

Twenty Five Years of Drug Discovery at the University of California Targeting Kinetoplastid Parasites

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Abstract

This review summarizes 25 years of screening compounds against three major kinetoplastid parasites, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* spp. The work was carried out at two University of California campuses by a consortium of scientists. The history of this effort is summarized beginning with DARPA and NIAID TDRU projects. The compound collections that were screened came from both academic and industry sources. To facilitate screening, high throughput or high content microtiter plate-based assays were developed. Three approaches to discovery of new drugs for kinetoplastid diseases are presented. These include structure-based drug design against specific parasite molecular targets, repurposing of already approved drugs, and screening of marine natural products. As similar screening efforts against other molecular targets or with other compound libraries are ongoing, one conclusion is that the current bottleneck in drug development for neglected tropical diseases is downstream of compound screening and hit to lead. More medicinal chemistry efforts for lead optimization and more preclinical package work needs to be done. For more information see (www.cdipd.org).

Keywords: Drug Discovery; Kinetoplastid parasites; *Trypanosoma brucei*; *Trypanosoma cruzi*; *Leishmania* spp

Background

The three major kinetoplastid parasites are *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* spp. These pathogens are responsible for human African trypanosomiasis (HAT) also known as Sleeping Sickness, Chagas Disease, and cutaneous or visceral leishmaniasis. These are designated “neglected tropical diseases” (NTDs) because they primarily affect poor people in poor regions of the world. Therefore there is little or no economic incentive for the pharmaceutical industry to develop new drugs, vaccines, or diagnostics. Over the past 25 years, a consortium of scientists at two University of California campuses have screened, optimized and advanced compounds obtained from both academic and industry sources to identify new therapies for kinetoplastid diseases. This review will summarize the history of this effort, its current status, and suggestions for future development.

History

Work at the University of California San Francisco began in the late 1980's and early 1990's supported by DARPA (Defense Advanced Research Projects Agency) and a Tropical Disease Research Unit program project grant from the NIAID (National Institute of Allergy and Infectious Diseases). The DARPA project was led by computational biologist Fred Cohen. It focused on the development of computational approaches to drug discovery and design following up on the pioneering molecular design work of Irwin “Tack” Kuntz. While the original focus of the DARPA project was on other tropical diseases (malaria and schistosomiasis), the compounds produced were cross-screened against kinetoplastids [1-4].

The original research on *Trypanosoma cruzi* and Chagas Disease at UCSF was supported by the Tropical Disease Research Unit grant. The first report identifying an inhibitor of the major protease of *Trypanosoma cruzi* as a drug lead was published in 1993 [5]. Research over the succeeding two decades was primarily supported by funding from the NIAID, the Sandler Family Foundation, and most recently the European Union (Kindred, FP7). Supplemental funding came from the American Heart Association, the World Health Organization, DNDi (Drugs for Neglected Diseases Initiative), the Department of Veterans Affairs, and the Burroughs Wellcome Fund. As detailed in the References, this funding supported assay development, structure-based drug design, HTS, and HCS. In 2014, much of the kinetoplastid drug discovery research shifted from UCSF to the University of California San

Diego (UCSD). Drug discovery research continued at the UCSD site with addition of new HTS and HCS screens (www.cdipd.org). Furthermore a Drug Development Pipeline was launched (<http://cddi.ucsd.edu/resources/drug-pipeline.html>) that provides the pre-clinical package of assays required to move a compound “hit” to a clinical candidate. This is the key for NTD drug development that must usually be done without support from an industry partner.

The compound collections (“libraries”) that were screened against the three kinetoplastid parasites came from both academic and industry sources. Over 20 companies donated compounds to be tested. Transfer and testing was carried out through material transfer agreements (MTAs). To facilitate screening, high throughput or high content microtiter plate-based assays were developed [6-8]. These were compatible with robotic liquid handling and automated imaging equipment.

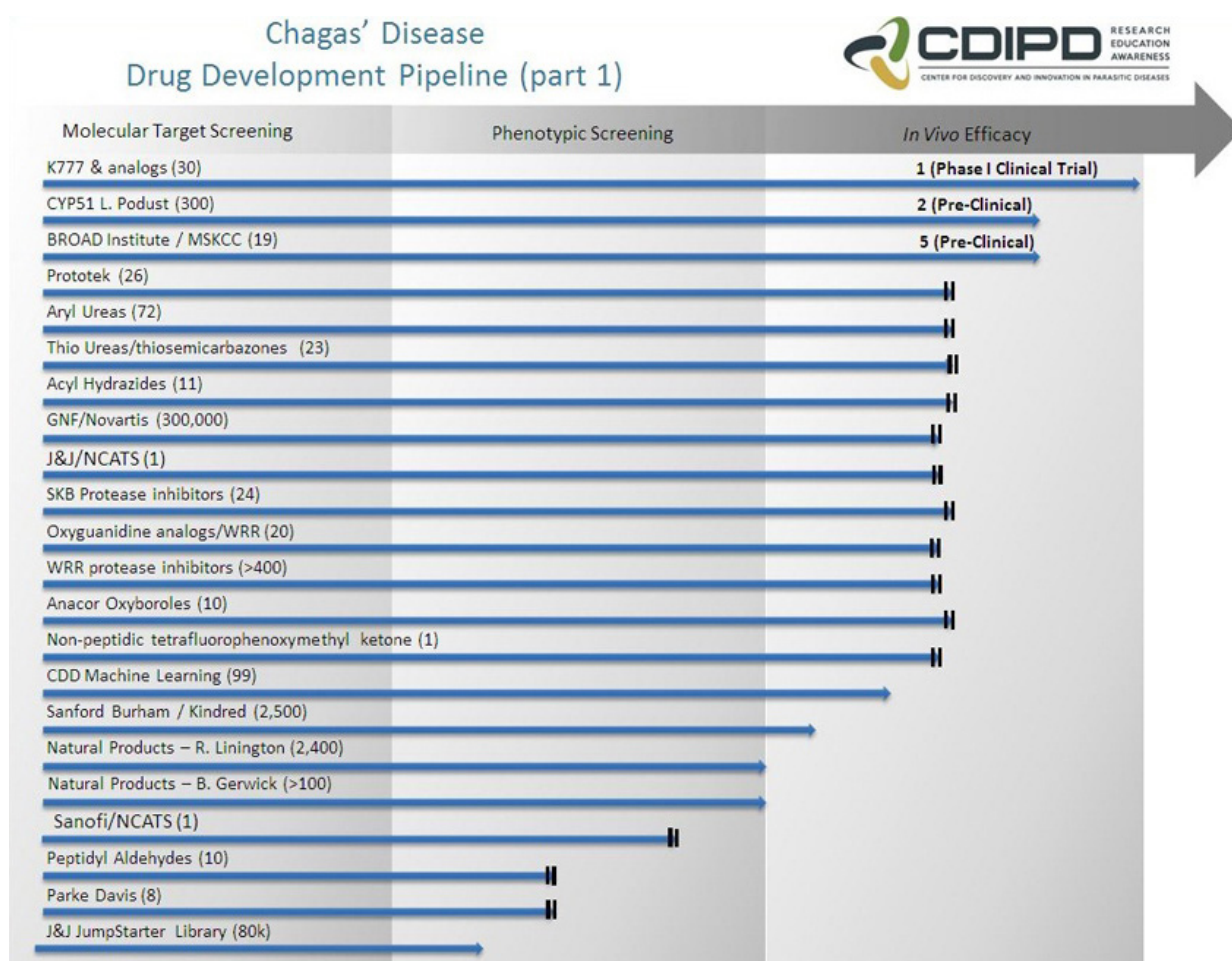
Results

A summary of the approximate number of compounds screened, and how far they progressed, is given in Figures 1, 2, 3 and 4. A more comprehensive review of all the compound series tested can be found in the over 70 publications that resulted from this work as listed in References [1-70].

Notable molecular targets

Both phenotypic screens (against the parasites themselves) as well as screens against specific molecular targets were carried out. Phenotypic screens have the advantage of identifying hits directly against the relevant parasite stage [6-8]. Target-based screens have more capacity and are more focused. That is they can use inhibitor libraries that might have been produced and validated against homologous enzyme or receptor targets. The molecular targets screened against at the two UC campuses were by no means comprehensive, but rather reflected the research interests of collaborators and whether they had been shown to be “druggable”. “Druggable” targets are herein defined as proteins (usually enzymes) which have homologues (usually human) successfully targeted by approved drugs in current clinical use. Two notable molecular targets in Figures 1, 2, 3 and 4 are proteases [29,38,48,66] and sterol biosynthesis enzymes [60,61] both of which represent drug target families for which multiple drugs are in clinical use. In some cases compounds were de-prioritized for PK/PD reasons but helped identify new targets like cytochrome b [67,68].

From phenotypic screens the most advanced hit was an oxyborole from Anacor Pharmaceuticals that is now in clinical trials for HAT. SCYX-7158 was identified as a promising hit by Zachary Mackey in the original HTS assay [6] and subsequently optimized at Scynexis for blood-brain barrier penetration [49].



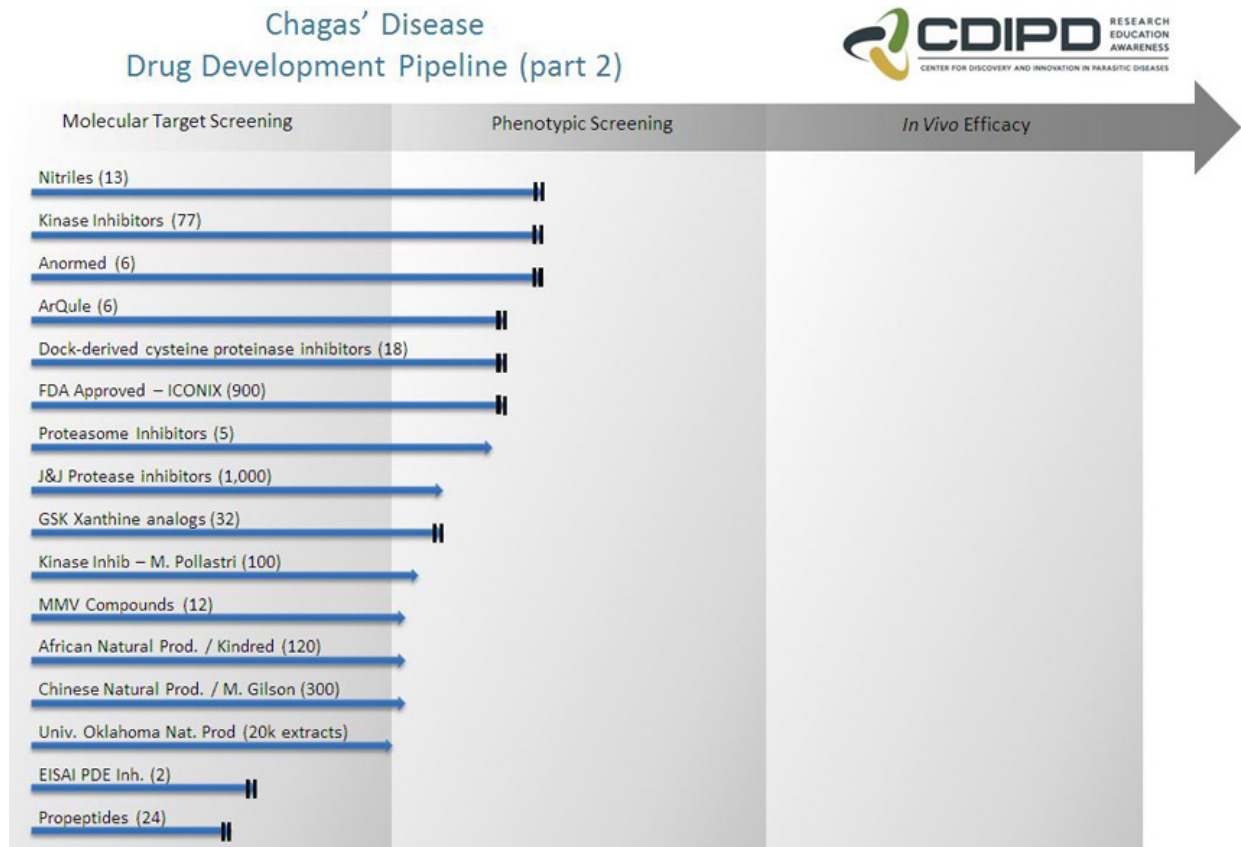


Figure 1: “Screening Molecular Target” refers to those compounds with known molecular target so the first HTS screen was against the target itself (Eg: kinases, proteases)

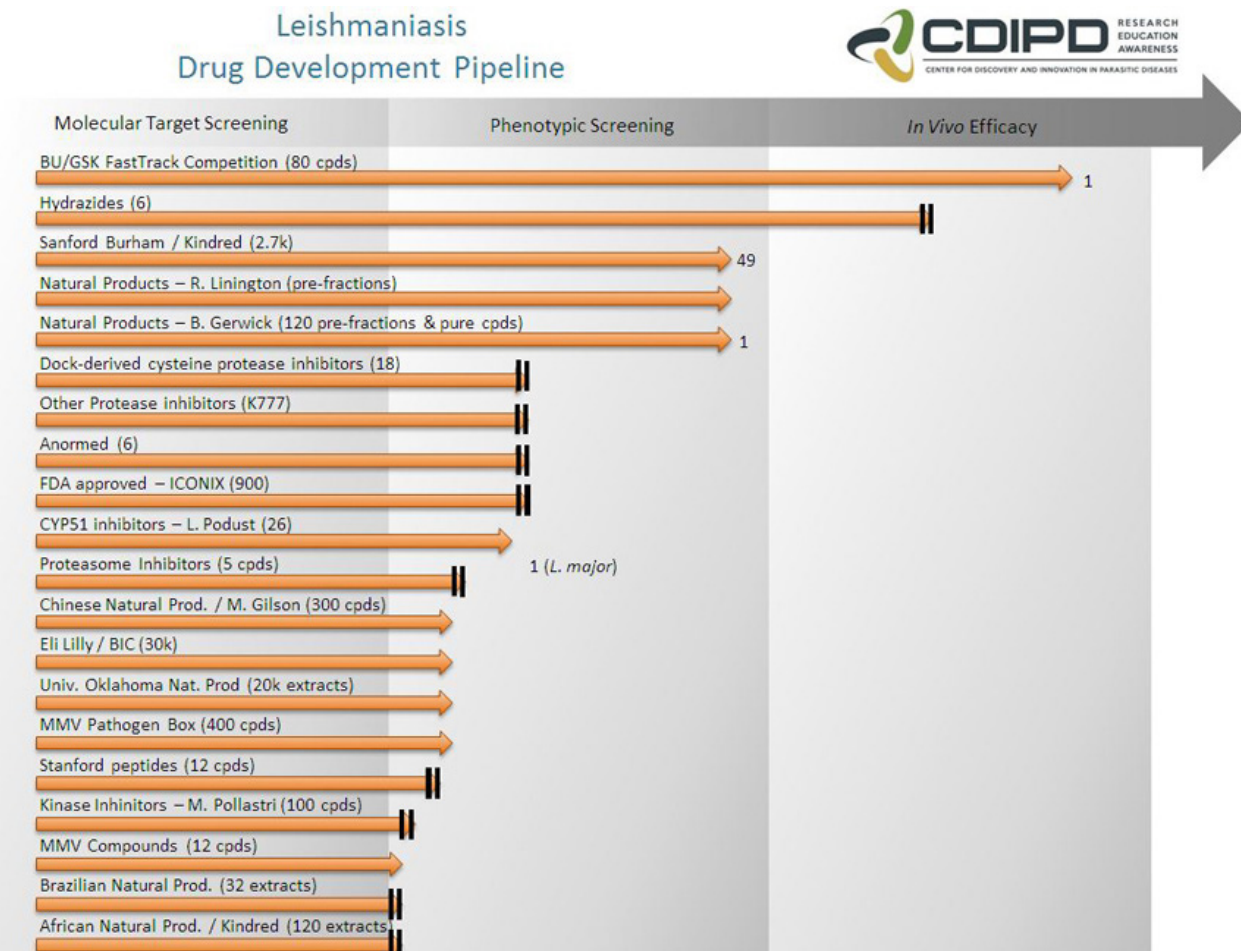


Figure 2: Chemical class is given for each compound library except in some cases where compounds from companies were not disclosed for IP reasons

Human African Trypanosomiasis Drug Development Pipeline (part 1)

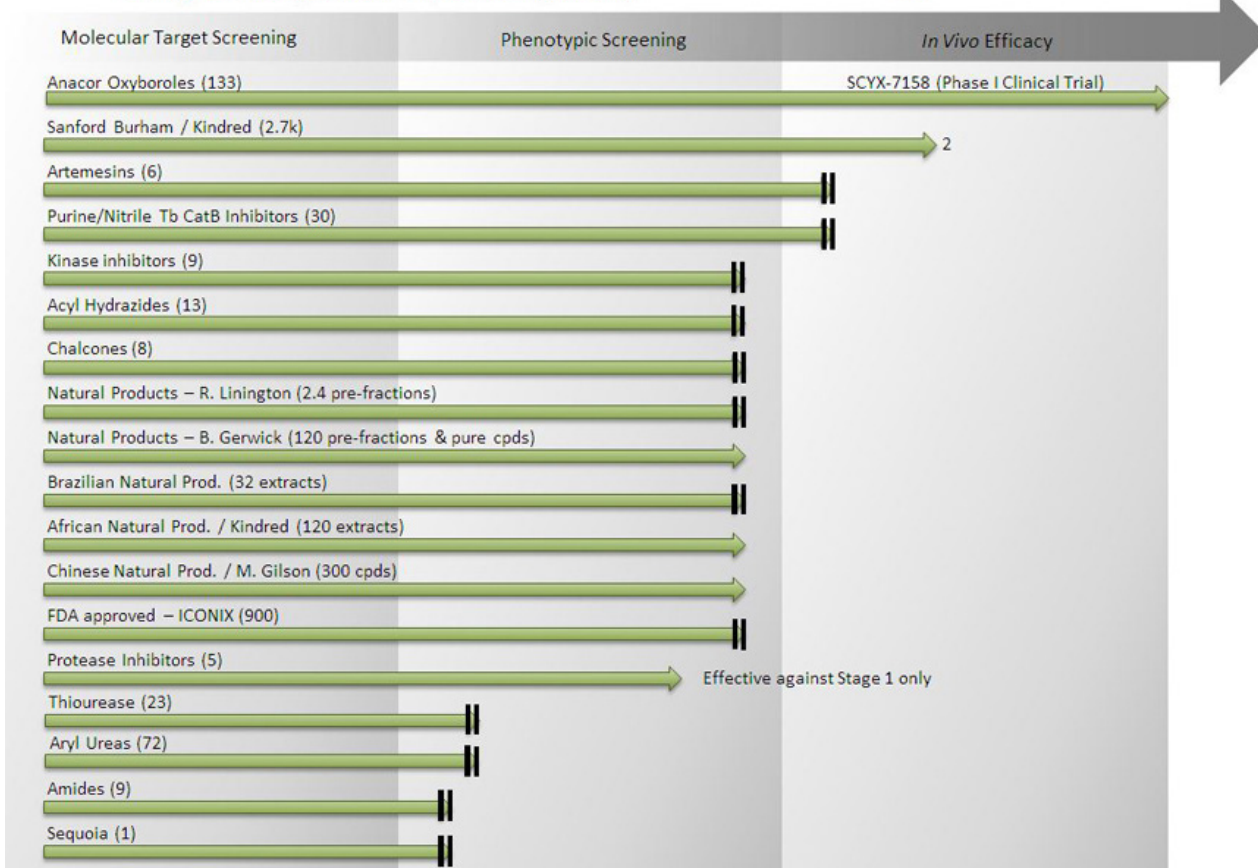


Figure 3: “Phenotypic Screening” refers to testing against the parasites themselves

Human African Trypanosomiasis Drug Development Pipeline (part 2)

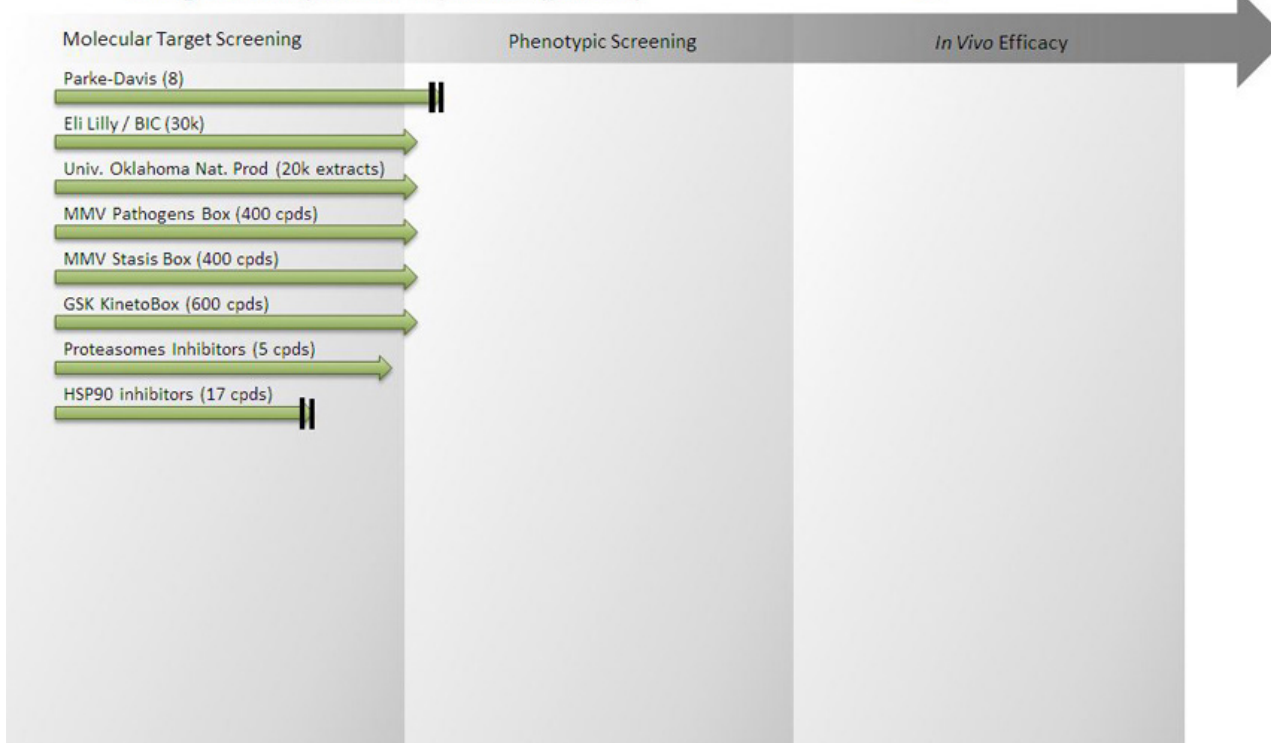


Figure 4: “Phenotypic Screening” refers to testing against the parasites themselves

Figures show numbers of compounds tested and how far they progressed for each of the three kinetoplastids assayed. Double bars indicate that a program was stopped because of lack of efficacy at that stage of screening or issues with ADME, toxicity, or pharmacokinetics.

Arrow heads indicate programs still in progress.

When available and relevant, Reference numbers are given in brackets []

Other promising phenotypic screen hits included compounds from Collaborative Drug Design [65] and the Memorial Sloan Kettering Cancer Center (manuscript in preparation).

Repurposed drugs

One potentially promising and cost effective approach to identifying drugs for NTDs is “repurposing” drugs already approved and in clinical use targeting other diseases (ncats.nih/preclinical/repurpose). Through the efforts of Teri Melese, a 900 compound library was donated in 2009 by ICONIX to James McKerrow. This library was screened [7,8] and later increased in size to include all FDA approved drugs with consultation by Michele Arkin of the UCSF SMDC (www.smdc.ucsf.edu), and support from the Bill and Melinda Gates Foundation. A second library of 2700 compounds, including some still in clinical trials, was recently obtained from the Prebys Sanford Burnham Institute. While a promising clinical candidate for amebiasis and filarid worm diseases was identified in these libraries, no promising drug candidate was identified for kinetoplastid diseases.

Marine Natural Products

One of the most promising sources of potential drugs is marine natural products provided by several laboratories. Natural products are the origin of 60% of drugs approved by the FDA. Most of the antibiotics now used for bacterial infections are natural products. But industry has until recently abandoned natural product development for reasons of complex synthesis challenges and scale-up issues. These issues have been addressed with new technology in HPLC, mass spectrometry, and genome sequencing. As Figures 1, 2, 3 and 4 indicate, several marine natural products are still being evaluated and represent some of the most effective “hits” to date [51,53]. In some cases the parasite molecular target of a specific marine natural product is now known [58,59].

Conclusion

Twenty five years of drugs screening has resulted in the incorporation of new HTS technology for assays involving both specific protein targets as well as kinetoplastid parasites themselves [1-70]. Molecular targets and natural products have yielded a higher hit rate than small synthetic molecule libraries. Many laboratories around the world are now carrying out similar screens with other molecular targets and other compound libraries. The bottleneck in drug development for NTDs is therefore “downstream” of screening. More medicinal chemistry help is needed for lead optimization and more compounds need to be pushed through the pre-clinical pipeline steps.

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