Large-scale screening effort to identify new chemical starting points for visceral leishmaniasis using a novel *in vitro* screening cascade

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A key issue in visceral leishmaniasis (VL) drug discovery is the difficulty finding new chemical starting points. Poor translation between the developed insect stage promastigote or axenic amastigote assays and the “gold-standard” intracellular assays has often been observed. The work presented here shows how some of these issues can be overcome by redeveloping existing assays and combining assays in screening cascades. The screening cascade presented was used to screen a large number of compounds from various pharma and commercial sources. We reconfigured our existing axenic amastigote assay to only detect compounds that are cytocidal. This was achieved by improving the detection limit of the assay and increasing the starting cell density. A screening cascade was developed using the “cidal” axenic amastigote assay as a primary screening platform, followed by potency and selectivity determination and a human counter-screen assay, and finally by assessment in a high-content intracellular amastigote assay. This cascade was used for the screening of >700,000 compounds. Our results show that the cidal axenic amastigote assay is a better predictor of intracellular amastigote activity compared to its non-cidal format. It provides a valuable primary screening platform for large scale screens, especially since throughput limitations do not allow the use of the intracellular assay. The screening cascade used here was successful in identifying several new hit series for VL drug discovery. Results from a single screening campaign will be presented. This work shows that there is value in using axenic *Leishmania* screening assays, and that the way these are configured, in particular with respect to the detection limit, has a major impact on the hits identified, and their translation to intracellular amastigote activity. Through the use of a rational and pragmatic screening cascade we have been able to identify much needed new chemical starting points for VL drug discovery.