC and CPX alone against amastigotes and trypomastigotes, respectively.

COMBINATION STUDIES

The sum of the fractional inhibitory concentration (FIC) was calculated for combinations of CPX:NFX and CPX:BZ, where an IC_{50} value could be calculated (Figure 4). As defined by Bell (2005)[3], the combination of CPX and either NFX or BZ resulted in an additive effect. There was no cytotoxicity displayed at the combinations tested.

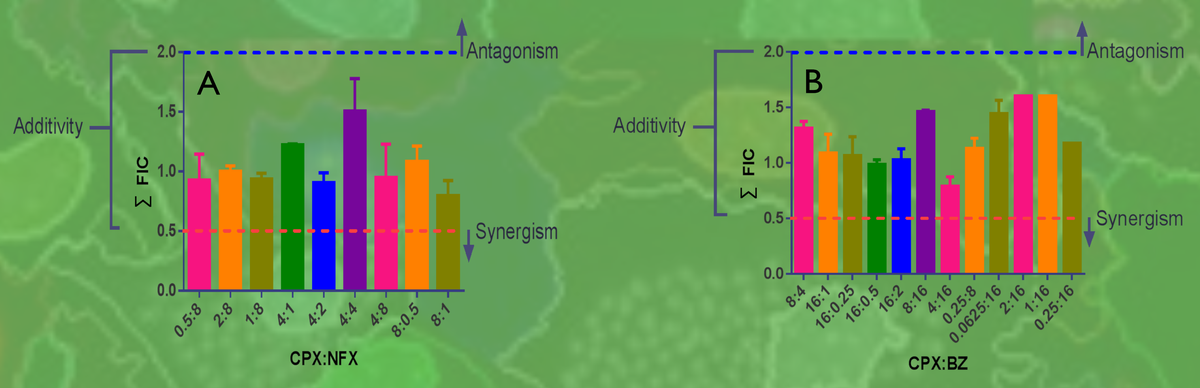

Figure 4. The sum of the fractional inhibitory concentration of combinations of ciclopirox olamine (CPX) with either (A) nifurtimox (NFX) or (B) benznidazole (BZ). Definition of additive, synergistic and antagonistic combinations are shown. Synergistic =∑FIC<0.5; Antagonistic =∑FIC>2; Additive =∑FIC>0.5<2.

CONCLUSIONS

Iron decreased the IC_{50} value of CPX approximately 10 fold in the trypomastigote and amastigote assays, suggesting a mode of action involving iron chelation. As DFO was less active than CPX and external Fe supplementation did not decrease activity as significantly, CPX may be more effective at intracellular iron depletion. Combinations of CPX with BZ or NFX displayed an additive effect. As it is believed that a positive pharmacologic outcome may be associated with synergistic combinations[4], further investigations will determine if known compounds with previously determined activity against *T. cruzi* are synergistic in combination with CPX.

REFERENCES AND AKNOWLEDGEMENTS

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