**INTRODUCTION**

Chagas disease (CD), caused by the protozoan parasite *Trypanosoma cruzi* (Figure 1) is endemic to South and Central America and estimated to cause 14,000 deaths per year. Treatment for infection relies upon two available drugs, nifurtimox and benznidazole. These drugs are effective in treating the acute stage of CD, however there are numerous side effects and efficacy in the chronic stage is limited, possibly due to poor pharmacokinetics. There remains a need for new chemotherapeutic agents against *T. cruzi*. Assays for the identification of active compounds against *T. cruzi* particularly the amastigote, thought to be persistent in the chronic phase, are important continuing developments. We have developed a 384 well imaging assay for estimation of compound activity against amastigotes of the Tulahuen strain in 3T3 fibroblasts.

**MATERIALS AND METHODS**

**Assay protocol**

3T3 fibroblasts at 1000 cells/well in RPMI +10% FCS were added to 384 well assay plates (PerkinElmer CellCarrier; Collagen I coated). After 24 hours, 2x10⁴ trypanamastigotes/well were added in 10µL of media. Following 24 hours, wells were washed with PBS on a Bravo liquid handling platform (Agilent) and 50µL of media added. Compounds/ DMSO in 10µL of media were added with a Minitrak™ liquid handler. After 48 hours, wells were fixed with parafomaldehyde and stained with Hoechst and HCS CellMask Green (Life Technologies).

**Analysis**

Plates were analysed on an Operetta® high content imaging system at a 20x magnification and images analysed with the Harmony® software package (PerkinElmer). Results were compared for 3T3 cell passages and for 3 and 5 images per well.

**Compound screening**

A library of 741 compounds were screened in the final assay format. Compounds were either FDA approved or are of interest for varying disease indications. Nifurtimox was used as the standard for *T.cruzi* and puromycin for host cells.

**RESULTS**

**Image analysis**

Figure 2 shows images of *T.cruzi* infected cells treated with DMSO (A) nifurtimox (D) and puromycin (G). A host cell cytoplasm containing >10 parasite nuclear spots was defined infected. These criteria successfully identified infected cells (infected are shown as green, red are non infected; C), identifying non infected cell populations (nifurtimox treatment: F) and no cells in wells treated with puromycin (I).

**Compound activity**

741 compounds were screened at 20, 2 and 0.2µM. The Z prime for the parasite was 0.55±0.06 and host cells 0.58±0.16. The cut of activity at 3x S.D was >50% (2µM), with <50% at 2µM for 3T3 cells. Six were FDA approved; or were used for treatments of other disease indications (Table 1). Those already identified active on *T.cruzi* amastigotes have shown lack of efficacy in human/animal models.

**CONCLUSIONS**

Compounds of interest, with no reports in the literature of *T. cruzi* activity (5) will be sourced for further analysis. This includes IC₅₀ determination and giemsa staining post 48 hr incubation to determine if action is static or cidal. Previously a 7 day assay for estimation of cidal/static compound activity was pursued, however it was found that liberation of trypomastigotes and external amastigotes from host cells made spot analysis of internalised parasites difficult to determine (results not shown). Amastigote assays for the Y strain parasite will also be developed.