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Methods Article

METHODS ARTICLE

# Optimization and biological validation of an *in vitro* assay using the transfected Dm28c/pLacZ *Trypanosoma cruzi* strain

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## Abstract

There is an urgent need to develop safer and more effective drugs for Chagas disease, as the current treatment relies on benznidazole (BZ) and nifurtimox (NFX). Using the *Trypanosoma cruzi* Dm28c strain genetically engineered to express the *Escherichia coli* $\beta$-galactosidase gene, lacZ, we have adapted and validated an easy, quick and reliable *in vitro* assay suitable for high-throughput screening for candidate compounds with anti-*T. cruzi* activity. *In vitro* studies were conducted to determine trypomastigotes sensitivity to BZ and NFX from Dm28c/pLacZ strain by comparing the conventional labour-intensive microscopy counting method with the colourimetric assay. Drug concentrations producing the lysis of 50% of trypomastigotes (lytic concentration 50%) were 41.36 and 17.99 μM for BZ and NFX, respectively, when measured by microscopy and 44.74 and 38.94 μM, for the colourimetric method, respectively. The optimal conditions for the amastigote development inhibitory assay were established considering the parasite–host relationship (i.e. multiplicity of infection) and interaction time, the time for colourimetric readout and the incubation time with the $\beta$-galactosidase substrate. The drug concentrations resulting in 50% amastigote development inhibition obtained with the colourimetric assay were 2.31 μM for BZ and 0.97 μM for NFX, similar to the reported values for the Dm28c wild

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strain (2.80 and 1.5 μM, respectively). In summary, a colourimetric assay using the Dm28c/pLacZ strain of *T. cruzi* has been set up, obtaining biologically meaningful sensibility values with the reference compounds on both trypomastigotes and amastigotes forms. This development could be applied to high-throughput screening programmes aiming to identify compounds with anti-*T. cruzi in vitro* activity.

*Keywords:* *Trypanosoma cruzi*; *in vitro* drug screening; high-throughput screening

## Introduction

American trypanosomiasis (Chagas disease) is an endemic disease in Latin America caused by the haemoflagellate protozoa *Trypanosoma cruzi* that affects ∼6–7 million people worldwide [1].

The complex life cycle of *T. cruzi* involves over 150 mammalian hosts and triatomine blood-sucking bugs. Typically, vectorial transmission occurs when infected bug faeces contaminate the bite site or through intact mucous membranes. Oral infection is usually linked with the ingestion of food and/or drinks with infected triatomine faeces [2].

Non-vectorial routes of infection include blood transfusions or organ transplants with non-tested donors, laboratory accidents and vertical transmission by transplacental passage, which is currently responsible for most new cases in urban areas [3]. Chagas disease is becoming and emergent health burden in non-endemic regions due to the migrant population and non-vectorial transmission routes [4].

Current treatment options for Chagas disease are limited to nifurtimox (NFX) and benznidazole (BZ), developed in 1966 and 1970, respectively [5]. While the efficacy of both drugs in the acute stage of infection is clearly demonstrated, etiological treatment during the chronic phase remains in dispute, according to recent clinical trials [6, 7]. Additionally, the long duration of therapy (i.e. 60 days) and severe frequent side effects often hamper treatment adherence [8]. In this context, there is an urgent need to identify new and better options to improve Chagas disease chemotherapy.

*In vitro* drug screening efforts have primarily involved labour-intensive microscopy counting of parasites to determine compound activity [9]. More recently, several biotechnology tools were applied to accelerate *in vitro* evaluation of new compounds, such as high-throughput [10, 11] or high-content screening [12, 13].

The establishment of a *T. cruzi* transfected strain with the *Escherichia coli* β-galactosidase gene (lacZ) was first developed by Buckner *et al.* [14]. In this model, the C4 clone of the Tulahuen strain and the B5 clone of the CL strain [both from discrete typing unit (DTU) VI] express an enzyme that hydrolyses the chromogenic substrate chlorophenol-red-β-D-galactopyranoside (CPRG) to chlorophenol red, catalyzing a colourimetric reaction [15].

In this work, we describe the development, optimization and biological validation of an *in vitro* assay using a β-galactosidase-transfected Dm28c *T. cruzi* strain (DTU I) for colourimetric high-throughput screening system to assess the efficacy on both trypomastigotes and intracellular amastigotes.

## Materials and methods

### Test compounds and reagents

To set the new assay up, the drugs currently marketed for the chemotherapy of Chagas disease were selected as reference compounds: BZ and NFX. Pure drugs were provided by Roche, Brazil, Bayer and Germany, respectively, and they were dissolved in dimethyl sulfoxide (DMSO; Stanton®, Buenos Aires, Argentina) in stock solutions at 20 mg/ml. DMSO concentration never exceeded 0.2% in plate wells, with no effect on the host cell viability nor parasite replicative growth [16]. Roswell Park Memorial Institute (RPMI)-1640 with or without phenol red (Sigma; R6504 or R8755, respectively). Sodium bicarbonate (Emeve®; P1551-500). Gentamicin (Sigma; G1397). Penicillin–streptomycin (Sigma; P4333). Resazurin solution (Sigma, R7017). CPRG (Roche Diagnostics GmbH; 10 884 308 001). Nonidet™ P-40 substitute (Roche Diagnostics GmbH; 11 754 599 001). Heat-inactivated, UV-irradiated foetal bovine serum (FBS) (Natocor® or Internegocios S.A, Argentina). Geneticin (G418; Life Technologies).

### Parasite and cell culture

*Trypanosoma cruzi* Dm28c epimastigotes were cultured at 28°C in LIT medium (5 g/l liver infusion, 5 g/l bacto-tryptose, 68 mM NaCl, 5.3 mM KCl, 22 mM $Na_2HPO_4$, 0.2% (w/v) glucose and 0.002% (w/v) hemin supplemented with 10% (v/v) FBS.

Vero C-76 cell line [purchased in the American Type Culture Collection (ATCC) certified Asociación Banco Argentino de Células] (passages 32–42) and monolayers for obtaining *T. cruzi* cell-culture derived trypomastigotes were maintained at 37°C and 5% $CO_2$. Trypomastigotes were harvested according to a published protocol [17]. Cultures to be assayed for β-galactosidase activity were grown in RPMI-1640 medium without phenol red supplemented with 2.5 g/l sodium bicarbonate, 5% FBS and a periodical antibiotic switching (100 UI/ml of penicillin and 50 μg/ml of streptomycin or 25 μg/ml of gentamicin).

### Transfection assay

The Dm28c/pLacZ strain of *T. cruzi* was generated from a plasmid generously provided by Dr Frederick S. Buckner [14]. Briefly, epimastigotes were cultured in LIT medium at 28°C to a final concentration of $4 \times 10^7$ parasites per transfection. Then, parasites were harvested by centrifugation at 1500*g* for 10 min at room temperature, washed once with phosphate-buffered saline (PBS) and resuspended in 0.4 ml BSF transfection buffer (5 mM KCl, 0.15 mM $CaCl_2$ 90 mM, $Na_2HPO_4$ 50 mM HEPES pH 7.3). Nucleofection (Nucleofector 2B, Lonza) was performed in a 0.2 cm gap cuvette (Bio-Rad) with ∼20 μg of pLacZ plasmid DNA added to a final volume of 400 μl. The parasite–DNA mixture was kept on ice for 20 min prior to nucleofection with programme X-014. After nucleofection, cells were transferred into 3 ml of LIT medium containing 20% FBS, maintained at room temperature for 15 min and then incubated at 28°C. Geneticin was added at a concentration of 200 μg/ml, and parasites were incubated at 28°C. Transgenic parasites were obtained after 4 weeks of selection.

### β-galactosidase activity assay

To assess whether the β-galactosidase activity was associated with parasite burden, $2 \times 10^5$ cell-cultured derived β-galactosidase-expressing trypomastigotes from Dm28c/pLacZ strain of *T. cruzi* were seeded in 90 µl RPMI-1640 medium without phenol red and supplemented with 5% FBS per well. Two-fold serial dilutions were plated in each well, and then 10 µl of cell culture medium containing substrate solution (CPRG and Nonidet P-40, at 100 µM and 0.1% of final concentration, respectively) was added. After incubation with CPRG at 2, 4, 6 and 24 h at 37°C and 5% $CO_2$, the plates were read in an Enzyme-Linked ImmunoSorbent Assay (ELISA) reader at 570 nm (Multiskan EX, Thermo Scientific®).

### Trypomastigote lysis assay

Cell-cultured derived trypomastigotes ($1 \times 10^5$ parasites) were plated in 50 µl RPMI-1640 without phenol red and 10% FBS and incubated with 4-fold serially diluted concentrations of BZ or NFX (160–0.039 µM) at 37°C and 5% $CO_2$ for 24 h in 96-well tissue culture plates (Nest®; 701001) in 100 µl as final volume. Then, 50 µl of a CPRG solution (100 µM final concentration) was added to each well and incubated at 37°C in an atmosphere of 5% $CO_2$ for 4 h. The β-galactosidase activity was quantitated at 570 nm in an ELISA reader. Each drug concentration was evaluated by triplicate, and non-treated trypomastigotes were included as a control.

An identical assay was carried out in parallel, and the final readout was determined by counting the motile trypomastigotes in KOVA Glasstic slides® in optic microscopy. There were performed three independents replicates for both assays.

The lytic concentration 50% ($LC_{50}$), defined as the drug concentration that resulted in a 50% reduction of motile trypomastigotes compared with the untreated control, was estimated by non-linear regression analysis [18].

### Amastigote development inhibition assay

Ninety-six-well tissue culture plates (Nest®; 701001) were seeded with Vero C-76 cell line at $10^4$ per well in 100 µl of RPMI-1640 and 5% FBS and incubated overnight at 37°C and 5% $CO_2$. Then, wells were rinsed with sterile PBS 1X, and *T. cruzi* Dm28c strain β-galactosidase-expressing trypomastigotes were added in a multiplicity of infection (MOI) equal to 1, 5 or 10 per well in 100 µl of RPMI-1640 and 5% FBS. After 2, 6 and 24 h of interaction, plates were washed, and treatment was added in 200 µl of RPMI-1640 and 5% FBS.

At 3, 4 or 5 days post-infection (dpi), when expanded numbers of trypomastigotes were easily visible in control wells, the plates were washed with sterile PBS 1X and filled with 100 µl of RPMI-1640 without phenol red, CPRG (100 µM final concentration) and Nonidet P-40 (0.1% final concentration). Plates were incubated for 2, 4, 6 or 24 h at 37°C and 5% $CO_2$. The β-galactosidase activity was quantitated at 570 nm in an ELISA reader.

BZ was added to infected plates at 10 µM and incubated during the different times, as stated above. After the assay conditions were selected, drug compounds were added in serial dilutions in 200 µl of RPMI-1640 and 5% FBS. Each drug concentration was evaluated by triplicate, and infected non-treated and non-infected non-treated cells were included in each replicate.

The non-parametric Kolmogorov–Smirnov test allowed the establishment of optimal assay conditions, detecting the differences between the non-treated wells and BZ treatment (10 µM) by providing the *D* value. The null hypothesis was that both groups were sampled from populations with identical distributions. Geometrically, the *D* value measures the maximum vertical distance between the empirical cumulative distribution function (ECDF) of the sample and the cumulative distribution function of the reference distribution. Thus, *D* ranges between 0 and 1, where 0 occurs if the two ECDFs are identical and 1 if the two samples are completely distinct [19].

### Cell toxicity

To assess the drug toxicity on the Vero C-76 cell line, $10^4$ cells/well were seeded in 96-well plates and incubated overnight at 37°C in an atmosphere of 5% $CO_2$. Then, wells were rinsed and incubated in the presence of BZ or NFX at 4-fold serially diluted concentrations (160–0.156 µM). After 5 days, plates were washed, and 100 µl of RPMI + 5% FBS and 10% of a 3 mM resazurin solution were added to each well. The plates were incubated at 37°C with a 5% $CO_2$ atmosphere for 4 h and read at 570–595 nm in an ELISA reader (MultiskanEX, Thermo Scientific®) to obtain a dose–response curve [20]. Each drug concentration was evaluated by triplicate, and non-treated cells were included in each replicate. The cytotoxic concentration 50 ($CC_{50}$), defined as the concentration of compound capable of damaging 50% of the cells, was calculated using GraphPad Software, San Diego, CA, USA (www.graphpad.com).

## Results

### β-galactosidase activity assay

Figure 1 shows the relationship between the coloured product and the concentration of cell culture-derived trypomastigotes plated in each well. The β-galactosidase activity increased proportionally with parasite concentration. The best curve fitting was achieved when trypomastigotes were incubated with the substrate solution for 4 h ($R^2 = 0.9816$), and β-galactosidase activity was linear in the range of 3 150–100 000 trypomastigotes per well (i.e. 31 500–1 000 000 trypomastigotes/ml). An illustrative culture plate is included as Supplementary material (Fig. S1).

### Trypomastigote lysis assay

The *in vitro* activity of NFX and BZ on trypomastigotes of Dm28c/pLacZ strain from *T. cruzi* was assessed with the previously established colourimetric method, and the $LC_{50}$ values were compared with the $LC_{50}$ obtained by direct microscopic counting (Table 1 and Fig. 2).

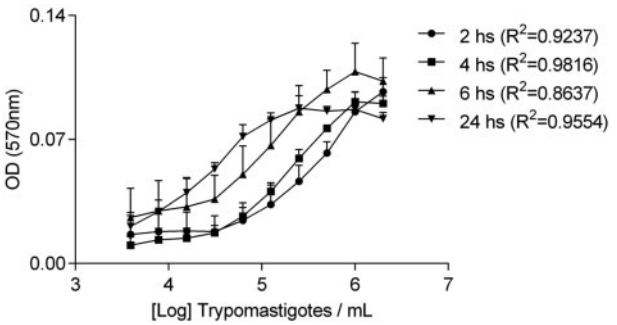


**Figure 1:** β-galactosidase activity (OD at 570 nm) with different concentrations of cell-culture derived trypomastigotes from Dm28c/pLacZ strain of *T. cruzi* after incubation at 37°C and 5% of $CO_2$ at different time points. Each value represents the mean and the standard deviation of three independent replicates. The dark continuous line represents the curve fit.

**Table 1:** *In vitro* activity of benznidazole and nifurtimox on trypomastigotes of Dm28c/pLacZ strain of *Trypanosoma cruzi*, obtained with direct microscopic counting or with the colorimetric method.

| Compound | Direct microscopic counting $LC_{50}$ (95% CI) | $R^2$ | Colourimetric method $LC_{50}$ (95% CI) | $R^2$ |
|---|---|---|---|---|
| BZ | 41.36 (35.50; 48.18) | 0.98 | 44.74 (40.93; 48.91) | 0.99 |
| NFX | 17.99 (12.32; 26.26) | 0.90 | 38.94 (37.05; 40.92) | 0.99 |

Mean values (95% CI) are expressed in μM.

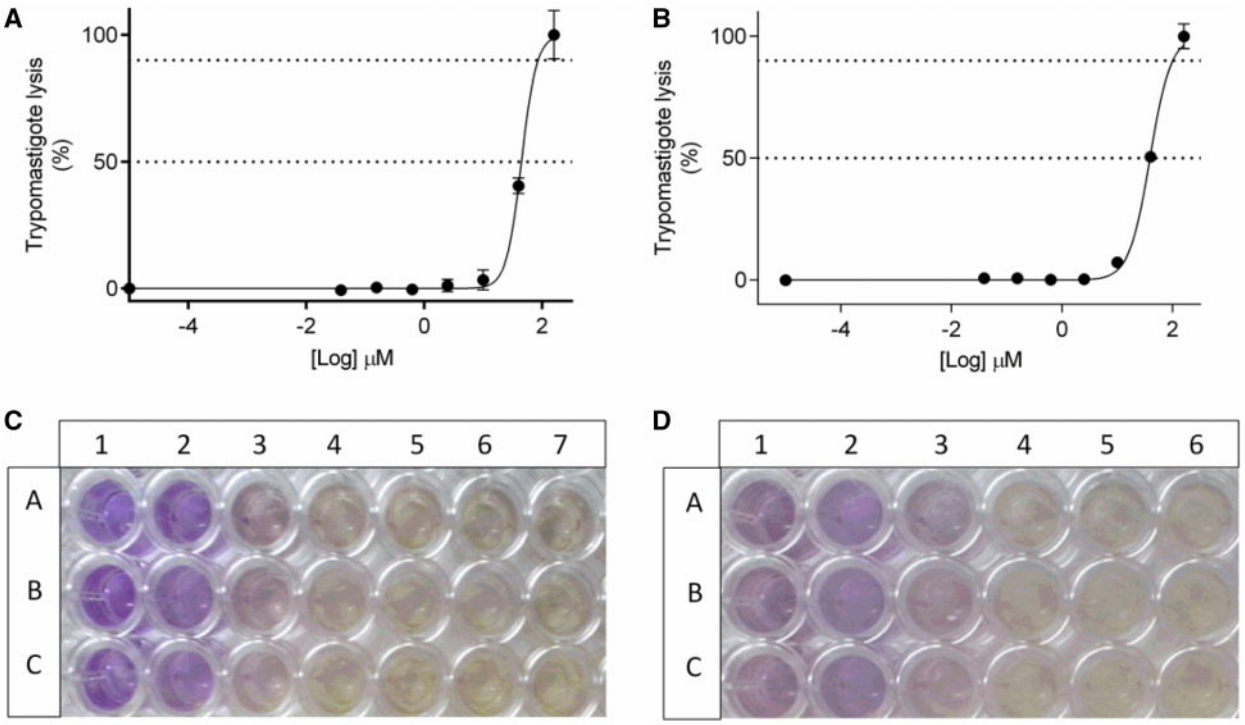


**Figure 2:** Concentration–response curves for (A) BZ and (B) NFX and culture plates corresponding to the colourimetric assay to determine (C) BZ $LC_{50}$ and (D) NFX $LC_{50}$ on the Dm28c/pLacZ strain of *T. cruzi* trypomastigotes. Each value represents the mean and the standard deviation of three independent replicates. The dark continuous line represents the curve fit. Dotted lines indicate 50 and 90% trypomastigote lysis. References: (C) BZ: A1-C1: 160 μM; A2-C2: 40 μM; A3-C3: 10 μM; A4-C4: 2.5 μM; A5-C5: 0.625 μM; A6-C6: 0.156 μM; A7-C7: NT (non-treated). (D) NFX: A1-C1: 160 μM; A2-C2: 40 μM; A3-C3: 10 μM; A4-C4: 2.5 μM; A5-C5: 0.625 μM; A6-C6: NT (non-treated).

Table 1 shows the $LC_5$ values for NFX and BZ obtained both by the direct microscopic counting and colourimetric methods. A correlation higher than 0.9 and narrow confidence intervals (CIs) were obtained for both screening methods. Regarding BZ, the obtained $LC_{50}$ values were similar with both methods. However, the $LC_{50}$ value for NFX obtained with the microscopic counting method nearly doubled the $LC_{50}$ value calculated with the colourimetric method.

## Amastigote development inhibition colourimetric assay optimization

We have studied 27 possible combinations, among the different conditions: parasite–cell relationship (MOI = 1, 5 or 10); time for parasite–cell interaction (2, 6, 24 h), amastigote development (3, 4 or 5 dpi) and incubation time (2, 4, 6 or 24 h). The non-parametric Kolmogorov–Smirnov test allowed to establish the optimal conditions in which the differences were significantly greater comparing treated (BZ, 10 μM) and non-treated wells. Table 2 and Fig. 3 indicate the experimental conditions with the most significant differences. Results from all tested combinations can be observed in Supplemental Table S1.

**Table 2:** Experimental conditions for the colorimetric assay with the Dm28c/pLacZ strain of *Trypanosoma. cruzi*.

| Experimental conditions | MOI | Interaction time | dpi | *D* value | *P*-value |
|---|---|---|---|---|---|
| Combination 2 | 1 | 2 | 4 | 0.75 | 0.0023 |
| Combination 3 | 1 | 2 | 5 | 0.83 | 0.0005 |
| Combination 4 | 1 | 6 | 3 | 0.75 | 0.0023 |
| Combination 5 | 1 | 6 | 4 | 0.75 | 0.0023 |
| Combination 11 | 5 | 2 | 4 | 0.75 | 0.0023 |

*D* value ranges between 0 and 1, where 0 occurs if the two ECDF are identical and 1 if the two samples are completely distinct.
*P*-values <0.05 were considered significant.
MOI: multiplicity of infection.

To move forward with the high-throughput colourimetric system optimization, the experimental conditions that

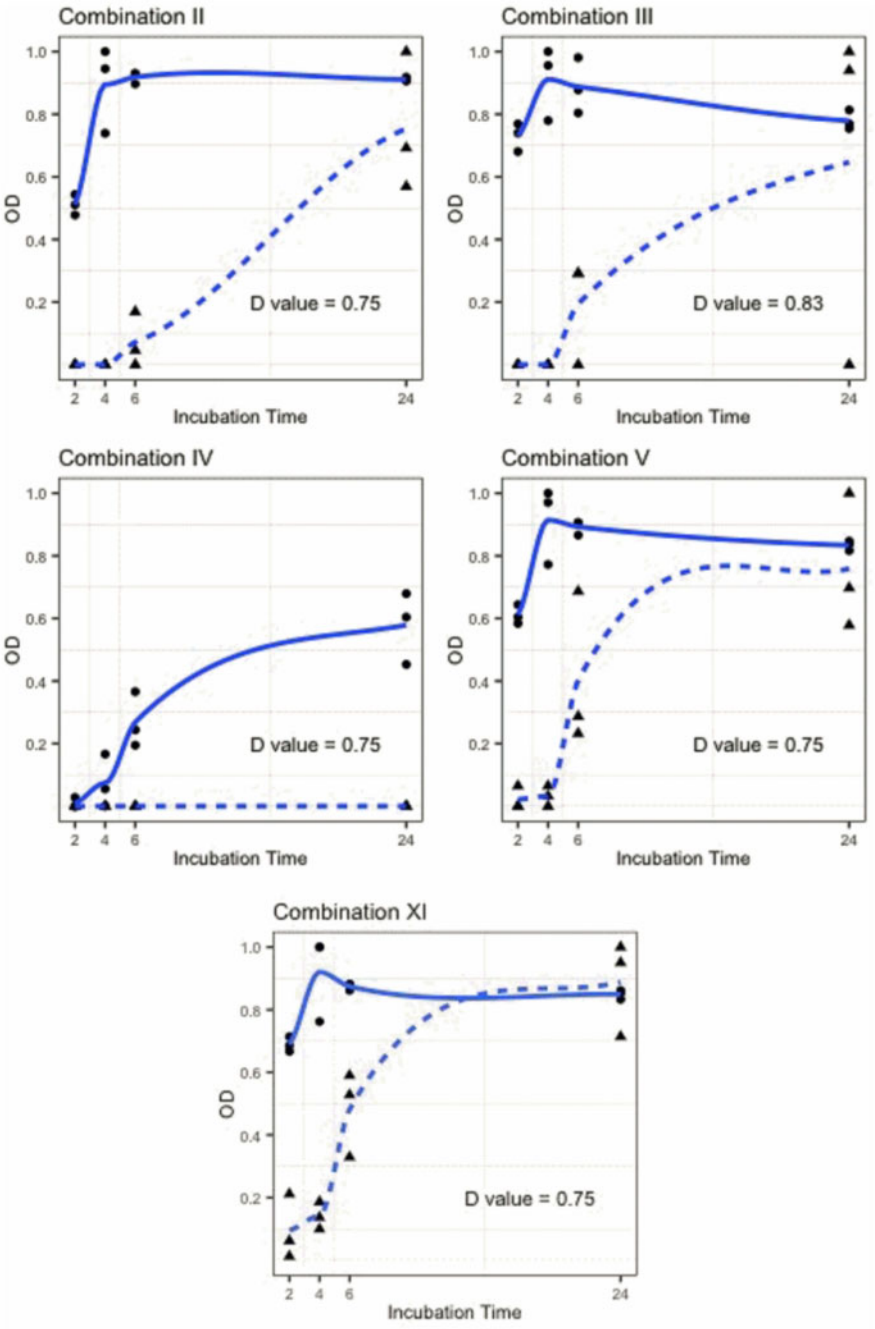


**Figure 3:** OD versus incubation time (2, 4, 6 and 24 h) with the substrate solution. Average OD values (normalized to 1) are indicated for non-treated wells (continuous blue line) and wells treated with 10 μM of BZ (dashed blue line).

exhibited higher *D*-value at the shortest interaction time and less dpi at the time of the substrate incubation were selected.

Accordingly, the optimal assay conditions were established as follows: Vero C-76 cells ($10^4$/well) were seeded in 100 μl of RPMI + 10% FBS in 96-well culture plates. After 24 h, wells were washed with sterile PBS 1X, and cells were infected with culture-derived trypomastigotes from the Dm28c/pLacZ strain of *T. cruzi*, with MOI=5 and 2 h of interaction. Then, wells were washed with sterile PBS 1X to remove unbound cells and parasites. Later, seven serial dilutions (160–0.039 μM) of the reference drugs (i.e. BZ or NFX) in RPMI + 10% FBS were dispensed to the infected cells in a final volume of 200 μl per well. Infected non-treated and non-infected non-treated cells were included in each replicate. At 4 dpi, wells were washed with sterile PBS 1X, and then cell culture media (RPMI with 5% FBS without phenol red) with substrate solution (CPRG at 100 μM and Nonidet P-40 at 0.1%, final concentration) was added. The plates were read in an ELISA reader at 570 nm after 4 h of incubation at 37°C and 5% $CO_2$.

## Amastigote development inhibition assay

The 50% inhibitory concentration ($IC_{50}$) values for BZ and NFX for the *T. cruzi* Dm28c/pLacZ strain obtained with the

**Table 3:** *In vitro* activity of benidazole and nifurtimox on intracellular amastigotes development from Dm28c/pLacZ strain of *Trypanosoma cruzi*.

| Compound | $IC_{50}$ (95% CI) | $IC_{90}$ (95% CI) | $R^2$ | $CC_{50}$ (95% CI) | SI |
|---|---|---|---|---|---|
| BZ | 2.31 (2.14; 2.49) | 8.60 (5.99; 12.30) | 0.99 | >160 | >69 |
| NFX | 0.97 (0.90; 1.05) | 3.51 (2.09; 5.69) | 0.99 | >160 | >165 |

Vero C-76 cells infected with cell-culture derived trypomastigotes from Dm28c/pLacZ strain of *T. cruzi* in a MOI = 5, 2 h of interaction and 4 days of treatment. SI: selectivity index. SI= $CC_{50}/IC_{50}$.

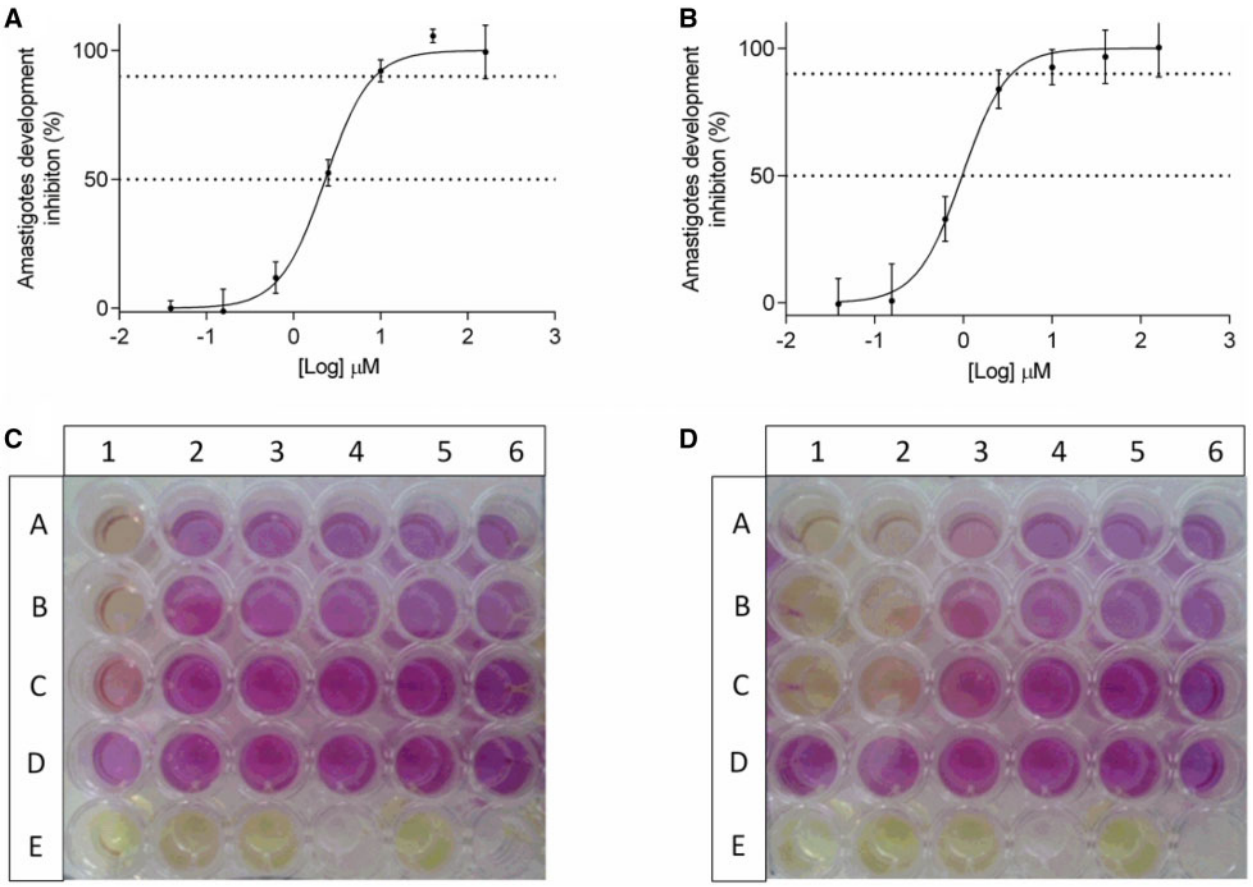


**Figure 4:** Concentration–response curves for (A) BZ and (B) NFX and culture plates corresponding to the colourimetric assay to determine (C) BZ $IC_{50}$ value and (D) NFX $IC_{50}$ value on the Dm28c/pLacZ strain of *Trypanosoma cruzi* amastigotes. Dotted lines indicate 50 and 90% inhibition of amastigote development. Each value represents the mean and the standard deviation of three independent replicates. References: (C) Representative plate for the amastigote inhibitory activity assay with BZ treatment. A1-C1: 160 µM, A2-C2: 40 µM; A3-C3: 10 µM; A4-C4: 2.5 µM; A5-C5: 0.625 µM; A6-C6: 0.156 µM; D1-D3: 0.039 µM; D4-D6: infected non-treated cells Vero-C76 cells; E1-E3: non-infected non-treated Vero-C76 cells; E4: RPMI-1640 + 5% FBS; E5: RPMI-1640 without phenol red + 5% FBS+ CPRG 100 µM + Nonidet P-40, 0.1%; E6: empty well. (D) Representative plate for the amastigote inhibitory activity assay with NFX treatment. A1-C1: 160 µM, A2-C2: 40 µM; A3-C3: 10 µM; A4-C4: 2.5 µM; A5-C5: 0.625 µM; A6-C6: 0.156 µM; D1-D3: 0.039 µM; D4-D6: infected non-treated Vero-C76 cells. D4-D6: infected non-treated cells Vero-C76 cells; E1-E3: non-infected non-treated Vero-C76 cells; E4: RPMI-1640 + 5% FBS; E5: RPMI-1640 without phenol red + 5% FBS+ CPRG 100 µM + Nonidet P-40, 0.1%; E6: empty well.

colourimetric screening method are indicated in Table 3. The concentration–response curves are shown in Fig. 4.

## Discussion

An *in vitro* colourimetric method was standardized and biologically validated to establish a suitable model for high-throughput drug screening using the Dm28c/pLacZ strain of *T. cruzi*.

The *T. cruzi* Dm28c strain was originally isolated from a common opossum (*Didelphys marsupialis*) in Carabobo, Venezuela, and it belongs to the DTU TcI [21]. The Dm28c strain was characterized both *in vitro* and *in vivo* [22], and its genome was recently sequenced [23].

Several *T. cruzi* strains have been previously genetically engineered: Tulahuen, CL, Peru, Sonja, VL2067, among others and are also available at the ATCC [24].

Briefly, the parasites have been genetically modified to express the *E. coli* lacZ. This enzyme is able to catalyze a reaction with CPRG as a substrate, and the generated product is directly proportional to the transfected parasites burden, allowing to quantify it by colourimetric readers [18].

The MOI (i.e. the parasite:cell infection ratio), the interaction time and the incubation time with the substrate are some of the critical steps to establish a successful *in vitro* infection and to correctly evaluate the chosen readout to assess the efficacy of candidate compounds on *T. cruzi* [25].

The optimal experimental conditions for this assay using the Dm28c/pLacZ strain (DTU TcI) were similar to the previously established protocol with CL and Tulahuen strains (DTU TcVI) [18, 26, 27].

The development of *in vitro* high-throughput screening platforms with similar experimental conditions and readouts would allow comparing the obtained results in different strains, thus explaining the differences in drug sensitivity due to intraspecific variety among the *T. cruzi* complex. As these differences were previously documented, high-throughput screening should consider the *T. cruzi* strain variability [28].

The optimization of the colourimetric assay to assess the amastigote development inhibition was tested with BZ since it is the reference compound for drug screening [29]. Among the different tested experimental conditions, the protocol that led to better outcomes was: a MOI= 5; 2 h of cell–parasite interaction, readout at 4 dpi and plate reading after 4 h of incubation with the substrate. Under these conditions, results were obtained in a total of 5 days of experimental work. Despite the larger number of trypomastigotes needed per well, the optical densities differences between treated and untreated wells were wide, allowing to obtain accurate $IC_{50}$ values.

Combination II (MOI = 1; 2 h of cell–parasite interaction and readout at 4 dpi) was not selected despite the statistical support since MOI = 1 would require a very high infection rate which may not be achieved in all experiments. Furthermore, combination III (MOI = 1; 2 h of cell–parasite interaction and readout at 5 dpi) was discarded despite its higher *D* value since experiments would take longer to obtain results.

Coloured compounds might interfere with readout when using colourimetric readings [30]. To overcome this difficulty in the amastigote development inhibition assay, we modified the original protocol from Buckner *et al.* [14] by removing the supernatant from the wells before adding the substrate in a cell culture medium without phenol red. This intermediate step would avoid the interference in colourimetric readings due to the absorbance of coloured compounds [24].

Furthermore, this additional step allowed discarding the trypomastigotes that would have emerged from the infected cells, generating a higher coloured signal and possibly underestimating the compound activity.

For all the transfection process, drug sensitivity from Dm28c/pLacZ strain remained similar to wild-type strain. The obtained $IC_{50}$ values for BZ and NFX on intracellular amastigotes development were biologically significant, with similar BZ values as reported with the Dm28c wild strain in a high-content screening method. Likewise, the value obtained for NFX on the Dm28c/pLacZ strain was slightly lower than BZ and in the same order of magnitude as reported for the wild Dm28c strain [31].

The effect of the reference drugs on trypomastigotes from the Dm28c/pLaZ was studied with the colourimetric method and the microscopic method as well. For BZ, the obtained $LC_{50}$ value was very similar between both methods. In contrast, the obtained $LC_{50}$ value for NFX with the standard microscopic counting method was 15 μM less than the colourimetric method. This difference could be explained due to the naturally coloured of the compound, which could interfere with the colourimetric reading at similar optical density (OD).

In summary, this colourimetric assay using the Dm28c/pLacZ strain of *T. cruzi* has been set up to improve and optimize drug screening capacity, obtaining biologically meaningful values from BZ and NFX sensitivity. This development can be included in a panel of several *T. cruzi* strains and applied in a high-throughput screening programme of large drug libraries to identify compounds with anti-*T. cruzi* activity.

## Supplementary data

Supplementary data is available at *Biology Methods and Protocols* online.

## Data availability

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

## Funding

The authors received no funding for this work.

*Conflict of interest statement*. None declared.

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