Trypanosoma cruzi, an intracellular protozoan of the Trypanosomatidae family, is the etiological agent of the Chagas disease that is a tropical neglected illness that is the leading cause of heart disease in Latin America where it affects approximately 12 million people living in very poor social conditions. Today, it still represents a serious public health problem in these affected areas claiming for care and resolution of its current challenges, including the imperative need to sustain public policies related to the transmission control and the requirement for new chemotherapeutic agents. Pentamidine and related di-cations are DNA minor groove-binders with broad-spectrum anti-protozoal activity. In this context, our aim was to investigate the tripanocidal activity in vitro of six di-cationic compounds – DB1582, DB1627, DB1645, DB1646, DB1651 and DB1670 – against bloodstream trypomastigotes (BT) of Trypanosoma cruzi and their cellular targets by fluorescence microscopy. Our results demonstrated that DB1645, DB1582 and DB1651 were the most active against BT showing IC50 values ranging between 0.15 and 6.9 µM. The compounds displayed toxicity after 24 h of incubation with 96 µM less than 20%. Following treatment for 72 h, DB1627, DB1645, DB1651 and DB1670 resulted in 36, 21, 45 and 22% loss of cellular viability at 96 µM drug concentration, while both DB1582 and DB1646 gave a <20% reduction. DB1645, DB1582 and DB1651 were also the most effective against intracellular parasites, with IC50 values ranging between 7.3 and 13.3 µM. Due to the characteristics of the tested compounds, blue fluorescence is emitted when excited by UV light, the amidines could be localized in both cellular structures of both BT and amastigotes through fluorescence microscopy. When the parasites were treated for 1 h with 10 µg/ml of each compound, we observed that in the treated parasites the diamidines presented a striking localization within the kDNA (asterisk) and at much lower levels in the parasite and host cardiac cells nuclei (thick white arrow). Interestingly, DB1582 and DB1651 were also localized in several punctated non-DNA containing organelles (thin white arrows) distributed within the BT and intracellular forms, which is related to acidic compartments (acidocalcisomes) localization and morphology (thin arrow). Our data suggest that the localization of these amidines in these organelles may be a consequence of their intracellular accumulation in these cellular sites (storage sites) and/or due to their primary or secondary drug targets. These studies may aid future design and synthesis of novel agents that could be used for Chagas disease therapy.

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Fig. 1: Intracellular localization of the compounds in bloodstream (A–E, G) and amastigote (F) forms of Trypanosoma cruzi after 1 h of incubation of each diamidine. DB1582, DB1627, DB1645, DB1646, DB1651 and DB1670.