# Computational approaches for drug discovery against trypanosomatid caused diseases

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#### Abstract.

During three decades, only about 20 new drugs have been developed for malaria. tuberculosis and all neglected tropical diseases (NTDs). This critical situation was reached because NTDs represents only 10% of health research investments, however, they comprise about 90% of the global disease burden. Computational simulations applied in virtual screening (VS) strategies are very efficient tools to identify pharmacologically active compounds or new indications for drugs already administered for other diseases. One of the advantages of this approach is the low time-consuming and low-budget first stage, which filters for testing experimentally a group of candidate compounds with high chances of binding to the target and present trypanocidal activity. In this work, we review the most common VS strategies that have been used for the identification of new drugs with special emphasis on those applied to trypanosomiasis and leishmaniasis. Computational simulations based on the selected protein targets or their ligands are explained, including the method selection criteria, examples of successful VS campaigns applied to NTDs, a list of validated molecular targets for drug development, and repositioned drugs for trypanosomatid caused diseases. Thereby, here we present the state-of-the-art of VS and drug repurposing to conclude pointing out the future perspectives in the field.

#### 1. Trypanosomatid caused diseases and treatments.

Trypanosomatids are unicellular flagellate organisms, belonging to the clade Trypanosomatida, most of them pathogenic for other organisms including mammals, insects, and plants (Adl *et al.*, 2019; Marchese *et al.*, 2018; Menna-Barreto, 2019). Among trypanosomatids, two genera comprise known species pathogenic to humans: *Trypanosoma* and *Leishmania*. The first one includes two human-infecting species: *Trypanosoma cruzi*, causing the American trypanosomiasis or Chagas disease (Chagas, 1909) (<u>https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)</u>), and *Trypanosoma brucei*, the etiological agent of the human African trypanosomiasis (HAT) or sleeping sickness (Steverding, 2008) (<u>https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)</u>). The genus *Leishmania* includes more than 20 species causing a variety of human diseases generically known as leishmaniasis ((Maxfield & Crane, 2019) (<u>https://www.who.int/news-room/fact-sheets/detail/leishmaniasis</u>).

Trypanosomatids have a series of peculiarities concerning their cellular organization, control of gene expression, and metabolism (Marchese *et al.*, 2018). But, despite these unique characteristics offering a myriad of potential targets for drugs, most of the treatments for trypanosomatid caused diseases remain unsatisfactory, and even in those cases in which new alternatives have been developed, the emergence of resistant strains is foreseeable (Menna-Barreto, 2019).

Chagas disease affects approximately 8 million people, and an estimated 70 million at risk of contracting the infection (Perez-Molina & Molina, 2018). The disease presents two major phases: acute and chronic. The acute phase happens immediately after infection and is usually asymptomatic. In cases in which clinical symptoms manifest, they are mild and unspecific as presented in Table 1. The acute

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phase is characterized by a high parasitemia and the absence of humoral immune response (Bern, 2015; Perez-Molina & Molina, 2018). After the acute phase, which can last for up to two months, follows the chronic phase that lasts for the rest of their life. The chronic phase is characterized by the absence of evident parasitemia and a robust immune humoral response, and presents several clinical forms that can be divided in the indeterminate form, which is asymptomatic and accounts for approximately 70% of the patients; and the symptomatic forms, affecting the remaining 30% of the infected population (Perez-Molina & Molina, 2018; Rassi *et al.*, 2010). The chronic clinical features are mentioned in Table 1. The treatment for Chagas disease consists of only two drugs approved for human use half a century ago: benznidazole (1) and nifurtimox (2). Both drugs are efficient in the acute phase, but frequently fail in the chronic phase when most of the patients are diagnosed (Boscardin *et al.*, 2010; Hall *et al.*, 2011).

HAT is considered mostly under control (Bottieau & Clerinx, 2019), in the last two decades it has been observed a dramatic drop of nearly 85% in the number of reported new cases. However, it still threatens 65 million people living in endemic areas. HAT presents two stages: during the first (or early) stage the parasites proliferate in the blood and lymphatic system, causing mild and unspecific symptoms, as shown in Table 1. After a variable time, the parasites can cross the brain-blood barrier, reaching the central nervous system, initiating the second (or brain) stage. While the central nervous system infection progresses, neurological and psychological symptoms can be observed (Table 1) (Mogk *et al.*, 2017). If left untreated, sleeping sickness can cause death within several months or several years, depending on which *T. brucei* subspecies caused the infection (Buscher *et al.*, 2017). The treatment of stage one is mostly based on the administration of suramin

(3), the first-line drug, and upon failure pentamidine (4), the second-line treatment. Both are ineffective for stage two since they do not cross the blood-brain barrier. Thus, for the second stage of the disease, melarsoprol (5) has been used since the 1940s. It has the advantage of being useful for both *T. brucei* sub-species causing HAT, however, it is extremely toxic, and in some cases, it could be fatal. Eflornithine (6; difluoromethylornithine) is less toxic than melarsoprol (5), but is ineffective against *T. brucei rhodesiense*. More recently, eflornithine (6) was indicated to be used in combination with nifurtimox (2), which made the therapy more efficient (Babokhov *et al.*, 2013). Finally, fexinidazole (7) was approved for being distributed via World Health Organization (WHO) since 2019 in *T. brucei* endemic countries to treat HAT first and second stages when caused by the subspecies *T. brucei gambiense* (which is responsible for 98% of human reported cases) (Deeks, 2019; Mesu *et al.*, 2018).

Leishmaniasis constitutes a broad spectrum of diseases with different severity, ranging from self-cure skin lesions to visceral damage that can lead to death (Aronson *et al.*, 2017). The disease is endemic of at least 100 countries mostly located in the tropical and sub-tropical belt of the planet and it is estimated that 12 million people are affected. Three main forms of leishmaniasis can be recognized, depending on the *Leishmania* species involved in the infection: visceral (VL), cutaneous (CL) and mucocutaneous (ML) (Burza *et al.*, 2018). VL can be asymptomatic, however, when symptoms appear they can develop within 2 weeks and several years after the infection. If left untreated, VL can be fatal. CL is the most common form of leishmaniasis, consisting of exposed lesions of the skin or, in a small number of cases, sub-dermal diffuse papules. ML is much more aggressive than CL, usually causing partial or complete destruction of mucous tissues. CL and

ML have serious consequences due to severe disabilities, opportunistic infections, and social stigma, producing negative psychological effects. Table 1 shows the main clinical features of the three forms of leishmaniasis. The strategies to treat and manage leishmaniasis must take into account several factors such as parasite species, geographic location, and co-infections. Classically, the treatment of VL consists of two pentavalent antimonials: sodium stibogluconate (8; Sb(V)) or meglumine antimoniate (9). Their toxicity and the increasing emergence of resistance, led to the search for alternatives. For example in North Bihar, India, where VL caused by *L. donovani* is endemic, a widespread primary failure to Sb(V) has been reported and its use is not recommended anymore (Croft et al., 2006; Ponte-Sucre et al., 2017). In the last two decades, some drugs were launched to be used as single-treatment or in combination: an oral formulation of miltefosine (10), which constitutes now the first-line treatment in most of Asian endemic countries (Pinto-Martinez et al., 2018), and later an injectable formulation of paromomycin (11), followed by a liposomal formulation of amphotericin B (12) (Alves et al., 2018; Burza et al., 2018; van Griensven & Diro, 2019). Most of CL lesions are self-cured in a period between 2 and 18 months in immunocompetent patients. However, accelerating the cure is desirable to reduce the risk of dissemination or progression to ML. The treatments used can be local, such as intralesional injections of sodium stibogluconate (8), physical therapies like cryotherapy or thermotherapy, or topical application of agents such as paromomycin (11). Currently, a combination of locally applied antimonials and cryotherapy are considered the first-line treatment in Asia and African endemic countries (Aronson & Joya, 2019; Burza et al., 2018).

The precise mode of action of the drugs mentioned in this section are not determined, except for effornithine (6), that functions as an irreversible inhibitor of

ornithine decarboxylase (ODC), an enzyme involved in the polyamine biosynthesis (Wilkinson & Kelly, 2009).

Table 1 summarizes the clinical and epidemiological characteristics and the available drugs for the treatment of these trypanosomatid diseases.

The molecular structures of all the mentioned drugs are presented in Fig. 1.

#### 2. Molecular targets for trypanosomiasis and leishmaniasis drug development.

Traditionally, the way to identify drug targets relied exclusively in comparative biochemistry and genetics. The completion of the genome projects for humaninfecting trypanosomatids is a breakthrough that allows the identification of an increasing number of possible molecular targets, usually enzymes, proteins, or biochemical pathways. Strictly, there are three features an ideal target must satisfy: it has to be absent or strikingly different from it homolog in the mammalian hosts, being druggable, and essential for the parasite survival (Hughes et al., 2011; Wyatt et al., 2011). The first criterion denotes target selectivity in order to differentially direct to the parasite a given drug. With the availability of trypanosomatid genomes (Berna et al., 2018; Berriman et al., 2005; El-Sayed et al., 2005a; El-Sayed et al., 2005b) more accurate databases (https://tritrypdb.org/tritrypdb/; and https://www.genedb.org/) supporting the computational background with biochemical data, it should be straightforward to verify if a given gene product is absent from the mammalian counterpart, or the degree of divergence they present. But, in practice, a real target does not always meet selectivity, for example, the ODC which is a valid target against African trypanosomiasis. In these cases, selectivity should be provided by improving the affinity of the drug towards the parasite target (Kawasaki & Freire, 2011). The term druggability refers to the capacity of a given target to be affected by

a drug, in essence, the target must bind a molecule that modulates its activity (Abi Hussein et al., 2017). This information can be obtained during the preliminary stage of a drug discovery project by accessing accurate computational druggability prediction methods. The Special Programme for Research and Training in Tropical Diseases (TDR) has developed the TDR targets database, which is a very useful tool that facilitates the identification and prioritization of candidate drug targets for the "Tritryp" genomes among other pathogens (Magarinos et al., 2012) (http://tdrtargets.org/). Likewise, more recently, the Target-Pathogen database (Sosa et al., 2018) (http://target.sbg.qb.fcen.uba.ar/patho) was designed and developed as an online resource. This plaform has integrated and weighed protein information, such as structural properties including druggability and essentiality, one of the most important steps in the validation of a given target. Nowadyas many drug discovery programs consider the genetic validation a critical point because it reflects the loss of function attributable to therapeutic intervention. This implies made genes inoperative by knockout or knock-down procedures which are particularly effective in T. brucei. However, when working with T. cruzi and Leishmania, the situation is more critic because T. cruzi and most species of Leishmania do not possess the RNA interference (RNAi) and the traditional genetic knockouts in many cases they are not successful (Burle-Caldas Gde et al., 2015). Also, gene disruption experiments are mainly carried out in the insect stage of the parasite because their easy culture and manipulation (Barrett et al., 1999) and the results not always reflect the biological effect observed in other stages. To avoid target misestimation, the mutants should be also tested for their ability to progress through the life cycle and survive in vivo and in vitro. It is possible that the gene is lethal for the other life cycle-stages or generates a conditional lethal phenotype (Barrett et al., 1999). The mentioned

limitations have been recently evidenced by Jones *et al.*, who published an overview of the genetic assessments of suitable targets in *Leishmania* and *T. cruzi* (Jones *et al.*, 2018). Noteworthy, to date, 65 out of 200 knocked out genes in *Leishmania* are essential and only 16 out of 36 in *T. cruzi* (Jones *et al.*, 2018; Osorio-Mendez & Cevallos, 2018); whereas *T. brucei* has been widely subjected to high-throughput genetic screens covering the whole genome. CRISPR-Cas9 has become one of the most promising methodologies for the genetic validation of trypanosomatid targets (Lander *et al.*, 2016; Soares Medeiros *et al.*, 2017), and it is expected to make further valuable contributions to this field.

An alternative strategy to the genetic validation is the pharmacological validation, but, evidence of essentiality are preferred to be supported by both criteria (Field *et al.*, 2017; Gilbert, 2013).

In the evaluation of which targets are better, the fundamentals of metabolic control analysis and metabolic modeling offer new insights into target prioritization. This methodology allows studying the control of cellular metabolic pathways regardless of whether it is a two-step or multiple-steps pathway, showing that enzymes with the highest pathway control are the most convenient targets for therapeutic intervention. This idea is suppoted based on the fact that in any essential pathway, removing an enzyme by genetic manipulations would lead to the same essential phenotype, providing a number of potential drug targets equal or similar to that of the total components (Bakker *et al.*, 2000; Gonzalez-Chavez *et al.*, 2015; Hornberg *et al.*, 2007; Olin-Sandoval *et al.*, 2012). Therefore, this approach emphasizes the point that proving a gene is essential, specific and druggable is no guarantee that it encodes a valid drug target.

Several biochemical pathways that are common to pathogenic trypanosomes and exclusive to them are supposed to be the most promising for drug discovery, for example, mitochondrial metabolic pathways, sterol biosynthesis, the thiol-polyamine metabolism, and glycolysis, among others (Alberca *et al.*, 2016; Avilan *et al.*, 2011; Burri & Brun, 2003; Dietrich *et al.*, 2018; Khare *et al.*, 2015; Leroux & Krauth-Siegel, 2016; Lu *et al.*, 2013; Menzies *et al.*, 2018; Morillo *et al.*, 2017; Nowicki *et al.*, 2008; Reigada *et al.*, 2018; Reigada *et al.*, 2017; Sharlow *et al.*, 2010a; Sharlow *et al.*, 2010b; Torrie *et al.*, 2009; Urbina, 2015; Vazquez *et al.*, 2017). In Table 2 are summarized some targets regarding these pathways, some of them are introduced in section 5 of this manuscript.

Finally, in addition to finding a good target, when thinking in a possible therapy, the biological differences between parasite intracellular and extracellular stages inside the host should be considered. Contrary to *T. brucei*, which is only extracellular, *T. cruzi* and *Leishmania* spp. possess intracellular forms, so the *in vivo* accessibility of a drug is different for each of them. Drugs need to overcome additional barriers to meet its target such as host plasmatic membranes, parasitophorous vacuoles, host metabolism, among others. Drugs must be active in these different environment conditions.

### 3. Drug development for trypanosomatid caused diseases.

Along with the history of drug development for trypanosomatids related diseases, many strategies have been implemented. Through different programs, an initial chemotherapy arsenal to treat leishmaniasis and trypanosomiasis was introduced and remained unaltered for decades. Different approaches have been implemented to identify new drug candidates. Classical methods to find and optimize new chemical entities (NCE) have been based mainly by new compounds synthesis (*de novo* drug discovery) and bio-guided fractionation and isolation of natural products.

The first one has been included in many classical drug discovery pipelines, being a high-cost and usually very long and time-consuming approximation. The second provided NCE with either a known or a new scaffold, which can be structurally complex. To be able to use those compounds as a starting point in a drug discovery program, it is necessary to develop a complete synthetic route to perform a Structure-Activity Relationship (SAR) and preclinical studies. Alternatively, the complete biosynthetic pathway has to be elucidated to produce adequate amounts of a natural product, and the heterologous expression of the biosynthetic genes should be optimized (Luo *et al.*, 2015).

On the other hand, short-term approaches have been introduced to speed up the process of candidates' identification. One of those strategies involved drug combinations (Sun *et al.*, 2016) that has been explored for leishmaniasis and trypanosomiasis treatment. Those approaches were implemented looking to increase drug efficacy, shorten the treatments, and decrease the administered doses (Alcântara *et al.*, 2018; Nwaka *et al.*, 2009). Additionally, drug combination therapy is a well-established approximation to avoid resistance in pathogenic organisms, being a valuable approach that optimize the resources and know-how to produce improved therapies with better properties (Walvekar *et al.*, 2019). Tolerability can be also increased, because if the combined drugs can be administered below their individually prescribed dose limits, their side effects would be significantly reduced. One leading case example of that approach is the nifurtimox (2) - effornithine (6)

combination therapy (NECT), which can be safely used as first-line treatment for the second-stage of human African trypanosomiasis caused by *T. brucei gambiense* (Kansiime *et al.*, 2018; Priotto *et al.*, 2009).

Pharmaceutical companies have recently recovered their historical role in drug development against NTDs (Aerts *et al.*, 2017). Over the last decades, GlaxoSmithKline (GSK), Johnson & Johnson, Merck KGaA, MedPharm, Merck & Co, and Pfizer reassumed the leadership as drugs provision for NTDs. Those companies have donated billions of tablets to treat some NTDs in addition to direct procurement. Beyond those efforts, it is clear that there are not enough investments for NTDs yet from the pharmaceutical industries nor participation of non-governmental organizations (NGOs). The WHO is critical to make the medication available for the patients on the endemic regions (Hollingsworth, 2018), working with the public and private sectors, international agencies, and NGOs in order guarantee adequate free of charge medication for millions of people.

Recently, one new approach have been consolidated, the partnership of large pharmaceutical companies with non-profit organizations like the Drugs for Neglected Diseases initiative (DND*i*), Wellcome Trust, or the Academia. Those partnerships have been actively working on campaigns to characterize new NCE with leishmanicidal and trypanocidal activity. Such efforts included the screening of millions of compounds against TriTryps parasites. In particular, partnerships with GSK and Novartis (Khare *et al.*, 2016). GSK Tres Cantos has also integrated a collaborative research network for more than a decade with the Drug Discovery Unit (DDU, University of Dundee) and Wellcome Trust to discover new candidate drugs for VL and Chagas disease ((Wyllie *et al.*, 2019). Thanks to that endeavors, many hit compounds have been identified. In general, the approach involves the screening of

druglike libraries against the etiological agents of these diseases to identify compounds that kill the parasites. This approach provides compounds able to cross the cell membranes and kill the parasite within the parasitophorous vacuole. One logical and important disadvantage is that usually this approach is set to be very stringent; providing few bioactive compounds per campaign. Another obvious disadvantage is that the molecular targets have to be elucidated, having to specifically design strategies to identify them.

There are some successful examples of new structures that have been identified from phenotypic screening of big libraries on *Leishmania* parasites. One of them is the identification of GNF6702 by Novartis (Khare *et al.*, 2016) and another is the "Leish- Box" of inhibitors by GSK (Lamotte *et al.*, 2019), just to mention a few.

It is also important to understand an experimental compound's mode of action as this can enable an assessment of the likelihood of resistance mechanisms evolving in the parasite. Strategies of target deconvolution are therefore required to identify the molecular target of a hit compound obtained by phenotypic screening. The usual approach involves a combination of genetic and/or metabolomic approaches or pull-down experiments, that afterward must be genetically validated. That is a long and laborious process, even with today's advances such as CRISPR/Cas9 (Beneke *et al.*, 2017; Duncan *et al.*, 2017).

An alternative strategy to the very costly, time-consuming, and usually very inefficient phenotypic screening campaigns is the target-based drug discovery approach, which is the most commonly used in the pharmaceutical industry. This strategy has also been applied to drug discovery against trypanosomatid caused diseases in Academia. In this approach, a validated protein target is selected, requiring a well-developed biochemical or biophysical assay that can be used to

identify inhibitors. Big pharmaceutical companies and some well-equipped academic institutions have performed high-throughput screening (HTS) campaigns looking for new hits. Those hits should eventually go through a hit-to-lead process where they are chemically optimized to improve their properties in terms of potency, selectivity, and bioavailability.

Over the last decades, the knowledge on the trypanosomatids biochemistry has allowed the identification of many putative drug targets that can potentially provide the validated hits for drug development. Nevertheless, only a few of them have been extensively explored.

Thiol-polyamine metabolism of trypanosomatids was one of the first examples of enzymes used as target-based drug discovery. The most studied enzyme on that matter is trypanothione reductase. Since the early reports of the activity, substrate specificity and kinetics of *T. cruzi* trypanothione reductase in the late '80s ((Krauth-Siegel *et al.*, 1987) the activity of hundreds of compounds have been reported on the enzyme (Tiwari *et al.*, 2018).

Recently, a high throughput screening campaign to find new inhibitors of *T. brucei* tryparedoxin peroxidase have been reported (Fueller *et al.*, 2012). On that work, nearly 80,000 compounds were analyzed, with only 32 displaying activity. Further studies revealed that the compounds not only targeted the enzyme *in vitro* but also in the intact parasite, validating the target. Trypanothione synthetase is another enzyme of thiol-polyamine metabolism that has been explored. Benítez and co-workers have studied the potential of that target by assaying 144 compounds, mostly obtained by chemical synthesis and some natural products (Benitez *et al.*, 2016). Different inhibitors have been found, being paulone derivatives the most promising

scaffold, nevertheless, some 5-Substituted 3-chlorokenpaullone derivatives were offtarget (Orban *et al.*, 2016).

An article reported by Prof. Gelb in 2003 highlighted the potential of protein farnesyl and N-myristoyl transferases as piggy-back medicinal chemistry targets for the development of anti-trypanosomatids (Gelb *et al.*, 2003). Those enzymes that produce the co- and post-translational protein modification were studied for drug development in other eukaryotic systems, in particular mainly looking for new anticancer agents. The studies on protein farnesyltransferase as target of screening libraries against the parasitic enzyme did not produce any interesting compounds to develop new medications.

CYP51 (sterol 14α-demethylase cytochrome P450) has been proposed as a possible target for antikinetoplastids drug discovery. That enzyme is the target of azole drugs in clinical practice. In general, the activity of antifungal drugs is often different on the parasitic orthologs, requiring the optimization of existing structures or introducing new chemical entities to achieve the required selectivity. Many different structures have been prepared and assayed *in vitro* against parasitic CYP51. Those differences require the optimization of existing structures or the introduction of new chemical entities that were more potent and selective. Between those structures, there are substrate analogs, mostly sterol derivatives, indomethacin amides,(Konkle *et al.*, 2009) imidazoles modified from a collection of vitamin D hydroxylase inhibitors. Interesting examples are imidazolyl benzamides (called VNI) that have been through a hit-to-lead optimization process, (Friggeri *et al.*, 2018; Lepesheva *et al.*, 2007) that have been able to cure acute and chronic forms of Chagas disease in mice models (Villalta *et al.*, 2013). Other examples are 4-aminopyridyl derivatives (Calvet *et al.*, 2017; Choi *et al.*, 2013) and the tipifarnib modified structures (Kraus *et al.*, 2010).

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Another enzyme that has been usefully used on target-directed antikinetoplastids drug discovery is the N-myristoyl transferase (NMT). This enzyme has been genetically and experimentally validated in *Leishmania* spp. Once its essentiality on the parasite biology was established, *in vitro* HTS of a diverse subset of the Pfizer corporate collection against *Ld*NMT, *Plasmodium falciparum* NMT, and the two human isoforms (*Hs*NMT) led to the discovery of new and potent inhibitors (Bell *et al.*, 2012). The compounds were subsequently resynthesized and validated leading to a *compound 43* that is a potent and neutral NMT inhibitor and a promising candidate for antileishmanial drug development (Hutton *et al.*, 2014).

An initiative led by the Novartis Institute for Tropical Diseases screened 3 million compounds in proliferation assays on *L. donovani*, *T. cruzi*, and *T. brucei*. That campaign provided GNF5343, that was later optimized preparing nearly 3000 new analogs that led to GNF6702, a compound 400-fold more active in intra-macrophage *L. donovani*. Later, the parasite proteasome was identified by different strategies as the target of the lead compound. GNF6702 shown to be able to eradicate parasites in mouse disease models (Khare *et al.*, 2016). Besides the tremendous work behind that report, there is a remarkable example of wide-spectrum antikinetoplastid drug development.

Despite the extensive work and the profound improvement on the drug discovery and development process over the last decades, there are many gaps in the process and only a few targets have been progressed to preclinical development. The involvement of pharmaceutical companies has improved the process and the budget, but there are still financial and material resources limitations. Consequently, the approaches of drug repurposing and the inclusion of computational resources in the analysis of the ever-growing amount of biochemical and genetic data appear as a logical and convenient approach to optimize the process.

## 4. An overview of the computational/Virtual Screening techniques.

Similarly to HTS, a virtual screening (VS) employs computer-generated models to search in libraries of small molecules those with chances of binding a molecular target, commonly, but not restricted to, an enzyme or receptor (Rester, 2008).

Computer-aided drug discovery is hugely advantageous; allowing to test bigger compound libraries at negligible costs. Molecules that are not yet synthesized to expand the chemical space can be also added (Rodriguez *et al.*, 2016) without to prepare compounds that most likely will not have the desired biological activity (Gasteiger, 2015; Schneider, 2010).

When using digital means in the search of bioactive molecules the options and strategies are plentiful (Haga *et al.*, 2016), and the factors to take into account when deciding which ones to employ and how to combine them are addressed below.

## 4.1. The starting point.

The first step before planning a VS workflow should always be performing an extensive bibliographical research about the target that one is trying to find drugs for (Gimeno *et al.*, 2019); aspects as, for example, its biological function, availability of techniques to measure its activity, natural ligands, known inhibitors, catalytic mechanism, structure, known homologues and their ligands.

While the results of the literature review will determine what kind of computational tools can be used, every strategy shares the need of a compound library to screen. The confection of the screening library will greatly depend on the specific goals of the VS. There are different small molecule databases available for virtual screening.

The ChEMBL (https://www.ebi.ac.uk/chembl/) (Gaulton et al., 2017), PubChem (https://pubchem.ncbi.nlm.nih.gov/) 2019). ZINC (Kim et al., and (https://zinc.docking.org/) (Sterling & Irwin, 2015) are databases with hundreds of millions of compounds and useful search tools. The SWEETLEAD (https://simtk.org/projects/sweetlead) (Novick et al., 2013), and the DrugBank (https://www.drugbank.ca/) (Wishart et al., 2018) databases contain drugs approved for human administration. Also, some compounds vendors, like Enamine (https://enamine.net/) and Asinex (http://www.asinex.com/) offer screening libraries of their products.

As the goal of a VS strategy is finding molecules to test against a molecular target, it is wise to filter out compounds that could give false positives in the binding assays. These compounds, known as Pan-Assay Interference Compounds (PAINs) (Dahlin *et al.*, 2015), can give false results by reacting non-specifically with the target, with several other targets, or interfering with the measurement assays (Baell & Walters, 2014). Some chemical groups are shared by many known PAINs, which make it possible to previously remove any molecule containing said groups (Baell & Holloway, 2010).

An estimated 50% of the tested drug candidates fail because of inefficiencies in Absorption, Distribution, Metabolism, Excretion, and/or Toxicity (ADME/Tox) (Li, 2001). Based on the physical chemical characteristics of known drugs, Lipinski *et al.* developed the "rule of five" for orally available drugs (H-bond donors  $\leq$  5, H-bond acceptors  $\leq$  10, molecular weight  $\leq$  500 Da, logP  $\leq$  5) (Lipinski *et al.*, 2001). There are computational tools that predict ADME/Tox characteristics, but many of them rely on the Lipinski's rules, excluding administration routes other than oral (Scior *et al.*,

2012), they also have a low predictive performance on more complex properties, *e.g* carginogenesis (Stouch *et al.*, 2003).

In this sense, at the stage of filtering the screening library one could take into account the pharmacokinetic characteristics of the compounds to be screened, and three scenarios are possible (Oprea, 2002). Some strategies focus on first obtaining high-affinity lead compounds that later would be optimized for good pharmacokinetic properties, modifications to achieve better ADME/Tox could be detrimental to the target binding, leading to a trial-error optimization that consume time and resources. Another scenario is filtering compounds before the screening, in an attempt to obtain lead compounds with good ADME/Tox properties and later optimize the potency, which can reduce ADME characteristics, but would be a less consuming process towards optimal structures. A third and highly recommended strategy is to simultaneously follow changes that increase affinity and ADME/Tox characteristics (Drews, 1998).

Known ligands of a target can be the starting point in a VS campaing. Also, using when experimental data of the molecular structure of the target or a homolog a receptor-based approximation can be performed (Ghemtio *et al.*, 2012).

#### 4.2. Ligand-based virtual screening.

Johnson and Maggiora (Johnson *et al.*, 1990) introduced the concept that similar molecules exhibit similar behaviors, an assumption extended to their biological activity. Based on this principle, if there is knowledge of compounds with the desired effect, finding molecules similar to them is a reasonable starting point in the search of new drugs. However, "similarity" is a tricky concept, to determine if two or more

compounds are similar, different characteristics, methods of comparison, and metrics that allow such contrasts can be used.

To compare molecules for ligand-based VS, the first step is representing them in numerical terms. To this end, there are different mathematical models to denote different measurable properties of compounds in ways that are usable, these models are called molecular descriptors (Todeschini *et al.*, 2009). Descriptors used in VS can be classified as one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D), depending on the molecules information about that they represent. As not all descriptors correlate with the biological activity of the molecule, the selection of the descriptors and the methods used to compare them is crucial.

Because the ligand binding to its receptor will depend in great extent on the spatial interactions that can occur between them, 3D descriptors are considered a more reliable choice (Danishuddin & Khan, 2016; Mavridis *et al.*, 2007) that, when thoroughly used, enhances the chances of finding structurally diverse candidates (Brown & Jacoby, 2006). However, a molecule can have many 3D configurations, and comparing spatial data is more complex than comparing 2D descriptors, which translates in greater computational costs (Mavridis *et al.*, 2007).

On the other hand, 2D descriptors consume less computational resources, maintaining good performance, but missing key characteristics involved in the interaction (Fradera & Babaoglu, 2017).

**Fingerprint similarity** methods rely on the abstraction of molecular properties into bit sequences, where the bit value (0 or 1) at each position of the sequence represents the absence or presence of a particular descriptor in a molecule (Banegas-Luna *et al.*, 2018). The sequences can be compared at each position to obtain a metric on how similar are the compounds, given the compared descriptors.

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Many comparison algorithms exist, being the Tanimoto coefficient one of the most popular (Bajusz *et al.*, 2015). Regardless of the comparison metric selected, the results can be sorted from more to less similar to the known ligands. At this point comes the thorny choice of where to apply the cut-off after which compounds will be discarded, as there is no universal value for it, so the selectivity/sensitivity trade-off needs to be carefully determined from the retrieval of known actives and inactives (Fradera & Babaoglu, 2017). In addition, because similarity coefficients assign to all the compared bits an equal relevance, compounds similar in the bits important for the biological activity can end down in the list for not sharing enough of the non-important characteristics, and *vice versa* (Scior *et al.*, 2012).

**Machine learning** algorithms permit computer-aided drug discovery take a step further, by stop relying on explicit physical representations of what is needed for an expected biological activity, and allow the use of complex pattern recognition algorithms to construct mathematical models that take into account many molecular descriptors at the same time, as well as exploring bigger datasets with low computational costs (Lo *et al.*, 2018). These methods rely on databases of known active and inactive compounds, so the algorithms try to find a set of molecular descriptors that correlate with the desired activity, assigning a level of importance to each of them, and producing a model able to predict the activity of new compounds (Gimeno *et al.*, 2019).

As the algorithm will try and find any patterns, the initial or "training" dataset of active and inactive compounds is extremely important. When the training library is too small or with poor structural diversity, the produced model might be based in chance correlation or be biased towards similar characteristics not determining the biological activity (Ma *et al.*, 2009; Scior *et al.*, 2012), so it is preferred to count with a large and structurally diverse input set. As the amount of inactive compounds will always be greater than the actives (Schierz, 2009), it is important to balance both sets to avoid the production of a model biased towards the correct identification of inactive compounds, but being sub-optimal in the discrimination of true actives. Whenever possible, it is advisable to choose those inactive compounds that are structurally similar to the actives, so the model will have more chances of discriminate between them (Tropsha, 2010).

**Ligand-based pharmacophores** are ensembles of spatial and electrostatic features shared between a set of known active molecules. These models are used later to search for other candidates containing such features, assuming they are responsible for the interaction with the receptor (Gimeno *et al.*, 2019). In this way, the compounds retrieved can be structurally richer, as the matched features can be contained by a wider range of structures.

Though pharmacophore models can be constructed from one or a few ligands, it is always better to use large sets of known actives (Scior *et al.*, 2012) in order to identify which features seem to be critical to the binding, as well as finding as many important features as possible, that may not be shared by every ligand.

# 4.3. Receptor-based virtual screening.

Using known ligands of the target macromolecule to find hit compounds is very fast and computationally inexpensive. Nevertheless, for NTDs such as those caused by trypanosomatids, the amount of information available regarding experimentally demonstrated ligands of interesting targets can be scarce or even non-existing. Additionally, ligand-based methods tend to narrow the chemical space by retrieving only molecules similar to the known ligands, leaving out potentially good and structurally diverse hits. Although similar molecules tend to have similar activities, this is not necessarily true, as some of the chemical groups that make the hit different from the known ligands might be detrimental to the ligand-receptor interaction in what is known as activity cliff (Stumpfe & Bajorath, 2012).

Analysing the molecular target allows to discard hits that would be incompatible with the binding site, and allows finding structurally novel hits capable of fitting in and interacting with a given pocket in the receptor. Structural information of some targets can be find in the Protein Data Bank (PDB, <u>https://www.rcsb.org/</u>) (Berman *et al.*, 2000), a database containing experimentally determined protein structures, or in the ModBase (<u>https://modbase.compbio.ucsf.edu/</u>) (Pieper *et al.*, 2014), a database of comparative protein structure models.

A strong drawback of receptor-based techniques is that in many cases the target 3D structure has not been experimentally determined, especially in the case of trypanosomatids. This problem can be circumvented if there is structural data of molecules similar enough to the target to build a homology model.

A general rule of thumb is selecting the protein template with the highest sequence identity with the receptor of interest, particularly on the target pocket, and sequence identities lower than 30% will produce significantly less reliable models (Fiser, 2010). The quality of homology models must be assessed before using them in a Receptor-based VS, and while there are many ways of evaluating the quality of a homology model (Bhattacharya *et al.*, 2008; DasGupta *et al.*, 2015; di Luccio & Koehl, 2012; Eramian *et al.*, 2006; Shen & Sali, 2006), and most of the modelling tools include scores for quality assessment, it is important to know their capabilities and limits. Many assessment tools are biased towards the more known structures and may fail with proteins less represented in the databases, as is the case for membrane proteins (Benkert *et al.*, 2011; di Luccio & Koehl, 2012).

**Molecular Docking** is the receptor-based technique most extensively used in VS (Forli *et al.*, 2016; Sousa *et al.*, 2013) to predict if and how a library of ligands would interact with a receptor. It relies on randomly changing the spatial conformation of the ligand and calculating how well the generated poses would interact with the receptor, assigning an interaction score to each. This results in a set of conformations that scored as the most likely to represent the real binding mode. When a compound library is uded, it is possible to rank those molecules according to their biding scores, and obtaining their possible modes of interaction with the receptor.

While molecular docking algorithms give potential ways of a receptor-ligand interaction, it is not definitive proof of the mode of binding, or that there is binding at all. Thus the docked poses should be treated more as hypotheses to test experimentally. In fact, for some receptors and docking algorithm mode of interaction with known ligands might not be reproduced (Chaput *et al.*, 2016), for that reason it is extremely important, when possible, to validate whether the docking algorithm is capable of reproducing experimental results before using it to predict interactions with new compounds. The two most used methods of validation are re-docking, when the co-crystalized ligand is removed from the protein and docked to test whether the produced pose is the same as in the crystal, and cross-docking, when different co-crystalized ligands are docked with the receptor (Jain, 2009).

One of the most important drawbacks of molecular docking is the treatment of the receptor as a rigid molecule, so compounds that would otherwise bind to a pocket that is different -or not present- in the rigid receptor will be wrongly targeted as non-binders. To manage the flexibility of the receptor (B-Rao *et al.*, 2009), an option would be using all known conformations of the receptor. Some algorithms make it

possible to allow some degree of overlapping between the ligand and the receptor, treating some key residues of the target as flexible, and even perform induced fit models (Xu & Lill, 2013); all of them requiring additional computational costs.

**Structure-based pharmacophore** can also be obtained from the receptor as a set of spatial features capable of interacting with the residues on the binding site. This can be done directly by analyzing the electrostatic distribution on the pocket, or by doing molecular docking of small fragments with varying molecular nature to probe the pocket and finding which features are more probable to interact with different parts of the binding site.

A very interesting strategy, although computationally expensive, is to perform molecular dynamic (MD) simulations of the receptor embedded in organic/aqueous mixed solvents containing different molecular features to find which areas of the receptor had more interactions with such solvents (Defelipe *et al.*, 2018). This approach results in a spatial distribution of preferential interactions in the protein surface while taking into account the flexibility of the protein in a span of time.

Fig. 2 shows a graph on how to choose among the different VS strategies, as well as their advantages and disadvantages.

## 4.4. Combined virtual screening strategies

Combinations of different techniques in a single VS workflow are strongly advised, taking advantage of their different strengths while minimizing the downsides they would have when used separately (Talevi *et al.*, 2009). The size of the compound library being used is another factor to take into account at the moment of designing a VS pipeline; as for bigger libraries it is preferable to start using methods computationally less expensive that allow discarding a huge volume of compounds and data. For smaller libraries or later steps in the workflow it is plausible to use

techniques that employ more computing power but also give more information about the possible mode of interaction that then can be used to bias the VS into finding compounds with such characteristics. As an example, receptor- and ligand-based pharmacophores can be used to adjust docking protocols to prefer the kind of interactions found in the model in what is known as "biased" or "guided" docking (Hu & Lill, 2014), which increases the performance of the docking algorithms.

Computational approaches are of great value in the drug discovery for trypanosomatid-caused diseases, by reducing the testable chemical space to a handful of promising compounds with high chances of having the desired biological activity, and by allowing to better exploit the growing information about the biology of these parasites. The available informatic tools are plentiful, whether the molecular target and its structure is known, or if there is a set of compounds interacting with a specific target in ways that might or might not be known; and combination of diverse tools is always the best choice to draw on their advantages while reducing their short-comes. The use of these approaches is not restricted to the search of active compounds, as the produced models can be harnessed to better understand the chemical characteristics of the ligand-target interaction. Finally, we must always keep in mind that these models have no value until they are experimentally tested, and feedback from the bench is critical for their betterment.

## 5. VS applied to trypanosomatid caused diseases.

During the last decade, there was a significant increase in the number of scientific publications about different VS techniques applied to the identification of drugs candidates for the treatment of NTDs. Probably one of the reasons for this emergent

trend is the power of VS techniques to select active compounds rapidly and with an accessible cost for any laboratory (Bellera *et al.*, 2019).

Many international organizations, such as DND*i* (Chatelain & loset, 2011), recommends repurposing drugs for the treatment of NTDs in order to reduce the economic cost and the time of implementation of new therapeutic alternatives. In this sense, one of the main approaches for drug repositioning is through the application of computer simulations or VS. These techniques can use libraries of approved drugs to find a molecule with the desired biological activity. Most common approaches usually include a first *in silico* step based on individual or combined VS campaign followed by *in vitro* enzymatic or cell viability assays (Kontoyianni, 2017). To illustrate the capabilities of VS, a few examples applied to drug discovery in NTDs will be detailed below.

Reigada *et al.* performed a VS strategy to repurpose drugs to inhibit the *T. cruzi* polyamine transporter *Tc*PAT12.The authors used the Tanimoto coefficient in LiSiCA v1.0 to search by 2D molecular similarity among 2924 compounds approved by the Food and Drug Administration (FDA) for its use in humans, employing retinol acetate as the reference since this molecule has been reported to decrease the intracellular polyamine concentration in *Leishmania*. A set of 7 retinoids of dermatological use was identified and subsequently used in molecular docking. Among these compounds, isotretinoin, a drug used to treat severe acne, obtained the lowest docking score (-10.78 kcal/mol), which was in the range of the reference molecule (10.02 kcal/mol) and three times higher than the scores obtained for its natural ligands, spermidine and putrescine. Because of this, isotretinoin was tested *in vitro*, inhibiting the polyamine transport in the parasite and showing a strong trypanocidal effect at nanomolar concentrations (Reigada *et al.*, 2017) (Fig. 3A).

Another approach to identify inhibitors of the same *T. cruzi* polyamine transporter, involved an anthracene-putrescine conjugate (Ant4) that blocks polyamine uptake in cancer cells. Ant4 was also found to inhibit the polyamine transport system in *T. cruzi* and produced a strong trypanocidal effect. Considering that Ant4 is not currently approved by the FDA, a similarity ligand-based VS using this compound as reference molecule was applied. Three tricyclic antipsychotic drugs, promazine, chlorpromazine and clomipramine, showed to be effective inhibitors of putrescine uptake, and also revealed a high trypanocidal activity against *T. cruzi* amastigotes and trypomastigotes with calculated IC<sub>50</sub>s between 1.3  $\mu$ M and 3.8  $\mu$ M (Reigada *et al.*, 2019)

These are interesting examples for trypanosomatid-caused diseases when little information about the target and its binding molecules is available. In the ligand-based approach it shows the capabilities of similarity search to find active molecules with high potency starting from a single compound, even when the antecedents are in another organism, a significant advantage in the case of these insufficiently studied organisms, but since similarity search highly depends on the input set, it is worthy to note the small quantity of retrieved compounds. It also highlights a potential problem that should be taken into account; if the molecular target is too similar to a human homolog it could bind to it as well, reason why the differences between the parasites and the host are a key aspect to observe in the drug search.

Regarding the receptor-based methods, membrane proteins are more readily accessible for drugs but less structural information about them is available. At the time of publication there was no crystal structure for a polyamine transporter in Tritryps , and the most related protein deposited in the PDB was an *E. coli* amino acid transporter (AdiC), with an identity of 30% with *Tc*PAT12, considered the lower

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limit in the production of a reasonable homology model. Having in mind the previously mentioned bias of the quality assessment towards soluble proteins, the authors had to rely only on a Ramachandran plot to check the produced model was worthy of using in docking assays. Nevertheless, the molecular docking worked on predicting the binding of isotretinoin that was later determined experimentally in the same work. An important thing to have in mind is that docking scores by themselves are not a good indicator of whether a ligand will be a good binder or not, as they do not represent actual binding energies, working only to rank the complementarity of a ligand inside a pocket and hinting to which molecules might be better ligands than the others, reason why the authors use retinoic acetate, spermidine, and putrescine as a reference for what a good score might be for this particular case.

Using the same protein target, Dietrich *et al.* identified other anti-T. *cruzi* polyamine transport inhibitor, cisapride, a drug withdrawn for human treatments currently used in veterinary medicine to stimulate the upper gastrointestinal tract. The authors screened the ZINC and DrugBank databases employing similarity search, quantitative structure-activity relationship (QSAR) models, and molecular docking based screening (Dietrich *et al.*, 2018).

For the similarity search they used six compounds that disrupted the putrescine uptake in *T. cruzi.* Two different cut-off values were employed, for the DrugBank database, comprising 8,261 molecules, those with a Tanimoto coefficient <0.5 were filtered out, while for the ZINC database, due to its greater size (17,900,742 compounds) they set a more stringent cut-off of 0.7, showing how its selection depends entirely on the researchers criteria about the desired quantity and structural diversity of the retrieved molecules. Because of the limitation of this strategy to find few compounds because of the quantity of input molecules, they complement the

strategy with a QSAR model designed to find polyamine analogs with trypanocidal activity in micromolar concentrations, whether or not their molecular target was known. Employing both strategies they find 594 candidates for further filtering by molecular docking.

The authors used the natural ligands and reported inhibitors of the transporter as reference molecules, and as negative controls a set of amino acids that do not bind to it. They compared Autodock 4.2 and Autodock Vina docking softwares, evaluate the performance of different scoring functions. Additionally, they performed a set of evaluations with rigid receptors, and other sets allowing flexibility on different residues determined by docking or mutagenesis to be involved in the binding of the natural ligands. Although Autodock Vina is reported to have better predictive power than Autodock 4.2 (Gaillard, 2018), in this case, the former ranked the inactive compounds higher than the natural ligands. From all the tested docking conditions, the rigid model with Autodock 4.2 performed the best on discriminating non-binders. The example shows the importance of testing various scoring functions and docking parameters, as their performance is specific to each receptor-ligand system.

By using a set of active and inactive compounds, the researchers could build a Receiving Operating Characteristic (ROC) curve to determine the score cut-off with the better trade-off between specificity and sensibility for the receptor-based filtering. Applying the mentioned model, 203 molecules were classified as possible binders; the top 10% were thoroughly analyzed for their physicochemical properties, structural diversity, and purchasability, leading to 4 compounds of which only cisapride inhibited the putrescine uptake *in vitro*. This illustrates how a richer input dataset can lead to better predictive models capable of process larger libraries and retrieve active compounds.

Recently, it was demonstrated that crystal violet, a colorant used as additive in blood banks to prevent transfusion-transmitted Chagas disease, inhibits the *T. cruzi* proline permease TcAAAP069. Using crystal violet as query for a drug repurposing ligand-based VS, loratadine, cyproheptadine, olanzapine and clofazimine were identified as structurally related compounds. All these already-approved drugs for clinical use inhibited TcAAAP069 activity with different efficacies, presented trypanocidal action in epimastigotes, trypomastigotes and amastigotes of different *T. cruzi* strains and also presented a synergistic effect in combination with benznidazole (Saye *et al.*, 2020)

Regarding the above mentioned examples, some properties of membrane transporters as targets for drug development are outlined in Fig. 3B.

Other approaches using molecular descriptors and QSAR models were applied to find natural products that inhibit the *de novo* pyrimidine biosynthetic pathway, specifically the enzyme dihydroorotate dehydrogenase (DHODH) from *L. major (Chibli et al., 2018)*. Similarly, inhibitors of the enzyme that reduce trypanothione were identified by linear discriminant analysis using molecular descriptors (Prieto *et al.,* 2006).

For *T. brucei*, the only fully validated molecular target is the protein ODC (*i.e.* its disruption is the known target of current clinic treatment for the disease) (Gilbert, 2014), in this sense, great efforts have been made in order to identify other compounds capable of inhibit its enzymatic activity. In a compelling example (Smithson *et al.*, 2010) the authors started from a commercially available library of compounds and used chemoinformatic tools to filter out potential PAINs, select molecules with good ADME properties, and generate clusters containing up to 20 compounds with maximum structural diversity. The generated clusters (with a total of

316,000 compounds) were used for high throughput screening against T. brucei and human ODC. They found a novel chemotype comprising 8 tested compounds that was a potent and selective inhibitor of the parasite ODC. Because both active sites have a high identity, the authors found unlikely that these compounds would be binding to the active site considering the high observed selectivity. Therefore, they used informatics tools to identify three other possible binding pockets for these inhibitors, followed by rigid docking simulations with the found active compounds and inactive chemical analogs in the identified pockets as well as the active site. The models predicted that only one of the pockets would bind better to the actives compared with the enzyme active site, also, the dockings in the same pocket yielded a better discrimination between actives and inactives. To evaluate the role of the predicted binding residues, the authors analyzed the differences in the predicted pocket between the human and parasite, and performed mutagenesis experiments, both analysis further supported their hypothesis. This is a fascinating example of how the feedback loop between computational models and the experimental results lead to a better understanding of the studied molecular systems.

Two promising drug targets for the treatment of HAT are the enzymes pteridine reductase and the N-acetyl-glucosaminyl-phosphatidylinositol deacetylase (GlcNAc-PI de-N-acetylase), involved in the essential pterin metabolism and GPI anchor biosynthesis of membrane proteins, respectively. Different chemical determinants of the *T. brucei* pteridine reductase activity were identified by pharmacophore mapping and subsequently used to database screening to find potential nanomolar range inhibitors (Dube *et al.*, 2014). A very similar approach was applied to discovery GlcNAc-PI de-N-acetylase inhibitors and two approved drugs were repositioned; the antibiotic ethambutol and the vasoconstrictor metaraminol (Rashmi & Swati, 2015).

A combined VS campaign was designed to find specific inhibitors of the L. donovani y-glutamylcysteine synthetase (Gcs), an enzyme of the trypanothione-based redox system. The receptor-based steps include the homology modeling of the enzyme structure and active site prediction. Using a database of 55,000 commercially available compounds obeying the Lipinski's rules (http://www.maybridge.com/), the authors used molecular docking with three different scoring functions and retrieved 5 compounds ranked by the three functions as better than L-buthionine-S, Rsulfoximine (BSO), a Gcs inhibitor that prolongs the survival in T. brucei mice infections but induces toxicity in the host. The predicted poses were evaluated by molecular dynamics using GROMACS (James Abraham, 2015). These simulations confirmed the stability of the predicted binding modes, allowing the authors better assess the residues important for the binding of these compounds, and to identify other residues in the active site that could be exploited in lead optimization to increase the binding affinity. However, including the docking pose of BSO would be a great addition to the work, as it would work as a positive control of the model and throw some light on what molecular determinants should be retained if an optimization of its toxicity would be carried in the future. The five ligands were successfully validated in vitro, four compounds had better enzymatic inhibition than BSO, dissociation constants comparable to it, and leishmanicidal activity, three of them having negligible toxicity in human cell lines (Agnihotri et al., 2017). These results are a clear example of following in parallel the binding affinity and the ADME/Tox properties, and how information obtained from the predicted models could be of use for further lead optimization.

Another example of combined ligand- and structure-based VS strategy employing similarity VS, molecular docking, and molecular dynamics was applied to found

putative *T. cruzi* enolase (TcENO) inhibitors. The enzyme substrates and two known enolase inhibitors were used as queries for the similarity VS using five different algorithms, resulting in six compounds of medical use (etidronate, pamidronate, fosfomycin, acetohydroxamate, triclofos, and aminohydroxybutyrate). Molecular docking simulations and pose re-scoring predicted that etidronate and pamidronate were the best candidates. Finally, using molecular dynamics calculations it was proposed that etidronate is the best potential TcENO inhibitor and described the molecular motifs to be taken into account in the repurposing or design of drugs targeting this enzyme active site (Valera-Vera *et al.*, 2020).

A novel approach based on the combination of proteomics and VS was used to identify potential drug targets to treat leishmaniasis. First, by proteome mining new drug targets essential for the parasite and with low identity to human homologs were detected. One of these proteins related to the N-glycan biosynthesis pathway, and a putative inhibitor, miglitol, were predicted *in silico* and validated *in vitro* (Chavez-Fumagalli *et al.*, 2019).

An important point that can be remarked from the previous examples of VS strategies is the need of sources of structural variability in the databases screened to increase the chances of finding a compound with the appropriate biological properties. In this sense, the databases of approved drugs used for the drug repositioning have only about 3,000 drugs. A widely used alternative are databases of small molecules either of natural or synthetic compounds that have > 100,000 structures to find lead compounds for further optimization, always reminding that compounds obtained by VS must be tested *in vitro* and *in vivo*, and that following the evolution of potency and ADME/Tox through the drug development is highly recommended. Although the different VS tools can be combined in diverse ways to

increase the efficiency in retrieved active compounds, the probability of success with this approach is completely uncertain until biological assays are performed on the protein, the target organism, and infection models, results that can in turn be used to the improve the predictive models. For the specific case of trypanosomatid-caused diseases there are, as yet, no treatments obtained from a VS strategy. Nevertheless, the enrichment in active molecules obtained from computational tools and the growing amount of information about potential targets and compounds binding to them make the discovery and development of chemotherapies against these parasites a more approachable task.

Additional examples of VS techniques applied to trypanosomiasis and leishmaniasis are listed in supplementary Table 1.

# 6. Drug repurposing, an advantageous alternative to new drugs in NTDs.

During the period 2016–2018, 130 new chemical entities and 78 drug line extensions, which are products based on a previously approved molecule, were approved and launched to global markets. That is almost 40% of the new treatments in the last years corresponds to new indications, new combinations or new formulations for already marketed drugs (Graul *et al.*, 2017; Graul *et al.*, 2019; Graul *et al.*, 2018). As previously mentioned, finding new indications for approved, withdrawn, abandoned or investigational drugs is called drug repurposing or drug repositioning, and in this section, we will present some advantages of this drug discovery strategy and also will provide examples of repositioned drugs to treat human pathologies, including trypanosomatid caused diseases.

The classic drug development approach usually takes between 10 to 17 years from target identification to be available in the market. All these years also imply a rough

investment of 0.8-2.3 billion dollars (DiMasi et al., 2016), and even then, drugs can fail and never get to the pharmacy. The main reasons for this failure are that the drugs are not as effective in humans as predicted by the preclinical assays, and/or that they are not safe for human administration. Drug repurposing can accelerate the time needed for a drug to reach the market and reduced the financial costs mainly because the preclinical and clinical assays can take advantage of the available safety, toxicity, and pharmacokinetics and pharmacodynamics data. This approach can take between 3 to 12 years and diminish the cost around 40% of the traditional development (Ashburn & Thor, 2004; Chong & Sullivan, 2007) (Drug Repurposing Workshop and Repositioning: Summary; https://www.nap.edu/read/18731/chapter/1). The potential repositioned compound can be identified through serendipity or rational approaches, including computational strategies, biological experimental strategies, or a combination of both (Xue et al., 2018). One of the most recognized examples of a successful repurposing story involves sildenafil which was first developed as an antihypertensive drug and then repurposed for the treatment of erectile dysfunction and pulmonary arterial hypertension (Ghofrani et al., 2006). Another example is the drug thalidomide that was originally developed for treating morning sickness and was withdrawn from the

market because of its teratogenic effects. However, this compound is now used to treat erythema nodosum leprosum and it is also employed in combination with dexamethasone for the treatment of newly diagnosed multiple myeloma (Gupta *et al.*, 2013; Singhal *et al.*, 1999; Zhou *et al.*, 2013). Inspired by these cases and many other success stories of repositioned drugs, several studies are underway to identifying new biological activities for existing drugs (Czech *et al.*, 2019; Ferreira & Andricopulo, 2016; Novac, 2013).

NTDs, like Chagas disease, HAT, and leishmaniasis, are usually associated with underdeveloped countries and poverty. Thus, big pharmaceutical companies are not generally interested in the development and production of treatments for these diseases because it is unlikely for them to recover the investment and even less probable to make a profit. In this regard, the drug repurposing approach turns out very appealing since the costs of the drug discovery process are greatly reduced.

Eflornithine (6), a polyamine synthesis inhibitor, constitutes a remarkable case of drug repositioning in trypanosomatid caused diseases since it was initially evaluated as an antitumor agent, but the clinical studies were discontinued due to adverse effects (Abeloff et al., 1986; Meyskens et al., 1986). However, in the late 1980s, eflornithine (6) was licensed as an orphan drug for treating HAT (Burri & Brun, 2003). Another example involves nifurtimox (2), used for Chagas diseases, which has been combined with eflornithine (6) for first-line treatment of second-stage T. brucei gambiense HAT (Priotto et al., 2009). Another drug tested was fexinidazole (7), which had been in preclinical development in the 1970s-1980s as a broad-spectrum antimicrobial agent (Raether & Seidenath, 1983). The molecule is a DNA synthesis inhibitor rediscovered by the DNDi in 2005 as having activity against African trypanosomes (Deeks, 2019). The DNDi, in collaboration with Sanofi, have demonstrated that fexinidazole (7) represents the first well-tolerated singlecompound oral therapy against first and second stage of HAT due to T. brucei gambiense (Deeks, 2019). The drug is currently undergoing Phase III clinical trials (https://www.dndi.org/diseasesfor treating this disease projects/portfolio/fexinidazole/).

Despite no drugs have been successfully repurposed for its use against Chagas disease yet, fexinidazole (7) also represents an important advance for drug

discovery in this parasitic disease. Its activity was investigated in vivo on several T. cruzi strains (susceptible, resistant or partially resistant to the current treatment benznidazole (1)) and it has been demonstrated its efficacy in suppressing parasitemia and preventing death in infected mice (Bahia et al., 2012). Another study revealed that fexinidazole (7) is more effective at curing chronic than acute T. cruzi infections in a similar mouse model (Francisco et al., 2016). This drug is currently being evaluated in clinical trials as а treatment for Chagas disease (https://www.dndi.org/diseases-projects/portfolio/fexinidazole-chagas/).

Most of the drugs that are active against leishmaniasis were repurposed from other indications. For instance, amphotericin B (12) was introduced as an antifungal agent obtained from Streptomyces nodosus. In 1997, liposomal amphotericin B (AmBisome) was the first drug approved for the treatment of visceral leishmaniasis (Meyerhoff, 1999). It binds to ergosterol, the predominant sterol in Leishmania (Roberts et al., 2003). Paromomycin (11), an aminoglycoside antibiotic, was isolated in the 1950s from Streptomyces krestomuceticus and it is active against bacteria as well as some protozoa and cestodes (Davidson et al., 2009). The antileishmanial activities of paromomycin (11) were recognized in the 1960s and it is used as an alternative treatment of both visceral and cutaneous leishmaniasis (Jain & Jain, 2013). One mechanism of action of paromomycin (11) involves inhibition of cytoplasmic and mitochondrial protein synthesis (Jhingran et al., 2009). The antileishmanial drug miltefosine (10), an alkylphosphocholine, was originally developed for the treatment of cutaneous cancers but was discontinued for this indication due to its adverse effects (Dorlo et al., 2012). Miltefosine (10) has reemerged as the only effective oral drug available to treat all of the clinical forms of leishmaniasis, however, it is limited by its relatively high cost and side effects

(Ortega *et al.*, 2017; Sindermann *et al.*, 2004). On the other hand, fexinidazole (7) was also effective in *L. donovani* infected mice, however, clinical trials in patients with visceral leishmaniasis have been discontinued due to lack of efficacy (Wyllie *et al.*, 2012) (<u>https://clinicaltrials.gov/ct2/show/NCT01980199</u>).

Many more cases are emerging for developing treatments for neglected diseases through drug repurposing. The computational strategies mentioned previously are becoming an important part of this process. They can be used for identifying potential repositioning candidates systematically and are an excellent complement to experimental techniques (Delavan *et al.*, 2018). These *in silico* approaches contribute to speed up the process of drug discovery at little extra cost (Ekins *et al.*, 2011). For example, VS methods offer a quick assessment of huge libraries compiling known drugs and reduce the number of compounds that need testing to discover novel treatments (Kontoyianni, 2017). This computer-aided strategy has been signaled as a relevant strategy to aid find new medications for neglected diseases (Ekins *et al.*, 2011; Pollastri & Campbell, 2011; Sardana *et al.*, 2011). Examples of drugs repositioned against trypanosomatid caused diseases are listed in Table 5.

# 7. Concluding remarks.

.Since the development of new drugs for neglected diseases is a hard task due to the low investment of resources and the lack of economic interest from most pharmaceutical companies, the use of VS techniques for drug repurposing is a good option. The advantages of this experimental approach are the low time-consuming first stage generating a group of candidate compounds for further testing *in vitro* and *in vivo*. In addition, working with drugs already approved for other diseases shortens the subsequent trials and the funds needed for implementing a new therapy against NTDs.

These applications are accessible to any laboratory since a large number of free open source software are available and in most cases can be used with standard personal computers. In addition, drugs used for other pathologies have available information about, for example, their toxicity, pharmacokinetics, pharmacodynamics, bioavailability, and half-life.

.In addition, in drug repurposing approaches side effects are not necessary exclusion factors since the main goal is the development of new therapeutics against deadly diseases and most of the current treatments for NTDs are not safe for the patients.

.There are numerous successful examples of drug development, involving virtual screening techniques, for the treatment of different diseases. Some examples are isoniazid (DrugBank ID: DB00951) approved as tuberculostatic, amprenavir (DrugBank ID: DB00701) approved for the treatment of HIV or flurbiprofen (DrugBank ID: DB00712) approved as non-steroidal anti-inflammatory agent with antipyretic and analgesic activity (Batool *et al.*, 2019). However, in the case of trypanosomiasis, these strategies were recently applied to the identification of new drugs and it will still be necessary to wait a few years to evaluate the first results in clinical trials.

.Finally, we encourage research groups that work with drug targets to try the VS techniques described in this review. As a very important initial tip we consider that most suitable and reliable approach is the use of a combined strategy. However, there are no predetermined schemes to establish the order or the techniques to use, they exclusively depend on each particular case.

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**Conflicts of Interest** 

None

**Ethical Standards** 

Not applicable

#### References.

- Abeloff, M. D., Rosen, S. T., Luk, G. D., Baylin, S. B., Zeltzman, M. and Sjoerdsma, A. (1986). Phase II trials of alpha-difluoromethylornithine, an inhibitor of polyamine synthesis, in advanced small cell lung cancer and colon cancer. *Cancer Treat Rep*, **70**, 843-845.
- Abi Hussein, H., Geneix, C., Petitjean, M., Borrel, A., Flatters, D. and Camproux, A. C. (2017). Global vision of druggability issues: applications and perspectives. *Drug Discov Today*, 22, 404-415. doi: S1359-6446(16)30449-4 [pii]

10.1016/j.drudis.2016.11.021.

- Adl, S. M., Bass, D., Lane, C. E., Lukes, J., Schoch, C. L., Smirnov, A., Agatha, S., Berney, C., Brown, M. W., Burki, F., Cardenas, P., Cepicka, I., Chistyakova, L., Del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A. A., Hoppenrath, M., James, T. Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D. J. G., Lara, E., Le Gall, L., Lynn, D. H., Mann, D. G., Massana, R., Mitchell, E. A. D., Morrow, C., Park, J. S., Pawlowski, J. W., Powell, M. J., Richter, D. J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F. W., Torruella, G., Youssef, N., Zlatogursky, V. and Zhang, Q. (2019). Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. J Eukaryot Microbiol, 66, 4-119. doi: 10.1111/jeu.12691.
- Aerts, C., Sunyoto, T., Tediosi, F. and Sicuri, E. (2017). Are public-private partnerships the solution to tackle neglected tropical diseases ? A systematic review of the literature. *Health policy*, **121**, 745-754. doi: 10.1016/j.healthpol.2017.05.005.
- Agnihotri, P., Mishra, A. K., Mishra, S., Sirohi, V. K., Sahasrabuddhe, A. A. and Pratap, J. V. (2017). Identification of Novel Inhibitors of Leishmania donovani gamma-Glutamylcysteine Synthetase Using Structure-Based Virtual Screening, Docking, Molecular Dynamics Simulation, and in Vitro Studies. J Chem Inf Model, 57, 815-825. doi: 10.1021/acs.jcim.6b00642.
- Aidas, K., Angeli, C., Bak, K. L., Bakken, V., Bast, R., Boman, L., Christiansen, O., Cimiraglia, R., Coriani, S., Dahle, P., Dalskov, E. K., Ekstrom, U., Enevoldsen, T., Eriksen, J. J., Ettenhuber, P., Fernandez, B., Ferrighi, L., Fliegl, H., Frediani, L., Hald, K., Halkier, A., Hattig, C., Heiberg, H., Helgaker, T., Hennum, A. C., Hettema, H., Hjertenaes, E., Host, S., Hoyvik, I. M., Iozzi, M. F., Jansik, B., Jensen, H. J., Jonsson, D., Jorgensen, P., Kauczor, J., Kirpekar, S., Kjaergaard, T., Klopper, W., Knecht, S., Kobayashi, R., Koch, H., Kongsted, J., Krapp, A., Kristensen, K., Ligabue, A., Lutnaes, O. B., Melo, J. I., Mikkelsen, K. V., Myhre, R. H., Neiss, C., Nielsen, C. B., Norman, P., Olsen, J., Olsen, J. M., Osted, A., Packer, M. J., Pawlowski, F., Pedersen, T. B., Provasi, P. F., Reine, S., Rinkevicius, Z., Ruden, T. A., Ruud, K., Rybkin, V. V., Salek, P., Samson, C. C., de Meras, A. S., Saue, T., Sauer, S. P., Schimmelpfennig, B., Sneskov, K., Steindal, A. H., Sylvester-Hvid, K. O., Taylor, P. R., Teale, A. M., Tellgren, E. I., Tew, D. P., Thorvaldsen, A. J., Thogersen, L., Vahtras, O., Watson, M. A., Wilson, D. J., Ziolkowski, M. and Agren, H. (2014). The Dalton quantum chemistry program system. *Wiley Interdiscip Rev Comput Mol Sci*, 4, 269-284. doi: 10.1002/wcms.1172.
- Alberca, L. N., Sbaraglini, M. L., Balcazar, D., Fraccaroli, L., Carrillo, C., Medeiros, A., Benitez, D., Comini, M. and Talevi, A. (2016). Discovery of novel polyamine analogs with anti-protozoal activity by computer guided drug repositioning. *J Comput Aided Mol Des*, **30**, 305-321. doi: 10.1007/s10822-016-9903-6
- 10.1007/s10822-016-9903-6 [pii].
- Alberca, L. N., Sbaraglini, M. L., Morales, J. F., Dietrich, R., Ruiz, M. D., Pino Martinez, A. M., Miranda, C. G., Fraccaroli, L., Alba Soto, C. D., Carrillo, C., Palestro, P. H. and Talevi, A. (2018). Cascade Ligand- and Structure-Based Virtual Screening to Identify New Trypanocidal Compounds Inhibiting Putrescine Uptake. Front Cell Infect Microbiol, 8, 173. doi: 10.3389/fcimb.2018.00173.
- Alcântara, L. M., Ferreira, T. C. S., Gadelha, F. R. and Miguel, D. C. (2018). Challenges in drug discovery targeting TriTryp diseases with an emphasis on leishmaniasis. *International Journal for Parasitology: Drugs and Drug Resistance*, **8**, 430-439. doi: 10.1016/j.ijpddr.2018.09.006.
- Alves, F., Bilbe, G., Blesson, S., Goyal, V., Monnerat, S., Mowbray, C., Muthoni Ouattara, G., Pecoul, B., Rijal, S., Rode, J., Solomos, A., Strub-Wourgaft, N., Wasunna, M., Wells, S., Zijlstra, E. E., Arana, B. and Alvar, J. (2018). Recent Development of Visceral Leishmaniasis Treatments: Successes, Pitfalls, and Perspectives. *Clin Microbiol Rev*, **31**. doi: e00048-18 [pii]

10.1128/CMR.00048-18

31/4/e00048-18 [pii].

- Allen, W. J., Balius, T. E., Mukherjee, S., Brozell, S. R., Moustakas, D. T., Lang, P. T., Case, D. A., Kuntz, I. D. and Rizzo, R. C. (2015). DOCK 6: Impact of new features and current docking performance. J Comput Chem, 36, 1132-1156. doi: 10.1002/jcc.23905.
- Aronson, N., Herwaldt, B. L., Libman, M., Pearson, R., Lopez-Velez, R., Weina, P., Carvalho, E., Ephros, M., Jeronimo, S. and Magill, A. (2017). Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg, 96, 24-45. doi: 10.4269/ajtmh.16-84256.
- Aronson, N. E. and Joya, C. A. (2019). Cutaneous Leishmaniasis: Updates in Diagnosis and Management. *Infect Dis Clin North Am*, **33**, 101-117. doi: S0891-5520(18)30091-6 [pii]

- Ashburn, T. T. and Thor, K. B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov*, **3**, 673-683. doi: 10.1038/nrd1468
- nrd1468 [pii].
- Avilan, L., Gualdron-Lopez, M., Quinones, W., Gonzalez-Gonzalez, L., Hannaert, V., Michels, P. A. and Concepcion, J. L. (2011). Enolase: a key player in the metabolism and a probable virulence factor of trypanosomatid parasites-perspectives for its use as a therapeutic target. *Enzyme Res*, 2011, 932549. doi: 10.4061/2011/932549.
- B-Rao, C., Subramanian, J. and Sharma, S. D. (2009). Managing protein flexibility in docking and its applications. *Drug Discov Today*, 14, 394-400. doi: 10.1016/j.drudis.2009.01.003

- Babokhov, P., Sanyaolu, A. O., Oyibo, W. A., Fagbenro-Beyioku, A. F. and Iriemenam, N. C. (2013). A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. *Pathog Glob Health*, **107**, 242-252. doi: 10.1179/2047773213Y.0000000105.
- Baell, J. and Walters, M. A. (2014). Chemistry: Chemical con artists foil drug discovery. Nature, 513, 481-483. doi: 10.1038/513481a
- 513481a [pii].
- Baell, J. B. and Holloway, G. A. (2010). New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem, 53, 2719-2740. doi: 10.1021/jm901137j.
- Bahia, M. T., de Andrade, I. M., Martins, T. A., do Nascimento, A. F., Diniz Lde, F., Caldas, I. S., Talvani, A., Trunz, B. B., Torreele, E. and Ribeiro, I. (2012). Fexinidazole: a potential new drug candidate for Chagas disease. *PLoS Negl Trop Dis*, 6, e1870. doi: 10.1371/journal.pntd.0001870

- Bajusz, D., Racz, A. and Heberger, K. (2015). Why is Tanimoto index an appropriate choice for fingerprintbased similarity calculations? *J Cheminform*, **7**, 20. doi: 10.1186/s13321-015-0069-3
- 69 [pii].
- Bakker, B. M., Westerhoff, H. V., Opperdoes, F. R. and Michels, P. A. (2000). Metabolic control analysis of glycolysis in trypanosomes as an approach to improve selectivity and effectiveness of drugs. *Mol Biochem Parasitol*, **106**, 1-10. doi: S0166-6851(99)00197-8 [pii].
- Banegas-Luna, A. J., Ceron-Carrasco, J. P. and Perez-Sanchez, H. (2018). A review of ligand-based virtual screening web tools and screening algorithms in large molecular databases in the age of big data. *Future Med Chem*, **10**, 2641-2658. doi: 10.4155/fmc-2018-0076.
- Barrett, M. P., Mottram, J. C. and Coombs, G. H. (1999). Recent advances in identifying and validating drug targets in trypanosomes and leishmanias. *Trends Microbiol*, 7, 82-88. doi: S0966-842X(98)01433-4 [pii].
- Batool, M., Ahmad, B. and Choi, S. (2019). A Structure-Based Drug Discovery Paradigm. Int J Mol Sci, 20. doi: E2783 [pii]

10.3390/ijms20112783

ijms20112783 [pii].

 Bell, A. S., Mills, J. E., Williams, G. P., Brannigan, J. A., Wilkinson, A. J., Parkinson, T., Leatherbarrow, R. J., Tate, E. W., Holder, A. A. and Smith, D. F. (2012). Selective inhibitors of protozoan protein Nmyristoyltransferases as starting points for tropical disease medicinal chemistry programs. *PLoS Negl Trop Dis*, 6, e1625. doi: 10.1371/journal.pntd.0001625

Bellera, C. L., Balcazar, D. E., Alberca, L., Labriola, C. A., Talevi, A. and Carrillo, C. (2013). Application of computer-aided drug repurposing in the search of new cruzipain inhibitors: discovery of amiodarone and bromocriptine inhibitory effects. *J Chem Inf Model*, **53**, 2402-2408. doi: 10.1021/ci400284v.

<sup>10.1016/</sup>j.idc.2018.10.004.

S1359-6446(09)00006-3 [pii].

PNTD-D-12-00357 [pii].

PNTD-D-11-01228 [pii].

- Bellera, C. L., Balcazar, D. E., Alberca, L., Labriola, C. A., Talevi, A. and Carrillo, C. (2014). Identification of levothyroxine antichagasic activity through computer-aided drug repurposing. *ScientificWorldJournal*, 2014, 279618. doi: 10.1155/2014/279618.
- Belllera, C. L., Sbaraglini, M. L., Alberca, L. N., Alice, J. I. and Talevi, A. (2019). In Silico Modeling of FDA-Approved Drugs for Discovery of Therapies Against Neglected Diseases: A Drug Repurposing Approach. In *In Silico Drug Design: Repurposing Techniques and Methodologies* (ed. Roy, K.), pp. 625-648. Academic Press.
- Beneke, T., Madden, R., Makin, L., Valli, J., Sunter, J. and Gluenz, E. (2017). A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. *R Soc Open Sci*, 4, 170095. doi: 10.1098/rsos.170095 rsos170095 [pii].
- Benitez, D., Medeiros, A., Fiestas, L., Panozzo-Zenere, E. A., Maiwald, F., Prousis, K. C., Roussaki, M., Calogeropoulou, T., Detsi, A., Jaeger, T., Sarlauskas, J., Peterlin Masic, L., Kunick, C., Labadie, G. R., Flohe, L. and Comini, M. A. (2016). Identification of Novel Chemical Scaffolds Inhibiting Trypanothione Synthetase from Pathogenic Trypanosomatids. *PLoS Negl Trop Dis*, 10, e0004617. doi: 10.1371/journal.pntd.0004617

- Benkert, P., Biasini, M. and Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, 27, 343-350. doi: 10.1093/bioinformatics/btq662 btq662 [pii].
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P.
   E. (2000). The Protein Data Bank. *Nucleic Acids Res*, 28, 235-242. doi: gkd090 [pii]
- 10.1093/nar/28.1.235.
- Bern, C. (2015). Chagas' Disease. N Engl J Med, 373, 456-466. doi: 10.1056/NEJMra1410150.
- Berna, L., Rodriguez, M., Chiribao, M. L., Parodi-Talice, A., Pita, S., Rijo, G., Alvarez-Valin, F. and Robello, C. (2018). Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi. *Microb Genom*, 4. doi: 10.1099/mgen.0.000177.
- Berneman, A., Montout, L., Goyard, S., Chamond, N., Cosson, A., d'Archivio, S., Gouault, N., Uriac, P., Blondel, A. and Minoprio, P. (2013). Combined approaches for drug design points the way to novel proline racemase inhibitor candidates to fight Chagas' disease. *PLoS One,* 8, e60955. doi: 10.1371/journal.pone.0060955
- PONE-D-12-31299 [pii].
- Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renauld, H., Bartholomeu, D. C., Lennard, N. J., Caler, E., Hamlin, N. E., Haas, B., Bohme, U., Hannick, L., Aslett, M. A., Shallom, J., Marcello, L., Hou, L., Wickstead, B., Alsmark, U. C., Arrowsmith, C., Atkin, R. J., Barron, A. J., Bringaud, F., Brooks, K., Carrington, M., Cherevach, I., Chillingworth, T. J., Churcher, C., Clark, L. N., Corton, C. H., Cronin, A., Davies, R. M., Doggett, J., Djikeng, A., Feldblyum, T., Field, M. C., Fraser, A., Goodhead, I., Hance, Z., Harper, D., Harris, B. R., Hauser, H., Hostetler, J., Ivens, A., Jagels, K., Johnson, D., Johnson, J., Jones, K., Kerhornou, A. X., Koo, H., Larke, N., Landfear, S., Larkin, C., Leech, V., Line, A., Lord, A., Macleod, A., Mooney, P. J., Moule, S., Martin, D. M., Morgan, G. W., Mungall, K., Norbertczak, H., Ormond, D., Pai, G., Peacock, C. S., Peterson, J., Quail, M. A., Rabbinowitsch, E., Rajandream, M. A., Reitter, C., Salzberg, S. L., Sanders, M., Schobel, S., Sharp, S., Simmonds, M., Simpson, A. J., Tallon, L., Turner, C. M., Tait, A., Tivey, A. R., Van Aken, S., Walker, D., Wanless, D., Wang, S., White, B., White, O., Whitehead, S., Woodward, J., Wortman, J., Adams, M. D., Embley, T. M., Gull, K., Ullu, E., Barry, J. D., Fairlamb, A. H., Opperdoes, F., Barrell, B. G., Donelson, J. E., Hall, N., Fraser, C. M., Melville, S. E. and El-Sayed, N. M. (2005). The genome of the African trypanosome Trypanosoma brucei. *Science*, 309, 416-422. doi: 309/5733/416 [pii]

10.1126/science.1112642.

Bhattacharya, A., Wunderlich, Z., Monleon, D., Tejero, R. and Montelione, G. T. (2008). Assessing model accuracy using the homology modeling automatically software. *Proteins*, **70**, 105-118. doi: 10.1002/prot.21466.

Boscardin, S. B., Torrecilhas, A. C., Manarin, R., Revelli, S., Rey, E. G., Tonelli, R. R. and Silber, A. M. (2010). Chagas' disease: an update on immune mechanisms and therapeutic strategies. *J Cell Mol Med*, **14**, 1373-1384. doi: 10.1111/j.1582-4934.2010.01007.x

JCMM1007 [pii].

Bottieau, E. and Clerinx, J. (2019). Human African Trypanosomiasis: Progress and Stagnation. *Infect Dis Clin North Am*, **33**, 61-77. doi: S0891-5520(18)30090-4 [pii]

PNTD-D-15-01732 [pii].

BIOVIA, D. S. (2017).

10.1016/j.idc.2018.10.003.

- Brown, N. and Jacoby, E. (2006). On scaffolds and hopping in medicinal chemistry. *Mini Rev Med Chem*, 6, 1217-1229. doi: 10.2174/138955706778742768.
- Burle-Caldas Gde, A., Grazielle-Silva, V., Laibida, L. A., DaRocha, W. D. and Teixeira, S. M. (2015). Expanding the tool box for genetic manipulation of Trypanosoma cruzi. *Mol Biochem Parasitol*, 203, 25-33. doi: 10.1016/j.molbiopara.2015.10.004
- S0166-6851(15)30035-9 [pii].
- Burri, C. and Brun, R. (2003). Eflornithine for the treatment of human African trypanosomiasis. *Parasitol Res,* 90 Supp 1, S49-52. doi: 10.1007/s00436-002-0766-5.
- Burza, S., Croft, S. L. and Boelaert, M. (2018). Leishmaniasis. *Lancet*, **392**, 951-970. doi: S0140-6736(18)31204-2 [pii]
- 10.1016/S0140-6736(18)31204-2.
- Buscher, P., Cecchi, G., Jamonneau, V. and Priotto, G. (2017). Human African trypanosomiasis. *Lancet*, **390**, 2397-2409. doi: S0140-6736(17)31510-6 [pii]
- 10.1016/S0140-6736(17)31510-6.
- Calvet, C. M., Choi, J. Y., Thomas, D., Suzuki, B., Hirata, K., Lostracco-Johnson, S., de Mesquita, L. B., Nogueira, A., Meuser-Batista, M., Silva, T. A., Siqueira-Neto, J. L., Roush, W. R., de Souza Pereira, M. C., McKerrow, J. H. and Podust, L. M. (2017). 4-aminopyridyl-based lead compounds targeting CYP51 prevent spontaneous parasite relapse in a chronic model and improve cardiac pathology in an acute model of Trypanosoma cruzi infection. *PLoS Negl Trop Dis*, **11**, e0006132. doi: 10.1371/journal.pntd.0006132

- Croft, S. L., Sundar, S. and Fairlamb, A. H. (2006). Drug resistance in leishmaniasis. *Clin Microbiol Rev*, **19**, 111-126. doi: 19/1/111 [pii]
- 10.1128/CMR.19.1.111-126.2006.
- Czech, T., Lalani, R. and Oyewumi, M. O. (2019). Delivery Systems as Vital Tools in Drug Repurposing. AAPS PharmSciTech, 20, 116. doi: 10.1208/s12249-019-1333-z
- 10.1208/s12249-019-1333-z [pii].
- **Chagas, C.** (1909). Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. *Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem, 1. doi: 1678-8060.*
- Chaput, L., Martinez-Sanz, J., Saettel, N. and Mouawad, L. (2016). Benchmark of four popular virtual screening programs: construction of the active/decoy dataset remains a major determinant of measured performance. J Cheminform, 8, 56. doi: 10.1186/s13321-016-0167-x

167 [pii].

- Chatelain, E. and Ioset, J. R. (2011). Drug discovery and development for neglected diseases: the DNDi model. *Drug Des Devel Ther*, **5**, 175-181. doi: 10.2147/DDDT.S16381.
- Chavez-Fumagalli, M. A., Lage, D. P., Tavares, G. S. V., Mendonca, D. V. C., Dias, D. S., Ribeiro, P. A. F., Ludolf, F., Costa, L. E., Coelho, V. T. S. and Coelho, E. A. F. (2019). In silico Leishmania proteome mining applied to identify drug target potential to be used to treat against visceral and tegumentary leishmaniasis. J Mol Graph Model, 87, 89-97. doi: S1093-3263(18)30590-4 [pii]

- Cheleski, J., Rocha, J. R., Pinheiro, M. P., Wiggers, H. J., da Silva, A. B., Nonato, M. C. and Montanari, C. A. (2010). Novel insights for dihydroorotate dehydrogenase class 1A inhibitors discovery. *Eur J Med Chem*, **45**, 5899-5909. doi: 10.1016/j.ejmech.2010.09.055
- S0223-5234(10)00704-X [pii].
- Chibli, L. A., Schmidt, T. J., Nonato, M. C., Calil, F. A. and Da Costa, F. B. (2018). Natural products as inhibitors of Leishmania major dihydroorotate dehydrogenase. *Eur J Med Chem*, **157**, 852-866. doi: S0223-5234(18)30701-3 [pii]

Choi, J. Y., Calvet, C. M., Gunatilleke, S. S., Ruiz, C., Cameron, M. D., McKerrow, J. H., Podust, L. M. and Roush, W. R. (2013). Rational development of 4-aminopyridyl-based inhibitors targeting Trypanosoma cruzi CYP51 as anti-chagas agents. J Med Chem, 56, 7651-7668. doi: 10.1021/jm401067s.

**Chong, C. R. and Sullivan, D. J., Jr.** (2007). New uses for old drugs. *Nature*, **448**, 645-646. doi: 448645a [pii] 10.1038/448645a.

PNTD-D-17-01639 [pii].

<sup>10.1016/</sup>j.jmgm.2018.11.014.

<sup>10.1016/</sup>j.ejmech.2018.08.033.

- D.A. Case, I. Y. B.-S., S.R. Brozell, D.S. Cerutti, T.E. Cheatham, III, V.W.D. Cruzeiro, T.A. Darden, R.E. Duke, D. Ghoreishi, M.K. Gilson, H. Gohlke, A.W. Goetz, D. Greene, R Harris, N. Homeyer, Y. Huang, S. Izadi, A. Kovalenko, T. Kurtzman, T.S. Lee, S. LeGrand, P. Li, C. Lin, J. Liu, T. Luchko, R. Luo, D.J. Mermelstein, K.M. Merz, Y. Miao, G. Monard, C. Nguyen, H. Nguyen, I. Omelyan, A. Onufriev, F. Pan, R. Qi, D.R. Roe, A. Roitberg, C. Sagui, S. Schott-Verdugo, J. Shen, C.L. Simmerling, J. Smith, R. SalomonFerrer, J. Swails, R.C. Walker, J. Wang, H. Wei, R.M. Wolf, X. Wu, L. Xiao, D.M. York and P.A. Kollman (2018). AMBER 2018. University of California, San Francisco.
- Dahlin, J. L., Nissink, J. W., Strasser, J. M., Francis, S., Higgins, L., Zhou, H., Zhang, Z. and Walters, M. A. (2015). PAINS in the assay: chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. J Med Chem, 58, 2091-2113. doi: 10.1021/jm5019093.
- Danishuddin and Khan, A. U. (2016). Descriptors and their selection methods in QSAR analysis: paradigm for drug design. *Drug Discov Today*, **21**, 1291-1302. doi: 10.1016/j.drudis.2016.06.013
- S1359-6446(16)30231-8 [pii].
- DasGupta, D., Kaushik, R. and Jayaram, B. (2015). From Ramachandran Maps to Tertiary Structures of Proteins. J Phys Chem B, 119, 11136-11145. doi: 10.1021/acs.jpcb.5b02999.
- Davidson, R. N., den Boer, M. and Ritmeijer, K. (2009). Paromomycin. *Trans R Soc Trop Med Hyg*, **103**, 653-660. doi: 10.1016/j.trstmh.2008.09.008
- S0035-9203(08)00392-1 [pii].
- de Souza, M. L., de Oliveira Rezende Junior, C., Ferreira, R. S., Espinoza Chavez, R. M., Ferreira, L. L. G., Slafer, B. W., Magalhaes, L. G., Krogh, R., Oliva, G., Cruz, F. C., Dias, L. C. and Andricopulo, A. D. (2019). Discovery of Potent, Reversible, and Competitive Cruzain Inhibitors with Trypanocidal Activity: A Structure-Based Drug Design Approach. *J Chem Inf Model*. doi: 10.1021/acs.jcim.9b00802.
- **Deeks, E. D.** (2019). Fexinidazole: First Global Approval. *Drugs,* **79**, 215-220. doi: 10.1007/s40265-019-1051-6 10.1007/s40265-019-1051-6 [pii].
- Defelipe, L. A., Arcon, J. P., Modenutti, C. P., Marti, M. A., Turjanski, A. G. and Barril, X. (2018). Solvents to Fragments to Drugs: MD Applications in Drug Design. *Molecules*, **23**. doi: E3269 [pii]
- 10.3390/molecules23123269
- molecules23123269 [pii].
- Delavan, B., Roberts, R., Huang, R., Bao, W., Tong, W. and Liu, Z. (2018). Computational drug repositioning for rare diseases in the era of precision medicine. *Drug Discov Today*, 23, 382-394. doi: S1359-6446(17)30158-7 [pii]
- 10.1016/j.drudis.2017.10.009.
- Demir, O., Labaied, M., Merritt, C., Stuart, K. and Amaro, R. E. (2014). Computer-aided discovery of Trypanosoma brucei RNA-editing terminal uridylyl transferase 2 inhibitors. *Chem Biol Drug Des*, 84, 131-139. doi: 10.1111/cbdd.12302.
- di Luccio, E. and Koehl, P. (2012). The H-factor as a novel quality metric for homology modeling. J Clin Bioinforma, 2, 18. doi: 10.1186/2043-9113-2-18
- 2043-9113-2-18 [pii].
- Dietrich, R. C., Alberca, L. N., Ruiz, M. D., Palestro, P. H., Carrillo, C., Talevi, A. and Gavernet, L. (2018). Identification of cisapride as new inhibitor of putrescine uptake in Trypanosoma cruzi by combined ligand- and structure-based virtual screening. *Eur J Med Chem*, **149**, 22-29. doi: S0223-5234(18)30128-4 [pii]

10.1016/j.ejmech.2018.02.006.

- DiMasi, J. A., Grabowski, H. G. and Hansen, R. W. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. *J Health Econ*, **47**, 20-33. doi: 10.1016/j.jhealeco.2016.01.012
- S0167-6296(16)00029-1 [pii].
- Dixon, S. L., Smondyrev, A. M., Knoll, E. H., Rao, S. N., Shaw, D. E. and Friesner, R. A. (2006). PHASE: a new engine for pharmacophore perception, 3D QSAR model development, and 3D database screening: 1. Methodology and preliminary results. *J Comput Aided Mol Des*, **20**, 647-671. doi: 10.1007/s10822-006-9087-6.
- Dorlo, T. P., Balasegaram, M., Beijnen, J. H. and de Vries, P. J. (2012). Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. *J Antimicrob Chemother*, **67**, 2576-2597. doi: 10.1093/jac/dks275

dks275 [pii].

Drews, J. (1998). Innovation deficit revisited: reflections on the productivity of pharmaceutical R&D. *Drugs Discovery Today*, **3**, 491-494. doi: 10.1016/S1359-6446(98)01252-5.

- Dube, D., Sharma, S., Singh, T. P. and Kaur, P. (2014). Pharmacophore Mapping, In Silico Screening and Molecular Docking to Identify Selective Trypanosoma brucei Pteridine Reductase Inhibitors. *Mol Inform*, 33, 124-134. doi: 10.1002/minf.201300023.
- Duncan, S. M., Jones, N. G. and Mottram, J. C. (2017). Recent advances in Leishmania reverse genetics: Manipulating a manipulative parasite. *Mol Biochem Parasitol*, **216**, 30-38. doi: S0166-6851(17)30079-8 [pii]

10.1016/j.molbiopara.2017.06.005.

Durrant, J. D., Hall, L., Swift, R. V., Landon, M., Schnaufer, A. and Amaro, R. E. (2010). Novel naphthalenebased inhibitors of Trypanosoma brucei RNA editing ligase 1. *PLoS Negl Trop Dis*, 4, e803. doi: 10.1371/journal.pntd.0000803

e803 [pii].

- Ekins, S., Williams, A. J., Krasowski, M. D. and Freundlich, J. S. (2011). In silico repositioning of approved drugs for rare and neglected diseases. *Drug Discov Today*, **16**, 298-310. doi: 10.1016/j.drudis.2011.02.016 S1359-6446(11)00067-5 [pii].
- El-Sayed, N. M., Myler, P. J., Bartholomeu, D. C., Nilsson, D., Aggarwal, G., Tran, A. N., Ghedin, E., Worthey, E. A., Delcher, A. L., Blandin, G., Westenberger, S. J., Caler, E., Cerqueira, G. C., Branche, C., Haas, B., Anupama, A., Arner, E., Aslund, L., Attipoe, P., Bontempi, E., Bringaud, F., Burton, P., Cadag, E., Campbell, D. A., Carrington, M., Crabtree, J., Darban, H., da Silveira, J. F., de Jong, P., Edwards, K., Englund, P. T., Fazelina, G., Feldblyum, T., Ferella, M., Frasch, A. C., Gull, K., Horn, D., Hou, L., Huang, Y., Kindlund, E., Klingbeil, M., Kluge, S., Koo, H., Lacerda, D., Levin, M. J., Lorenzi, H., Louie, T., Machado, C. R., McCulloch, R., McKenna, A., Mizuno, Y., Mottram, J. C., Nelson, S., Ochaya, S., Osoegawa, K., Pai, G., Parsons, M., Pentony, M., Pettersson, U., Pop, M., Ramirez, J. L., Rinta, J., Robertson, L., Salzberg, S. L., Sanchez, D. O., Seyler, A., Sharma, R., Shetty, J., Simpson, A. J., Sisk, E., Tammi, M. T., Tarleton, R., Teixeira, S., Van Aken, S., Vogt, C., Ward, P. N., Wickstead, B., Wortman, J., White, O., Fraser, C. M., Stuart, K. D. and Andersson, B. (2005a). The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease. *Science*, 309, 409-415. doi: 309/5733/409 [pii]

10.1126/science.1112631.

El-Sayed, N. M., Myler, P. J., Blandin, G., Berriman, M., Crabtree, J., Aggarwal, G., Caler, E., Renauld, H., Worthey, E. A., Hertz-Fowler, C., Ghedin, E., Peacock, C., Bartholomeu, D. C., Haas, B. J., Tran, A. N., Wortman, J. R., Alsmark, U. C., Angiuoli, S., Anupama, A., Badger, J., Bringaud, F., Cadag, E., Carlton, J. M., Cerqueira, G. C., Creasy, T., Delcher, A. L., Djikeng, A., Embley, T. M., Hauser, C., Ivens, A. C., Kummerfeld, S. K., Pereira-Leal, J. B., Nilsson, D., Peterson, J., Salzberg, S. L., Shallom, J., Silva, J. C., Sundaram, J., Westenberger, S., White, O., Melville, S. E., Donelson, J. E., Andersson, B., Stuart, K. D. and Hall, N. (2005b). Comparative genomics of trypanosomatid parasitic protozoa. *Science*, 309, 404-409. doi: 309/5733/404 [pii]

10.1126/science.1112181.

- Eramian, D., Shen, M. Y., Devos, D., Melo, F., Sali, A. and Marti-Renom, M. A. (2006). A composite score for predicting errors in protein structure models. *Protein Sci*, **15**, 1653-1666. doi: ps.062095806 [pii] 10.1110/ps.062095806.
- Ferreira, L. G. and Andricopulo, A. D. (2016). Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective. Drug Discov Today, 21, 1699-1710. doi: S1359-6446(16)30239-2 [pii]

10.1016/j.drudis.2016.06.021.

Field, M. C., Horn, D., Fairlamb, A. H., Ferguson, M. A. J., Gray, D. W., Read, K. D., De Rycker, M., Torrie, L. S., Wyatt, P. G., Wyllie, S. and Gilbert, I. H. (2017). Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need. *Nat Rev Microbiol*, **15**, 447. doi: 10.1038/nrmicro.2017.69

nrmicro.2017.69 [pii].

- Fiser, A. (2010). Template-based protein structure modeling. *Methods Mol Biol*, 673, 73-94. doi: 10.1007/978-1-60761-842-3\_6.
- Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S. and Olson, A. J. (2016). Computational proteinligand docking and virtual drug screening with the AutoDock suite. *Nat Protoc*, **11**, 905-919. doi: 10.1038/nprot.2016.051

nprot.2016.051 [pii].

- Fradera, X. and Babaoglu, K. (2017). Overview of Methods and Strategies for Conducting Virtual Small Molecule Screening. *Curr Protoc Chem Biol*, **9**, 196-212. doi: 10.1002/cpch.27.
- Francisco, A. F., Jayawardhana, S., Lewis, M. D., White, K. L., Shackleford, D. M., Chen, G., Saunders, J., Osuna-Cabello, M., Read, K. D., Charman, S. A., Chatelain, E. and Kelly, J. M. (2016).

Nitroheterocyclic drugs cure experimental Trypanosoma cruzi infections more effectively in the chronic stage than in the acute stage. *Sci Rep,* **6**, 35351. doi: 10.1038/srep35351

srep35351 [pii].

- Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A., Sanschagrin, P. C. and Mainz, D. T. (2006). Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem, 49, 6177-6196. doi: 10.1021/jm0512560.
- Friggeri, L., Hargrove, T. Y., Rachakonda, G., Blobaum, A. L., Fisher, P., de Oliveira, G. M., da Silva, C. F., Soeiro, M. N. C., Nes, W. D., Lindsley, C. W., Villalta, F., Guengerich, F. P. and Lepesheva, G. I. (2018). Sterol 14alpha-Demethylase Structure-Based Optimization of Drug Candidates for Human Infections with the Protozoan Trypanosomatidae. J Med Chem, 61, 10910-10921. doi: 10.1021/acs.jmedchem.8b01671.
- Fueller, F., Jehle, B., Putzker, K., Lewis, J. D. and Krauth-Siegel, R. L. (2012). High throughput screening against the peroxidase cascade of African trypanosomes identifies antiparasitic compounds that inactivate tryparedoxin. J Biol Chem, 287, 8792-8802. doi: 10.1074/jbc.M111.338285

- Gaillard, T. (2018). Evaluation of AutoDock and AutoDock Vina on the CASF-2013 Benchmark. J Chem Inf Model, 58, 1697-1706. doi: 10.1021/acs.jcim.8b00312.
- Gangwar, S., Baig, M. S., Shah, P., Biswas, S., Batra, S., Siddiqi, M. I. and Goyal, N. (2012). Identification of novel inhibitors of dipeptidylcarboxypeptidase of Leishmania donovani via ligand-based virtual screening and biological evaluation. *Chem Biol Drug Des*, **79**, 149-156. doi: 10.1111/j.1747-0285.2011.01262.x.
- Gasteiger, J. (2015). Cheminformatics: Computing target complexity. *Nat Chem*, **7**, 619-620. doi: 10.1038/nchem.2311
- nchem.2311 [pii].
- Gaulton, A., Hersey, A., Nowotka, M., Bento, A. P., Chambers, J., Mendez, D., Mutowo, P., Atkinson, F., Bellis, L. J., Cibrian-Uhalte, E., Davies, M., Dedman, N., Karlsson, A., Magarinos, M. P., Overington, J.
   P., Papadatos, G., Smit, I. and Leach, A. R. (2017). The ChEMBL database in 2017. Nucleic Acids Res, 45, D945-D954. doi: 10.1093/nar/gkw1074

gkw1074 [pii].

Gelb, M. H., Van Voorhis, W. C., Buckner, F. S., Yokoyama, K., Eastman, R., Carpenter, E. P., Panethymitaki,
 C., Brown, K. A. and Smith, D. F. (2003). Protein farnesyl and N-myristoyl transferases: piggy-back medicinal chemistry targets for the development of antitrypanosomatid and antimalarial therapeutics. *Mol Biochem Parasitol*, 126, 155-163. doi: S0166685102002827 [pii]

10.1016/s0166-6851(02)00282-7.

Ghemtio, L., Perez-Nueno, V. I., Leroux, V., Asses, Y., Souchet, M., Mavridis, L., Maigret, B. and Ritchie, D. W. (2012). Recent trends and applications in 3D virtual screening. *Comb Chem High Throughput Screen*, 15, 749-769. doi: CCHTS-EPUB-20120828-6 [pii]

10.2174/138620712803519707.

Ghofrani, H. A., Osterloh, I. H. and Grimminger, F. (2006). Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat Rev Drug Discov*, **5**, 689-702. doi: nrd2030 [pii]

10.1038/nrd2030.

- Gilbert, I. H. (2013). Drug discovery for neglected diseases: molecular target-based and phenotypic approaches. *J Med Chem*, 56, 7719-7726. doi: 10.1021/jm400362b.
- Gilbert, I. H. (2014). Target-based drug discovery for human African trypanosomiasis: selection of molecular target and chemical matter. *Parasitology*, **141**, 28-36. doi: 10.1017/S0031182013001017

S0031182013001017 [pii].

Gimeno, A., Ojeda-Montes, M. J., Tomas-Hernandez, S., Cereto-Massague, A., Beltran-Debon, R., Mulero, M., Pujadas, G. and Garcia-Vallve, S. (2019). The Light and Dark Sides of Virtual Screening: What Is There to Know? *Int J Mol Sci*, 20. doi: E1375 [pii]

10.3390/ijms20061375

ijms20061375 [pii].

Gonzalez-Chavez, Z., Olin-Sandoval, V., Rodiguez-Zavala, J. S., Moreno-Sanchez, R. and Saavedra, E. (2015). Metabolic control analysis of the Trypanosoma cruzi peroxide detoxification pathway identifies tryparedoxin as a suitable drug target. *Biochim Biophys Acta*, **1850**, 263-273. doi: 10.1016/j.bbagen.2014.10.029

S0304-4165(14)00365-1 [pii].

M111.338285 [pii].

Graul, A. I., Pina, P., Cruces, E. and Stringer, M. (2017). The year's new drugs & biologics 2016: Part I. *Drugs Today (Barc)*, **53**, 27-74. doi: 10.1358/dot.2017.53.1.2584699

Graul, A. I., Pina, P., Cruces, E. and Stringer, M. (2019). The year's new drugs and biologics 2018: Part I. *Drugs Today (Barc)*, **55**, 35-87. doi: 10.1358/dot.2019.55.1.2959663

Graul, A. I., Pina, P. and Stringer, M. (2018). The year's new drugs and biologics 2017: Part I. *Drugs Today* (*Barc*), 54, 35-84. doi: 10.1358/dot.2018.54.1.2766396

2766396 [pii].

Grosdidier, A., Zoete, V. and Michielin, O. (2011). SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic Acids Res*, **39**, W270-277. doi: 10.1093/nar/gkr366

gkr366 [pii].

Gupta, S. C., Sung, B., Prasad, S., Webb, L. J. and Aggarwal, B. B. (2013). Cancer drug discovery by repurposing: teaching new tricks to old dogs. *Trends Pharmacol Sci*, **34**, 508-517. doi: 10.1016/j.tips.2013.06.005

Haga, J. H., Ichikawa, K. and Date, S. (2016). Virtual Screening Techniques and Current Computational Infrastructures. *Curr Pharm Des*, 22, 3576-3584. doi: CPD-EPUB-74999 [pii]

10.2174/1381612822666160414142530.

Hall, B. S., Bot, C. and Wilkinson, S. R. (2011). Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J Biol Chem, 286, 13088-13095. doi: 10.1074/jbc.M111.230847

M111.230847 [pii].

 Harigua-Souiai, E., Abdelkrim, Y. Z., Bassoumi-Jamoussi, I., Zakraoui, O., Bouvier, G., Essafi-Benkhadir, K., Banroques, J., Desdouits, N., Munier-Lehmann, H., Barhoumi, M., Tanner, N. K., Nilges, M., Blondel, A. and Guizani, I. (2018). Identification of novel leishmanicidal molecules by virtual and biochemical screenings targeting Leishmania eukaryotic translation initiation factor 4A. *PLoS Negl Trop Dis*, 12, e0006160. doi: 10.1371/journal.pntd.0006160

PNTD-D-16-01452 [pii].

Herrera-Mayorga, V., Lara-Ramirez, E. E., Chacon-Vargas, K. F., Aguirre-Alvarado, C., Rodriguez-Paez, L., Alcantara-Farfan, V., Cordero-Martinez, J., Nogueda-Torres, B., Reyes-Espinosa, F., Bocanegra-Garcia, V. and Rivera, G. (2019). Structure-Based Virtual Screening and In Vitro Evaluation of New Trypanosoma cruzi Cruzain Inhibitors. Int J Mol Sci, 20. doi: E1742 [pii]

10.3390/ijms20071742

ijms20071742 [pii].

Herrmann, F. C., Lenz, M., Jose, J., Kaiser, M., Brun, R. and Schmidt, T. J. (2015). In Silico Identification and in Vitro Activity of Novel Natural Inhibitors of Trypanosoma brucei Glyceraldehyde-3-phosphatedehydrogenase. *Molecules*, 20, 16154-16169. doi: 10.3390/molecules200916154

molecules200916154 [pii].

- Hollingsworth, T. D. (2018). Counting Down the 2020 Goals for 9 Neglected Tropical Diseases: What Have We Learned From Quantitative Analysis and Transmission Modeling? *Clinical Infectious Diseases*, 66, S237-S244. doi: 10.1093/cid/ciy284.
- Hornberg, J. J., Bruggeman, F. J., Bakker, B. M. and Westerhoff, H. V. (2007). Metabolic control analysis to identify optimal drug targets. *Prog Drug Res*, 64, 171, 173-189.
- Hu, B. and Lill, M. A. (2014). PharmDock: a pharmacophore-based docking program. J Cheminform, 6, 14. doi: 10.1186/1758-2946-6-14

1758-2946-6-14 [pii].

- Hughes, J. P., Rees, S., Kalindjian, S. B. and Philpott, K. L. (2011). Principles of early drug discovery. Br J Pharmacol, 162, 1239-1249. doi: 10.1111/j.1476-5381.2010.01127.x.
- Humphrey, W., Dalke, A. and Schulten, K. (1996). VMD: visual molecular dynamics. *J Mol Graph*, **14**, 33-38, 27-38. doi: 0263785596000185 [pii]

10.1016/0263-7855(96)00018-5.

Hutton, J. A., Goncalves, V., Brannigan, J. A., Paape, D., Wright, M. H., Waugh, T. M., Roberts, S. M., Bell, A. S., Wilkinson, A. J., Smith, D. F., Leatherbarrow, R. J. and Tate, E. W. (2014). Structure-based design of potent and selective Leishmania N-myristoyltransferase inhibitors. J Med Chem, 57, 8664-8670. doi: 10.1021/jm5011397.

<sup>2584699 [</sup>pii].

<sup>2959663 [</sup>pii].

S0165-6147(13)00116-8 [pii].

Jain, A. N. (2009). Effects of protein conformation in docking: improved pose prediction through protein pocket adaptation. *J Comput Aided Mol Des*, 23, 355-374. doi: 10.1007/s10822-009-9266-3.

Jain, K. and Jain, N. K. (2013). Novel therapeutic strategies for treatment of visceral leishmaniasis. *Drug Discov Today*, **18**, 1272-1281. doi: 10.1016/j.drudis.2013.08.005

- James Abraham, T. M., Roland Schulz, Szilard Pall, Jeremy Smith, Berk Hess, Erik Lindahl (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, **1**, 19-25. doi: 10.1016/j.softx.2015.06.001.
- Jhingran, A., Chawla, B., Saxena, S., Barrett, M. P. and Madhubala, R. (2009). Paromomycin: uptake and resistance in Leishmania donovani. *Mol Biochem Parasitol*, 164, 111-117. doi: 10.1016/j.molbiopara.2008.12.007
- S0166-6851(08)00287-9 [pii].
- Johnson, M. A., Maggiora, G. M. and Meeting, A. C. S. (1990). Concepts and applications of molecular similarity, Wiley.
- Jones, G., Willett, P., Glen, R. C., Leach, A. R. and Taylor, R. (1997). Development and validation of a genetic algorithm for flexible docking. *J Mol Biol*, **267**, 727-748. doi: S0022-2836(96)90897-9 [pii]

10.1006/jmbi.1996.0897.

- Jones, N. G., Catta-Preta, C. M. C., Lima, A. and Mottram, J. C. (2018). Genetically Validated Drug Targets in Leishmania: Current Knowledge and Future Prospects. ACS Infect Dis, 4, 467-477. doi: 10.1021/acsinfecdis.7b00244.
- Kansiime, F., Adibaku, S., Wamboga, C., Idi, F., Kato, C. D., Yamuah, L., Vaillant, M., Kioy, D., Olliaro, P. and Matovu, E. (2018). A multicentre, randomised, non-inferiority clinical trial comparing a nifurtimoxeflornithine combination to standard eflornithine monotherapy for late stage Trypanosoma brucei gambiense human African trypanosomiasis in Uganda. *Parasites and Vectors*, **11**, 1-11. doi: 10.1186/s13071-018-2634-x.
- Kashif, M., Hira, S. K., Upadhyaya, A., Gupta, U., Singh, R., Paladhi, A., Khan, F. I., Rub, A. and Manna, P. P. (2019). In silico studies and evaluation of antiparasitic role of a novel pyruvate phosphate dikinase inhibitor in Leishmania donovani infected macrophages. *Int J Antimicrob Agents*, 53, 508-514. doi: S0924-8579(18)30381-9 [pii]
- 10.1016/j.ijantimicag.2018.12.011.
- Kawasaki, Y. and Freire, E. (2011). Finding a better path to drug selectivity. *Drug Discov Today*, **16**, 985-990. doi: 10.1016/j.drudis.2011.07.010
- S1359-6446(11)00247-9 [pii].
- Kelley, B. P., Brown, S. P., Warren, G. L. and Muchmore, S. W. (2015). POSIT: Flexible Shape-Guided Docking For Pose Prediction. J Chem Inf Model, 55, 1771-1780. doi: 10.1021/acs.jcim.5b00142.
- Khare, P., Gupta, A. K., Gajula, P. K., Sunkari, K. Y., Jaiswal, A. K., Das, S., Bajpai, P., Chakraborty, T. K., Dube, A. and Saxena, A. K. (2012). Identification of novel S-adenosyl-L-homocysteine hydrolase inhibitors through homology-model-based virtual screening, synthesis, and biological evaluation. J Chem Inf Model, 52, 777-791. doi: 10.1021/ci2005862.
- Khare, S., Nagle, A. S., Biggart, A., Lai, Y. H., Liang, F., Davis, L. C., Barnes, S. W., Mathison, C. J. N., Myburgh, E., Gao, M.-y., Gillespie, J. R., Liu, X., Tan, J. L., Stinson, M., Rivera, I. C., Ballard, J., Yeh, V., Groessl, T., Federe, G., Koh, H. X. Y., Venable, J. D., Bursulaya, B., Shapiro, M., Mishra, P. K., Spraggon, G., Brock, A., Mottram, J. C. and Buckner, F. S. (2016). Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. *Nature*, 537, 229-233. doi: 10.1038/nature19339.
- Khare, S., Roach, S. L., Barnes, S. W., Hoepfner, D., Walker, J. R., Chatterjee, A. K., Neitz, R. J., Arkin, M. R., McNamara, C. W., Ballard, J., Lai, Y., Fu, Y., Molteni, V., Yeh, V., McKerrow, J. H., Glynne, R. J. and Supek, F. (2015). Utilizing Chemical Genomics to Identify Cytochrome b as a Novel Drug Target for Chagas Disease. *PLoS Pathog*, **11**, e1005058. doi: 10.1371/journal.ppat.1005058

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J. and Bolton, E. E. (2019). PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res*, 47, D1102-D1109. doi: 10.1093/nar/gky1033

King-Keller, S., Li, M., Smith, A., Zheng, S., Kaur, G., Yang, X., Wang, B. and Docampo, R. (2010). Chemical validation of phosphodiesterase C as a chemotherapeutic target in Trypanosoma cruzi, the etiological agent of Chagas' disease. *Antimicrob Agents Chemother*, **54**, 3738-3745. doi: 10.1128/AAC.00313-10 AAC.00313-10 [pii].

S1359-6446(13)00268-7 [pii].

PPATHOGENS-D-15-00422 [pii].

<sup>5146201 [</sup>pii].

- Konkle, M. E., Hargrove, T. Y., Kleshchenko, Y. Y., von Kries, J. P., Ridenour, W., Uddin, M. J., Caprioli, R. M., Marnett, L. J., Nes, W. D., Villalta, F., Waterman, M. R. and Lepesheva, G. I. (2009). Indomethacin amides as a novel molecular scaffold for targeting Trypanosoma cruzi sterol 14alpha-demethylase. J *Med Chem*, **52**, 2846-2853. doi: 10.1021/jm801643b.
- Kontoyianni, M. (2017). Docking and Virtual Screening in Drug Discovery. Methods Mol Biol, 1647, 255-266. doi: 10.1007/978-1-4939-7201-2 18.
- Kraus, J. M., Tatipaka, H. B., McGuffin, S. A., Chennamaneni, N. K., Karimi, M., Arif, J., Verlinde, C. L., Buckner, F. S. and Gelb, M. H. (2010). Second generation analogues of the cancer drug clinical candidate tipifarnib for anti-Chagas disease drug discovery. J Med Chem, 53, 3887-3898. doi: 10.1021/jm9013136.
- Krauth-Siegel, R. L., Enders, B., Henderson, G. B., Fairlamb, A. H. and Schirmer, R. H. (1987). Trypanothione reductase from Trypanosoma cruzi. Purification and characterization of the crystalline enzyme. Eur J *Biochem*, **164**, 123-128. doi: 10.1111/j.1432-1033.1987.tb11002.x.
- Lamotte, S., Aulner, N., Spath, G. F. and Prina, E. (2019). Discovery of novel hit compounds with broad activity against visceral and cutaneous Leishmania species by comparative phenotypic screening. Sci Rep, 9, 438. doi: 10.1038/s41598-018-36944-6
- 10.1038/s41598-018-36944-6 [pii].
- Lander, N., Chiurillo, M. A. and Docampo, R. (2016). Genome Editing by CRISPR/Cas9: A Game Change in the Genetic Manipulation of Protists. J Eukaryot Microbiol, 63, 679-690. doi: 10.1111/jeu.12338.
- Lara-Ramirez, E. E., Lopez-Cedillo, J. C., Nogueda-Torres, B., Kashif, M., Garcia-Perez, C., Bocanegra-Garcia, V., Agusti, R., Uhrig, M. L. and Rivera, G. (2017). An in vitro and in vivo evaluation of new potential trans-sialidase inhibitors of Trypanosoma cruzi predicted by a computational drug repositioning method. Eur J Med Chem, 132, 249-261. doi: S0223-5234(17)30230-1 [pii]
- 10.1016/j.ejmech.2017.03.063.
- Lepesheva, G. I., Ott, R. D., Hargrove, T. Y., Kleshchenko, Y. Y., Schuster, I., Nes, W. D., Hill, G. C., Villalta, F. and Waterman, M. R. (2007). Sterol 14alpha-demethylase as a potential target for antitrypanosomal therapy: enzyme inhibition and parasite cell growth. Chem Biol, 14, 1283-1293. doi: S1074-5521(07)00362-6 [pii]
- 10.1016/j.chembiol.2007.10.011.
- Leroux, A. E. and Krauth-Siegel, R. L. (2016). Thiol redox biology of trypanosomatids and potential targets for chemotherapy. Mol Biochem Parasitol, 206, 67-74. doi: 10.1016/j.molbiopara.2015.11.003 S0166-6851(15)30039-6 [pii].
- Lesnik, S., Stular, T., Brus, B., Knez, D., Gobec, S., Janezic, D. and Konc, J. (2015). LiSiCA: A Software for Ligand-Based Virtual Screening and Its Application for the Discovery of Butyrylcholinesterase Inhibitors. J Chem Inf Model, 55, 1521-1528. doi: 10.1021/acs.jcim.5b00136.
- Li, A. P. (2001). Screening for human ADME/Tox drug properties in drug discovery. Drug Discov Today, 6, 357-366. doi: S1359-6446(01)01712-3 [pii]
- 10.1016/s1359-6446(01)01712-3.
- Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev, 46, 3-26. doi: S0169-409X(00)00129-0 [pii]
- 10.1016/s0169-409x(00)00129-0.
- Lo, Y. C., Rensi, S. E., Torng, W. and Altman, R. B. (2018). Machine learning in chemoinformatics and drug discovery. Drug Discov Today, 23, 1538-1546. doi: S1359-6446(17)30469-5 [pii]
- 10.1016/j.drudis.2018.05.010.
- Lu, J., Vodnala, S. K., Gustavsson, A. L., Gustafsson, T. N., Sjoberg, B., Johansson, H. A., Kumar, S., Tjernberg, A., Engman, L., Rottenberg, M. E. and Holmgren, A. (2013). Ebsulfur is a benzisothiazolone cytocidal inhibitor targeting the trypanothione reductase of Trypanosoma brucei. J Biol Chem, 288, 27456-27468. doi: 10.1074/jbc.M113.495101
- M113.495101 [pii].
- Luo, Y., Li, B. Z., Liu, D., Zhang, L., Chen, Y., Jia, B., Zeng, B. X., Zhao, H. and Yuan, Y. J. (2015). Engineered biosynthesis of natural products in heterologous hosts. Chemical Society Reviews, 44, 5265-5290. doi: 10.1039/c5cs00025d.
- Llanos, M. A., Sbaraglini, M. L., Villalba, M. L., Ruiz, M. D., Carrillo, C., Alba Soto, C., Talevi, A., Angeli, A., Parkkila, S., Supuran, C. T. and Gavernet, L. (2020). A structure-based approach towards the identification of novel antichagasic compounds: Trypanosoma cruzi carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem, 35, 21-30. doi: 10.1080/14756366.2019.1677638.

- Ma, X. H., Jia, J., Zhu, F., Xue, Y., Li, Z. R. and Chen, Y. Z. (2009). Comparative analysis of machine learning methods in ligand-based virtual screening of large compound libraries. *Comb Chem High Throughput Screen*, 12, 344-357. doi: 10.2174/138620709788167944.
- Magarinos, M. P., Carmona, S. J., Crowther, G. J., Ralph, S. A., Roos, D. S., Shanmugam, D., Van Voorhis, W.
   C. and Aguero, F. (2012). TDR Targets: a chemogenomics resource for neglected diseases. *Nucleic Acids Res*, 40, D1118-1127. doi: 10.1093/nar/gkr1053

- Maluf, F. V., Andricopulo, A. D., Oliva, G. and Guido, R. V. (2013). A pharmacophore-based virtual screening approach for the discovery of Trypanosoma cruzi GAPDH inhibitors. *Future Med Chem*, 5, 2019-2035. doi: 10.4155/fmc.13.166.
- Mansuri, R., Kumar, A., Rana, S., Panthi, B., Ansari, M. Y., Das, S., Dikhit, M. R., Sahoo, G. C. and Das, P. (2017). In Vitro Evaluation of Antileishmanial Activity of Computationally Screened Compounds against Ascorbate Peroxidase To Combat Amphotericin B Drug Resistance. *Antimicrob Agents Chemother*, **61**. doi: e02429-16 [pii]
- 10.1128/AAC.02429-16

AAC.02429-16 [pii].

- Marchese, L., Nascimento, J. F., Damasceno, F. S., Bringaud, F., Michels, P. A. M. and Silber, A. M. (2018). The Uptake and Metabolism of Amino Acids, and Their Unique Role in the Biology of Pathogenic Trypanosomatids. *Pathogens*, **7**. doi: E36 [pii]
- 10.3390/pathogens7020036
- pathogens7020036 [pii].
- Mavridis, L., Hudson, B. D. and Ritchie, D. W. (2007). Toward high throughput 3D virtual screening using spherical harmonic surface representations. J Chem Inf Model, 47, 1787-1796. doi: 10.1021/ci7001507.
- Maxfield, L. and Crane, J. S. (2019). Leishmaniasis. doi: NBK531456 [bookaccession].
- Meiering, S., Inhoff, O., Mies, J., Vincek, A., Garcia, G., Kramer, B., Dormeyer, M. and Krauth-Siegel, R. L. (2005). Inhibitors of Trypanosoma cruzi trypanothione reductase revealed by virtual screening and parallel synthesis. J Med Chem, 48, 4793-4802. doi: 10.1021/jm050027z.
- Menna-Barreto, R. F. S. (2019). Cell death pathways in pathogenic trypanosomatids: lessons of (over)kill. *Cell Death Dis*, **10**, 93. doi: 10.1038/s41419-019-1370-2

10.1038/s41419-019-1370-2 [pii].

- Menzies, S. K., Tulloch, L. B., Florence, G. J. and Smith, T. K. (2018). The trypanosome alternative oxidase: a potential drug target? *Parasitology*, **145**, 175-183. doi: 10.1017/S0031182016002109 S0031182016002109 [pii].
- Mesu, V., Kalonji, W. M., Bardonneau, C., Mordt, O. V., Blesson, S., Simon, F., Delhomme, S., Bernhard, S., Kuziena, W., Lubaki, J. F., Vuvu, S. L., Ngima, P. N., Mbembo, H. M., Ilunga, M., Bonama, A. K., Heradi, J. A., Solomo, J. L. L., Mandula, G., Badibabi, L. K., Dama, F. R., Lukula, P. K., Tete, D. N., Lumbala, C., Scherrer, B., Strub-Wourgaft, N. and Tarral, A. (2018). Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. *Lancet*, 391, 144-154. doi: S0140-6736(17)32758-7 [pii]

10.1016/S0140-6736(17)32758-7.

- Meyerhoff, A. (1999). U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. *Clin Infect Dis*, **28**, 42-48; discussion 49-51. doi: 10.1086/515085.
- Meyskens, F. L., Kingsley, E. M., Glattke, T., Loescher, L. and Booth, A. (1986). A phase II study of alphadifluoromethylornithine (DFMO) for the treatment of metastatic melanoma. *Invest New Drugs*, 4, 257-262.
- Mishra, A. K., Singh, N., Agnihotri, P., Mishra, S., Singh, S. P., Kolli, B. K., Chang, K. P., Sahasrabuddhe, A. A., Siddiqi, M. I. and Pratap, J. V. (2017). Discovery of novel inhibitors for Leishmania nucleoside diphosphatase kinase (NDK) based on its structural and functional characterization. J Comput Aided Mol Des, 31, 547-562. doi: 10.1007/s10822-017-0022-9

- Mogk, S., Bosselmann, C. M., Mudogo, C. N., Stein, J., Wolburg, H. and Duszenko, M. (2017). African trypanosomes and brain infection - the unsolved question. *Biol Rev Camb Philos Soc*, 92, 1675-1687. doi: 10.1111/brv.12301.
- Morillo, C. A., Waskin, H., Sosa-Estani, S., Del Carmen Bangher, M., Cuneo, C., Milesi, R., Mallagray, M., Apt, W., Beloscar, J., Gascon, J., Molina, I., Echeverria, L. E., Colombo, H., Perez-Molina, J. A., Wyss, F.,

gkr1053 [pii].

<sup>10.1007/</sup>s10822-017-0022-9 [pii].

Meeks, B., Bonilla, L. R., Gao, P., Wei, B., McCarthy, M. and Yusuf, S. (2017). Benznidazole and Posaconazole in Eliminating Parasites in Asymptomatic T. Cruzi Carriers: The STOP-CHAGAS Trial. *J Am Coll Cardiol*, **69**, 939-947. doi: S0735-1097(17)30115-8 [pii]

10.1016/j.jacc.2016.12.023.

- Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*, **30**, 2785-2791. doi: 10.1002/jcc.21256.
- Mpamhanga, C. P., Spinks, D., Tulloch, L. B., Shanks, E. J., Robinson, D. A., Collie, I. T., Fairlamb, A. H., Wyatt, P. G., Frearson, J. A., Hunter, W. N., Gilbert, I. H. and Brenk, R. (2009). One scaffold, three binding modes: novel and selective pteridine reductase 1 inhibitors derived from fragment hits discovered by virtual screening. J Med Chem, 52, 4454-4465. doi: 10.1021/jm900414x.
- Novac, N. (2013). Challenges and opportunities of drug repositioning. *Trends Pharmacol Sci*, **34**, 267-272. doi: 10.1016/j.tips.2013.03.004
- S0165-6147(13)00045-X [pii].
- Novick, P. A., Ortiz, O. F., Poelman, J., Abdulhay, A. Y. and Pande, V. S. (2013). SWEETLEAD: an in silico database of approved drugs, regulated chemicals, and herbal isolates for computer-aided drug discovery. *PLoS One*, 8, e79568. doi: 10.1371/journal.pone.0079568

PONE-D-13-17130 [pii].

Nowicki, M. W., Tulloch, L. B., Worralll, L., McNae, I. W., Hannaert, V., Michels, P. A., Fothergill-Gilmore, L. A., Walkinshaw, M. D. and Turner, N. J. (2008). Design, synthesis and trypanocidal activity of lead compounds based on inhibitors of parasite glycolysis. *Bioorg Med Chem*, 16, 5050-5061. doi: 10.1016/j.bmc.2008.03.045

S0968-0896(08)00244-7 [pii].

- Nwaka, S., Ramirez, B., Brun, R., Maes, L., Douglas, F. and Ridley, R. (2009). Advancing Drug Innovation for Neglected Diseases—Criteria for Lead Progression. *PLOS Neglected Tropical Diseases*, **3**, e440.
- Ochoa, R., Garcia, E., Robledo, S. M. and Cardona, G. W. (2019). Virtual and experimental screening of phenylfuranchalcones as potential anti-Leishmania candidates. J Mol Graph Model, 91, 164-171. doi: S1093-3263(19)30207-4 [pii]
- 10.1016/j.jmgm.2019.06.015.
- Ochoa, R., Rocha-Roa, C., Marin-Villa, M., Robledo, S. M. and Varela, M. R. (2018). Search of Allosteric Inhibitors and Associated Proteins of an AKT-like Kinase from Trypanosoma cruzi. *Int J Mol Sci*, **19**. doi: E3951 [pii]

10.3390/ijms19123951

ijms19123951 [pii].

Ochoa, R., Watowich, S. J., Florez, A., Mesa, C. V., Robledo, S. M. and Muskus, C. (2016). Drug search for leishmaniasis: a virtual screening approach by grid computing. *J Comput Aided Mol Des*, **30**, 541-552. doi: 10.1007/s10822-016-9921-4

10.1007/s10822-016-9921-4 [pii].

Olin-Sandoval, V., Gonzalez-Chavez, Z., Berzunza-Cruz, M., Martinez, I., Jasso-Chavez, R., Becker, I., Espinoza, B., Moreno-Sanchez, R. and Saavedra, E. (2012). Drug target validation of the trypanothione pathway enzymes through metabolic modelling. *FEBS J*, **279**, 1811-1833. doi: 10.1111/j.1742-4658.2012.08557.x.

Oprea, T. I. (2002). Virtual Screening in Lead Discovery: A Viewpoint. *Molecules*, 7.

Orban, O. C., Korn, R. S., Benitez, D., Medeiros, A., Preu, L., Loaec, N., Meijer, L., Koch, O., Comini, M. A. and Kunick, C. (2016). 5-Substituted 3-chlorokenpaullone derivatives are potent inhibitors of Trypanosoma brucei bloodstream forms. *Bioorg Med Chem*, 24, 3790-3800. doi: 10.1016/j.bmc.2016.06.023

S0968-0896(16)30446-1 [pii].

- Ortega, V., Giorgio, S. and de Paula, E. (2017). Liposomal formulations in the pharmacological treatment of leishmaniasis: a review. *J Liposome Res*, **27**, 234-248. doi: 10.1080/08982104.2017.1376682.
- **Osorio-Mendez, J. F. and Cevallos, A. M.** (2018). Discovery and Genetic Validation of Chemotherapeutic Targets for Chagas' Disease. *Front Cell Infect Microbiol,* **8**, 439. doi: 10.3389/fcimb.2018.00439.
- Palos, I., Lara-Ramirez, E. E., Lopez-Cedillo, J. C., Garcia-Perez, C., Kashif, M., Bocanegra-Garcia, V., Nogueda-Torres, B. and Rivera, G. (2017). Repositioning FDA Drugs as Potential Cruzain Inhibitors from Trypanosoma cruzi: Virtual Screening, In Vitro and In Vivo Studies. *Molecules*, 22. doi: E1015 [pii] 10.3390/molecules22061015

molecules22061015 [pii].

Parameswaran, S., Saudagar, P., Dubey, V. K. and Patra, S. (2014). Discovery of novel anti-leishmanial agents targeting LdLip3 lipase. *J Mol Graph Model*, **49**, 68-79. doi: 10.1016/j.jmgm.2014.01.007

S1093-3263(14)00010-2 [pii].

**Perez-Molina, J. A. and Molina, I.** (2018). Chagas disease. *Lancet,* **391**, 82-94. doi: S0140-6736(17)31612-4 [pii] 10.1016/S0140-6736(17)31612-4.

Pieper, U., Webb, B. M., Dong, G. Q., Schneidman-Duhovny, D., Fan, H., Kim, S. J., Khuri, N., Spill, Y. G., Weinkam, P., Hammel, M., Tainer, J. A., Nilges, M. and Sali, A. (2014). ModBase, a database of annotated comparative protein structure models and associated resources. *Nucleic Acids Res*, 42, D336-346. doi: 10.1093/nar/gkt1144

gkt1144 [pii].

- Pierce, B. G., Hourai, Y. and Weng, Z. (2011). Accelerating protein docking in ZDOCK using an advanced 3D convolution library. *PLoS One*, *6*, e24657. doi: 10.1371/journal.pone.0024657
- PONE-D-11-13547 [pii].
- Pinto-Martinez, A. K., Rodriguez-Duran, J., Serrano-Martin, X., Hernandez-Rodriguez, V. and Benaim, G. (2018). Mechanism of Action of Miltefosine on Leishmania donovani Involves the Impairment of Acidocalcisome Function and the Activation of the Sphingosine-Dependent Plasma Membrane Ca(2+) Channel. Antimicrob Agents Chemother, 62. doi: e01614-17 [pii]

10.1128/AAC.01614-17 AAC.01614-17 [pii].

- Pollastri, M. P. and Campbell, R. K. (2011). Target repurposing for neglected diseases. *Future Med Chem*, **3**, 1307-1315. doi: 10.4155/fmc.11.92.
- Ponte-Sucre, A., Gamarro, F., Dujardin, J. C., Barrett, M. P., Lopez-Velez, R., Garcia-Hernandez, R., Pountain, A. W., Mwenechanya, R. and Papadopoulou, B. (2017). Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. *PLoS Negl Trop Dis*, **11**, e0006052. doi: 10.1371/journal.pntd.0006052

- Prieto, J. J., Talevi, A. and Bruno-Blanch, L. E. (2006). Application of linear discriminant analysis in the virtual screening of antichagasic drugs through trypanothione reductase inhibition. *Mol Divers*, **10**, 361-375. doi: 10.1007/s11030-006-9044-2.
- Priotto, G., Kasparian, S., Mutombo, W., Ngouama, D., Ghorashian, S., Arnold, U., Ghabri, S., Baudin, E., Buard, V., Kazadi-Kyanza, S., Ilunga, M., Mutangala, W., Pohlig, G., Schmid, C., Karunakara, U., Torreele, E. and Kande, V. (2009). Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, noninferiority trial. *Lancet*, **374**, 56-64. doi: 10.1016/S0140-6736(09)61117-X

S0140-6736(09)61117-X [pii].

- Prokopczyk, I. M., Ribeiro, J. F., Sartori, G. R., Sesti-Costa, R., Silva, J. S., Freitas, R. F., Leitao, A. and Montanari, C. A. (2014). Integration of methods in cheminformatics and biocalorimetry for the design of trypanosomatid enzyme inhibitors. *Future Med Chem*, 6, 17-33. doi: 10.4155/fmc.13.185.
- Raether, W. and Seidenath, H. (1983). The activity of fexinidazole (HOE 239) against experimental infections with Trypanosoma cruzi, trichomonads and Entamoeba histolytica. *Ann Trop Med Parasitol*, **77**, 13-26. doi: 10.1080/00034983.1983.11811668.
- Rarey, M., Kramer, B., Lengauer, T. and Klebe, G. (1996). A fast flexible docking method using an incremental construction algorithm. *J Mol Biol*, 261, 470-489. doi: S0022-2836(96)90477-5 [pii]
   10.1006/imbi 1996.0477
- 10.1006/jmbi.1996.0477.
- Rashmi, M. and Swati, D. (2015). In silico drug re-purposing against African sleeping sickness using GlcNAc-PI de-N-acetylase as an experimental target. *Comput Biol Chem*, 59 Pt A, 87-94. doi: 10.1016/j.compbiolchem.2015.09.010
- S1476-9271(15)30172-9 [pii].
- Rassi, A., Jr., Rassi, A. and Marin-Neto, J. A. (2010). Chagas disease. *Lancet*, **375**, 1388-1402. doi: 10.1016/S0140-6736(10)60061-X
- S0140-6736(10)60061-X [pii].
- Reigada, C., Phanstiel, O. t., Miranda, M. R. and Pereira, C. A. (2018). Targeting polyamine transport in Trypanosoma cruzi. *Eur J Med Chem*, **147**, 1-6. doi: S0223-5234(18)30105-3 [pii]

10.1016/j.ejmech.2018.01.083.

Reigada, C., Saye, M., Phanstiel, O. t., Valera-Vera, E., Miranda, M. R. and Pereira, C. A. (2019). Identification of Trypanosoma cruzi Polyamine Transport Inhibitors by Computational Drug Repurposing. *Front Med* (*Lausanne*), **6**, 256. doi: 10.3389/fmed.2019.00256.

PNTD-D-17-01203 [pii].

- Reigada, C., Valera-Vera, E. A., Saye, M., Errasti, A. E., Avila, C. C., Miranda, M. R. and Pereira, C. A. (2017). Trypanocidal Effect of Isotretinoin through the Inhibition of Polyamine and Amino Acid Transporters in Trypanosoma cruzi. *PLoS Negl Trop Dis*, **11**, e0005472. doi: 10.1371/journal.pntd.0005472 PNTD-D-16-01527 [pii].
- **Rester, U.** (2008). From virtuality to reality Virtual screening in lead discovery and lead optimization: a medicinal chemistry perspective. *Curr Opin Drug Discov Devel*, **11**, 559-568.
- Roberts, C. W., McLeod, R., Rice, D. W., Ginger, M., Chance, M. L. and Goad, L. J. (2003). Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. *Mol Biochem Parasitol*, **126**, 129-142. doi: S0166685102002803 [pii].
- Rodriguez, D., Chakraborty, S., Warnick, E., Crane, S., Gao, Z. G., O'Connor, R., Jacobson, K. A. and Carlsson, J. (2016). Structure-Based Screening of Uncharted Chemical Space for Atypical Adenosine Receptor Agonists. ACS Chem Biol, 11, 2763-2772. doi: 10.1021/acschembio.6b00357.
- Rogers, K. E., Keranen, H., Durrant, J. D., Ratnam, J., Doak, A., Arkin, M. R. and McCammon, J. A. (2012). Novel cruzain inhibitors for the treatment of Chagas' disease. *Chem Biol Drug Des*, **80**, 398-405. doi: 10.1111/j.1747-0285.2012.01416.x.
- Ruda, G. F., Campbell, G., Alibu, V. P., Barrett, M. P., Brenk, R. and Gilbert, I. H. (2010). Virtual fragment screening for novel inhibitors of 6-phosphogluconate dehydrogenase. *Bioorg Med Chem*, **18**, 5056-5062. doi: 10.1016/j.bmc.2010.05.077
- S0968-0896(10)00516-X [pii].
- Sardana, D., Zhu, C., Zhang, M., Gudivada, R. C., Yang, L. and Jegga, A. G. (2011). Drug repositioning for orphan diseases. *Brief Bioinform*, **12**, 346-356. doi: 10.1093/bib/bbr021

bbr021 [pii].

- Saye, M., Gauna, L., Valera-Vera, E., Reigada, C., Miranda, M. R. and Pereira, C. A. (2020). Crystal violet structural analogues identified by in silico drug repositioning present anti-Trypanosoma cruzi activity through inhibition of proline transporter TcAAAP069. *PLoS Negl Trop Dis*, 14, e0007481. doi: 10.1371/journal.pntd.0007481
- PNTD-D-19-00768 [pii].
- Scior, T., Bender, A., Tresadern, G., Medina-Franco, J. L., Martinez-Mayorga, K., Langer, T., Cuanalo-Contreras, K. and Agrafiotis, D. K. (2012). Recognizing pitfalls in virtual screening: a critical review. J Chem Inf Model, 52, 867-881. doi: 10.1021/ci200528d.
- Schierz, A. C. (2009). Virtual screening of bioassay data. J Cheminform, 1, 21. doi: 10.1186/1758-2946-1-21.
- Schmidtke, P., Le Guilloux, V., Maupetit, J. and Tuffery, P. (2010). fpocket: online tools for protein ensemble pocket detection and tracking. *Nucleic Acids Res*, **38**, W582-589. doi: 10.1093/nar/gkq383 gkq383 [pii].

- Sharlow, E., Golden, J. E., Dodson, H., Morris, M., Hesser, M., Lyda, T., Leimgruber, S., Schroeder, C. E., Flaherty, D. P., Weiner, W. S., Simpson, D., Lazo, J. S., Aube, J. and Morris, J. C. (2010a). Identification of Inhibitors of Trypanosoma brucei Hexokinases. doi: NBK63599 [bookaccession].
- Sharlow, E. R., Lyda, T. A., Dodson, H. C., Mustata, G., Morris, M. T., Leimgruber, S. S., Lee, K. H., Kashiwada,
   Y., Close, D., Lazo, J. S. and Morris, J. C. (2010b). A target-based high throughput screen yields
   Trypanosoma brucei hexokinase small molecule inhibitors with antiparasitic activity. *PLoS Negl Trop Dis*, 4, e659. doi: 10.1371/journal.pntd.0000659.
- Shen, M. Y. and Sali, A. (2006). Statistical potential for assessment and prediction of protein structures. *Protein Sci*, 15, 2507-2524. doi: 15/11/2507 [pii]
- 10.1110/ps.062416606.
- Sindermann, H., Croft, S. L., Engel, K. R., Bommer, W., Eibl, H. J., Unger, C. and Engel, J. (2004). Miltefosine (Impavido): the first oral treatment against leishmaniasis. *Med Microbiol Immunol*, **193**, 173-180. doi: 10.1007/s00430-003-0201-2.
- Singh, J., Srivastava, A., Jha, P., Sinha, K. K. and Kundu, B. (2015). L-Asparaginase as a new molecular target against leishmaniasis: insights into the mechanism of action and structure-based inhibitor design. *Mol Biosyst*, **11**, 1887-1896. doi: 10.1039/c5mb00251f.
- Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeddis, J. and Barlogie, B. (1999). Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med, 341, 1565-1571. doi: 10.1056/NEJM199911183412102.

Schneider, G. (2010). Virtual screening: an endless staircase? *Nat Rev Drug Discov*, 9, 273-276. doi: 10.1038/nrd3139

nrd3139 [pii].

Smithson, D. C., Lee, J., Shelat, A. A., Phillips, M. A. and Guy, R. K. (2010). Discovery of potent and selective inhibitors of Trypanosoma brucei ornithine decarboxylase. J Biol Chem, 285, 16771-16781. doi: 10.1074/jbc.M109.081588

M109.081588 [pii].

Soares, M. B., Silva, C. V., Bastos, T. M., Guimaraes, E. T., Figueira, C. P., Smirlis, D. and Azevedo, W. F., Jr. (2012). Anti-Trypanosoma cruzi activity of nicotinamide. *Acta Trop*, **122**, 224-229. doi: 10.1016/j.actatropica.2012.01.001

S0001-706X(12)00002-2 [pii].

Soares Medeiros, L. C., South, L., Peng, D., Bustamante, J. M., Wang, W., Bunkofske, M., Perumal, N., Sanchez-Valdez, F. and Tarleton, R. L. (2017). Rapid, Selection-Free, High-Efficiency Genome Editing in Protozoan Parasites Using CRISPR-Cas9 Ribonucleoproteins. *MBio*, 8. doi: e01788-17 [pii] 10.1128/mBio.01788-17

mBio.01788-17 [pii].

Sosa, E. J., Burguener, G., Lanzarotti, E., Defelipe, L., Radusky, L., Pardo, A. M., Marti, M., Turjanski, A. G. and Fernandez Do Porto, D. (2018). Target-Pathogen: a structural bioinformatic approach to prioritize drug targets in pathogens. *Nucleic Acids Res*, 46, D413-D418. doi: 10.1093/nar/gkx1015

4584621 [pii].

- Sousa, S. F., Ribeiro, A. J., Coimbra, J. T., Neves, R. P., Martins, S. A., Moorthy, N. S., Fernandes, P. A. and Ramos, M. J. (2013). Protein-ligand docking in the new millennium--a retrospective of 10 years in the field. *Curr Med Chem*, **20**, 2296-2314. doi: CMC-EPUB-20130315-9 [pii].
- Spinks, D., Ong, H. B., Mpamhanga, C. P., Shanks, E. J., Robinson, D. A., Collie, I. T., Read, K. D., Frearson, J. A., Wyatt, P. G., Brenk, R., Fairlamb, A. H. and Gilbert, I. H. (2011). Design, synthesis and biological evaluation of novel inhibitors of Trypanosoma brucei pteridine reductase 1. *ChemMedChem*, 6, 302-308. doi: 10.1002/cmdc.201000450.
- Sterling, T. and Irwin, J. J. (2015). ZINC 15--Ligand Discovery for Everyone. J Chem Inf Model, 55, 2324-2337. doi: 10.1021/acs.jcim.5b00559.
- Stevanovic, S., Perdih, A., Sencanski, M., Glisic, S., Duarte, M., Tomas, A. M., Sena, F. V., Sousa, F. M., Pereira, M. M. and Solmajer, T. (2018). In Silico Discovery of a Substituted 6-Methoxy-quinalidine with Leishmanicidal Activity in Leishmania infantum. *Molecules*, 23. doi: E772 [pii]

10.3390/molecules23040772

molecules23040772 [pii].

Stevanovic, S., Sencanski, M., Danel, M., Menendez, C., Belguedj, R., Bouraiou, A., Nikolic, K., Cojean, S., Loiseau, P. M., Glisic, S., Baltas, M. and Garcia-Sosa, A. T. (2019). Synthesis, In Silico, and In Vitro Evaluation of Anti-Leishmanial Activity of Oxadiazoles and Indolizine Containing Compounds Flagged against Anti-Targets. *Molecules*, 24. doi: E1282 [pii]

10.3390/molecules24071282

molecules24071282 [pii].

Steverding, D. (2008). The history of African trypanosomiasis. *Parasit Vectors*, **1**, 3. doi: 10.1186/1756-3305-1-3

1756-3305-1-3 [pii].

- Stouch, T. R., Kenyon, J. R., Johnson, S. R., Chen, X. Q., Doweyko, A. and Li, Y. (2003). In silico ADME/Tox: why models fail. *J Comput Aided Mol Des*, **17**, 83-92. doi: 10.1023/a:1025358319677.
- Stumpfe, D. and Bajorath, J. (2012). Exploring activity cliffs in medicinal chemistry. J Med Chem, 55, 2932-2942. doi: 10.1021/jm201706b.
- Sun, W., Sanderson, P. E. and Zheng, W. (2016). Drug combination therapy increases successful drug repositioning. *Drug Discovery Today*, **21**, 1189-1195. doi: 10.1016/j.drudis.2016.05.015.
- Talevi, A., Gavernet, L. and Bruno-Blanch, L. E. (2009). Combined Virtual Screening Strategies. *Current Computer-Aided Drug Design*, **5**, 23-37. doi: <u>http://dx.doi.org/10.2174/157340909787580854</u>.

The PyMOL Molecular Graphics System, V. S., LLC.

Tiwari, N., Tanwar, N. and Munde, M. (2018). Molecular insights into trypanothione reductase-inhibitor interaction: A structure-based review. Arch Pharm (Weinheim), 351, e1700373. doi: 10.1002/ardp.201700373.

Todeschini, R., Consonni, V. and Gramatica, P. (2009). Chemometrics in QSAR. Comprehensive Chemometrics.

Torrie, L. S., Wyllie, S., Spinks, D., Oza, S. L., Thompson, S., Harrison, J. R., Gilbert, I. H., Wyatt, P. G., Fairlamb, A. H. and Frearson, J. A. (2009). Chemical validation of trypanothione synthetase: a potential drug target for human trypanosomiasis. J Biol Chem, 284, 36137-36145. doi: 10.1074/jbc.M109.045336 M109.045336 [pii].

- Tropsha, A. (2010). Best Practices for QSAR Model Development, Validation, and Exploitation. *Mol Inform*, 29, 476-488. doi: 10.1002/minf.201000061.
- Trott, O. and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem, 31, 455-461. doi: 10.1002/jcc.21334.
- Uran Landaburu, L., Berenstein, A. J., Videla, S., Maru, P., Shanmugam, D., Chernomoretz, A. and Aguero, F. (2019). TDR Targets 6: driving drug discovery for human pathogens through intensive chemogenomic data integration. *Nucleic Acids Res.* doi: gkz999 [pii]

10.1093/nar/gkz999

5611677 [pii].

- Urbina, J. A. (2015). Recent clinical trials for the etiological treatment of chronic chagas disease: advances, challenges and perspectives. *J Eukaryot Microbiol*, **62**, 149-156. doi: 10.1111/jeu.12184.
- Vainio, M. J., Puranen, J. S. and Johnson, M. S. (2009). ShaEP: molecular overlay based on shape and electrostatic potential. *J Chem Inf Model*, **49**, 492-502. doi: 10.1021/ci800315d

10.1021/ci800315d [pii].

Valera-Vera, E. A., Saye, M., Reigada, C., Miranda, M. R. and Pereira, C. A. (2020). In silico repositioning of etidronate as a potential inhibitor of the Trypanosoma cruzi enolase. J Mol Graph Model, 95, 107506. doi: S1093-3263(19)30584-4 [pii]

10.1016/j.jmgm.2019.107506.

Valera Vera, E. A., Saye, M., Reigada, C., Damasceno, F. S., Silber, A. M., Miranda, M. R. and Pereira, C. A. (2016). Resveratrol inhibits Trypanosoma cruzi arginine kinase and exerts a trypanocidal activity. *Int J Biol Macromol,* **87**, 498-503. doi: 10.1016/j.ijbiomac.2016.03.014

van Griensven, J. and Diro, E. (2019). Visceral Leishmaniasis: Recent Advances in Diagnostics and Treatment Regimens. Infect Dis Clin North Am, 33, 79-99. doi: S0891-5520(18)30092-8 [pii]

10.1016/j.idc.2018.10.005.

- Vazquez, C., Mejia-Tlachi, M., Gonzalez-Chavez, Z., Silva, A., Rodriguez-Zavala, J. S., Moreno-Sanchez, R. and Saavedra, E. (2017). Buthionine sulfoximine is a multitarget inhibitor of trypanothione synthesis in Trypanosoma cruzi. FEBS Lett, 591, 3881-3894. doi: 10.1002/1873-3468.12904.
- Villalta, F., Dobish, M. C., Nde, P. N., Kleshchenko, Y. Y., Hargrove, T. Y., Johnson, C. A., Waterman, M. R., Johnston, J. N. and Lepesheva, G. I. (2013). VNI cures acute and chronic experimental Chagas disease. J Infect Dis, 208, 504-511. doi: 10.1093/infdis/jit042

jit042 [pii].

- Walvekar, P., Gannimani, R. and Govender, T. (2019). Combination drug therapy via nanocarriers against infectious diseases. *European Journal of Pharmaceutical Sciences*, **127**, 121-141. doi: 10.1016/j.ejps.2018.10.017.
- Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R. and Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*, 46, W296-W303. doi: 10.1093/nar/gky427 5000024 [pii].
- Webb, B. and Sali, A. (2016). Comparative Protein Structure Modeling Using MODELLER. *Curr Protoc Protein Sci*, 86, 2 9 1-2 9 37. doi: 10.1002/cpps.20.
- Wiggers, H. J., Rocha, J. R., Fernandes, W. B., Sesti-Costa, R., Carneiro, Z. A., Cheleski, J., da Silva, A. B., Juliano, L., Cezari, M. H., Silva, J. S., McKerrow, J. H. and Montanari, C. A. (2013). Non-peptidic cruzain inhibitors with trypanocidal activity discovered by virtual screening and in vitro assay. *PLoS Negl Trop Dis*, 7, e2370. doi: 10.1371/journal.pntd.0002370

PNTD-D-12-00698 [pii].

Wilkinson, S. R. and Kelly, J. M. (2009). Trypanocidal drugs: mechanisms, resistance and new targets. *Expert Rev Mol Med*, **11**, e31. doi: 10.1017/S1462399409001252

S1462399409001252 [pii].

 Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., Pon, A., Knox, C. and Wilson, M. (2018). DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res, 46, D1074-D1082. doi: 10.1093/nar/gkx1037

4602867 [pii].

S0141-8130(16)30238-0 [pii].

- Wolber, G. and Langer, T. (2005). LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. *J Chem Inf Model*, **45**, 160-169. doi: 10.1021/ci049885e.
- Wyatt, P. G., Gilbert, I. H., Read, K. D. and Fairlamb, A. H. (2011). Target validation: linking target and chemical properties to desired product profile. *Curr Top Med Chem*, **11**, 1275-1283. doi: BSP/CTMC/E-Pub/-00065-11-9 [pii].
- Wyllie, S., Brand, S., Thomas, M., Rycker, M. D., Chung, C.-w., Pena, I., Shishikura, Y., Spinks, D., Stojanovski,
   L., Thomas, J., Thompson, S., Viayna, E., Martin, J., Gray, D. W., Miles, T. J., Gilbert, I. H., Read, K. D.,
   Marco, M. and Wyatt, P. G. (2019). Preclinical candidate for the treatment of visceral leishmaniasis
   that acts through proteasome inhibition. *Proceedings of the National Academy of Sciences*, 116, 9318-9323. doi: 10.1073/pnas.1820175116.
- Wyllie, S., Patterson, S., Stojanovski, L., Simeons, F. R., Norval, S., Kime, R., Read, K. D. and Fairlamb, A. H. (2012). The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis. *Sci Transl Med*, **4**, 119re111. doi: 10.1126/scitranslmed.3003326

4/119/119re1 [pii].

Xu, M. and Lill, M. A. (2013). Induced fit docking, and the use of QM/MM methods in docking. *Drug Discov Today Technol*, **10**, e411-418. doi: 10.1016/j.ddtec.2013.02.003

S1740-6749(13)00010-3 [pii].

Xue, H., Li, J., Xie, H. and Wang, Y. (2018). Review of Drug Repositioning Approaches and Resources. Int J Biol Sci, 14, 1232-1244. doi: 10.7150/ijbs.24612

ijbsv14p1232 [pii].

Yang, J. and Zhang, Y. (2015). I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res*, 43, W174-181. doi: 10.1093/nar/gkv342

gkv342 [pii].

Yang, X., Wu, X., Zhang, J., Zhang, X., Xu, C., Liao, S. and Tu, X. (2017). Recognition of hyperacetylated Nterminus of H2AZ by TbBDF2 from Trypanosoma brucei. *Biochem J*, 474, 3817-3830. doi: 10.1042/BCJ20170619

BCJ20170619 [pii].

- Yap, C. W. (2011). PaDEL-descriptor: an open source software to calculate molecular descriptors and fingerprints. J Comput Chem, 32, 1466-1474. doi: 10.1002/jcc.21707.
- Zhou, S., Wang, F., Hsieh, T. C., Wu, J. M. and Wu, E. (2013). Thalidomide-a notorious sedative to a wonder anticancer drug. *Curr Med Chem*, 20, 4102-4108. doi: CMC-EPUB-54980 [pii].

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Disease	Organism	Transmission	Epidemiology	Drugs	Main clinical manifestations
Chagas disease or American trypanosomiasis	Trypanosoma cruzi	Vectorial (triatomine insect) Mother-to-child transmission Organ transplant and blood transfusion Oral transmission through contaminated food Sexual transmission Laboratory accidents	Latin America 6-7 million infected people <40,000 new cases per year 70 million people at risk	Nifurtimox Benznidazole	Acute phase: Fever, inflammation at the inoculation site, increased lymph nodes, muscle pain, headaches <u>Chronic phase</u> : Cardiomyopathy (severe arrhythmia, heart muscle failures and embolism) and digestive forms (megaoesophagus, megacolon)
Human African trypanosomiasis or sleeping sickness	Trypanosoma brucei	Vectorial (tse-tse fly) Transmission through other blood-sucking insects Mother-to-child transmission Sexual transmission Laboratory accidents	Sub-Saharan Africa 300,000 infected people <2,500 new cases per year 60 million people at risk	Suramin Pentamidine Melarsoprol Eflornithine/ Nifurtimox Fexinidazole	<u>First or early stage</u> : Fever, headaches, muscle and joint pains, lymphadenopathy <u>Second or brain stage</u> : Neurological and psychological symptoms (sleep disorders, ataxia, sensory alterations, hallucinations, personality changes)
Leishmaniasis: -visceral (VL) -cutaneous (CL) -mucocutaneous (ML)	<i>Leishmania</i> spp.	Vectorial (sand fly) Mother-to-child transmission Organ transplant and blood transfusion Zooanthroponotic transmission Sexual transmission	Worldwide, except Australia and Antarctica 12 million infected people 2 million new cases per year 350 million people at risk	Miltefosine Amphotericin B Paromomycin	VL: persistent irregular fevers, splenomegaly, pancytopenia, hepatomegaly, hypergammaglobulinemia, weight loss CL: ulcerating lesions ML: destructive lesions of the nasal septum, lips, and palate

**Table 1.** Principal features of trypanosomatid-caused diseases.

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For more information about these diseases see: Chagas disease <u>https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis);</u> human African trypanosomiasis <u>https://www.who.int/news-room/fact-sheets/detail/chagas-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness);</u> leishmaniasis <u>https://www.who.int/news-room/fact-sheets/detail/chagas-sheets/detail/leishmaniasis.</u>

Target	Process	Localization	Organism	Drugs	Reference
Alternative oxidase	Electron transport chain	Mitochondria	T. brucei	Aromatic hydroxamates	Menzies et al., 2018
Cytochrome b	Electron transport chain	Mitochondria	T. cruzi	GNF7686	Khare <i>et al.</i> , 2015
Lanosterol 14- α- demethylase	Ergosterol biosynthesis	Mitochondria and endoplasmatic reticulum	T. cruzi	Posaconazole Ravuconazole	Morillo <i>et al.</i> , 2017; Urbina, 2015
Trypanothione synthase	Thiol metabolism	Cytoplasm	T. brucei T. cruzi Leishmania spp.	Revised in the references	Vazquez <i>et al.</i> , 2017; Leroux & Krauth- Siegel, 2016; <u>Torrie <i>et</i></u> <i>al.</i> , 2009
Trypanothione reductase	Thiol metabolism	Cytoplasm	T. brucei T. cruzi Leishmania spp.	Revised in the references	Leroux & Krauth- Siegel, 2016; Vazquez <i>et al.</i> , 2017; Lu <i>et al.</i> , 2013
Ornithine decarboxilase	Polyamine metabolism	Cytoplasm	T. brucei	DFMO	Burri & Brun, 2003
TcPAT12	Polyamine metabolism	Flagellar pocket	T. cruzi	Isotretinoin ANT4 Triclabendazole Sertaconazole Paroxetine Cisapride	Alberca <i>et al.</i> , 2016; Reigada <i>et al.</i> , 2018; Reigada <i>et al.</i> , 2017 <u>;</u> Dietrich <i>et al.</i> , 2018
Enolase	Glycolisis	Cytoplasm and cell surface	T. brucei T. cruzi Leishmania spp.	Phosphonoacetohydr oxamate	Avilan <i>et al.</i> , 2011
Piruvate kinase	Glycolisis	Cytoplasm	<i>Leishmania</i> spp.	Furanose sugar amino amides	Nowicki et al., 2008
Phosphofructokinase	Glycolisis	Glycosome	T. brucei T. cruzi	ML251 Furanose sugar amino amides	Brimacombe <i>et al.</i> , 2014; Nowicki <i>et al.</i> , 2008
Hexokinase	Glycolisis	Glycosome	T. brucei	EbSe Revised in the references	Lu <i>et al.</i> , 2013 <u>;</u> Sharlow <i>et al.</i> , 2010
	P				

# Table 2. Drug targets in *Trypanosoma* and *Leishmania* parasites.

Table 3. List of c	databases of	interest for	drug virtual	screening.

Database	Description	Web-link	Reference
SwissDock	Molecular docking with rigid target	http://www.swissdock.ch/	Sterling & Irwin, 2015
ChEMBL	1.9 million curated bioactive molecules including when known their activities, molecular targets, tissue absorption, indication, development and approval state, molecular assays, physico- chemical properties, and related genomic data	https://www.ebi.ac.uk/chembl/	Gaulton <i>et</i> <i>al.,</i> 2017
PubChem	102 million compounds with reported bioactivity, safety and toxicity, patents, citations, physico- chemical properties, and more. It includes a molecule drawing tool for search.	https://pubchem.ncbi.nlm.nih.gov/	Kim <i>et al.,</i> 2019
SWEETLEAD	Chemical structures of >9000 approved medicines, illegal drugs, and isolates from traditional medicinal herbs.	https://simtk.org/projects/sweetlead	Novick <i>et al.,</i> 2013
DrugBank	Contains>13400 entries including approved drugs, nutraceuticals, experimental and illicit drugs. Additionally, >5000 non-redundant proteins linked to the drug entries.	https://www.drugbank.ca/	Wishart <i>et</i> <i>al.,</i> 2018
Enamine	Compound librarias available for purchase in the	https://enamine.net/	-
Asinex	vondors wobsitos	http://www.asinex.com/	-
Maybridge	vendors websites.	http://www.maybridge.com/	-
TDR Targets	Contains information about genes and targets from 21 bacterial and eukaryotic tropical pathogens, phylogeny, >2 million bioactive compounds, and the possibility of specifying the search criteria to prioritize drug targets.	https://tdrtargets.org	Uran Landaburu <i>et al.,</i> 2019
Protein Data Bank	Curated and annotated archive about the experimentally determined 3D shapes of proteins, nucleic acids, and complex assemblies	https://www.rcsb.org/	Berman <i>et</i> <i>al.,</i> 2000
ModBase	Comparative protein structure models calculated by the ModPipe pipeline	https://modbase.compbio.ucsf.edu/	Pieper <i>et al.,</i> 2014

by the ModPipe pipeline

# Table 4. Software of interest for computer-aided drug discovery.

Software	Free license	Capabilities	Web-link	Reference
SwissDock	Yes	Molecular docking with rigid target	http://www.swissdock.ch/	Grosdidier <i>et al.,</i> 2011
AutoDock 4	Yes	Molecular docking with rigid target or allowing flexible residues	http://autodock.scripps.edu/	Morris <i>et</i> al., 2009
AutoDock Vina	Yes	Rigid docking, online server	<u>http://vina.scripps.edu/</u> I docking, ne server	
ZDOCK	Yes		https://zlab.umassmed.edu/zdock/	Pierce <i>et</i> <i>al.,</i> 2011
OEDocking	Trial	Molecular docking, flexible fitting, 2D and 3D similarity	https://www.eyesopen.com/oedocking	Kelley <i>et</i> <i>al.,</i> 2015
ООСК	Yes	Molecular docking with rigid target	http://dock.compbio.ucsf.edu/	Allen <i>et al.,</i> 2015
GOLD	No	Molecular docking with rigid target, side-chain flexibility, and ensemble docking	https://www.ccdc.cam.ac.uk/solutions/csd- discovery/components/gold/	Jones <i>et al.,</i> 1997
Glide	No	Protein	https://www.schrodinger.com/glide	Friesner <i>et</i> <i>al.,</i> 2006
FlexX	No	homology modelling	https://www.biosolveit.de/flexx/	Rarey <i>et</i> <i>al.,</i> 1996
SwissModel	Yes		https://swissmodel.expasy.org/	Waterhous e <i>et al.,</i> 2018
I-TASSER	Yes	Setting of docking	<u>https://zhanglab.ccmb.med.umich.edu/I-</u> <u>TASSER/</u>	Yang & Zhang, 2015
Modeller	Yes	parameters, docking results and molecular	https://salilab.org/modeller/	Webb & Sali, 2016
AutoDock Tools	Yes	visualization	http://autodock.scripps.edu/	Morris <i>et</i> al., 2009

Pymol	Yes	Molecular visualization	https://pymol.org/	The PyMOL Molecular Graphics System
VMD	Yes	Ligand and	https://www.ks.uiuc.edu/Research/vmd/	Humphrey et al., 1996
Ligand Scout	Trial	receptor-based pharmacophore s	http://www.inteligand.com/ligandscout/	Wolber & Langer, 2005
Discovery Studio	Visualizer only	Ligand and receptor-based pharmacophore, docking, ligand design, physico- chemical predictions, molecular graphics	https://www.3dsbiovia.com/	BIOVIA, 2017
Phase	No	Ligand and receptor-based pharmacophore s, 3D QSAR	https://www.schrodinger.com/phase/	Dixon <i>et</i> al., 2006
LiSiCA	Yes	2D and 3D similarity	http://insilab.org/lisica/	Lesnik <i>et</i> <i>al.,</i> 2015
ShaEP	Yes	3D small molecule alignment and similarity	http://users.abo.fi/mivainio/shaep/	Vainio <i>et</i> al., 2009
fPocket	Yes	Protein pocket prediction	http://fpocket.sourceforge.net/	Schmidtke <i>et al.,</i> 2010
Gromacs	Yes	Molecular dynamics	http://www.gromacs.org/	James Abraham, 2015
AMBER suite	No		https://ambermd.org/	D.A. Case, 2018
Dalton	Yes	Calculation of molecular	https://daltonprogram.org/	Aidas <i>et al.,</i> 2014
PaDEL	Yes	descriptors 1D, 2D, and 3D	http://www.yapcwsoft.com/dd/padeldescript or/	Yap, 2011

Drug/Structure	Original indication	Target	Organism	Repurposed/ Progress	Reference
Isotretinoin $\overset{H_{3}C}{\underset{H_{3}C}{\leftarrow}}\overset{H_{3}C}{\underset{H_{3}C}{\leftarrow}}\overset{OH}{\underset{H_{3}C}{\leftarrow}}$	Acute acne	Polyamine permease	T. cruzi	Effectiveness in animal model	Reigada <i>et al.</i> , 2017
Cisapride $f$	Gastroesophageal reflux	Polyamine permease	T. cruzi	Evaluation in <i>T.</i> <i>cruzi</i> clinically relevant forms	Dietrich <i>et al.</i> , 2018
Miglitol	Diabetes mellitus type 2	N-glycan biosynthesis (predicted)	L. amazonensis L. infantum	Effectiveness in animal model	Chávez-Fumagalli et al., 2019
Eflornithine (6) HO HO HO HO H2N F	Cancer	Polyamine metabolism	T. brucei	Human African trypanosomiasis	Burri & Brun, 2003
Nifurtimox (2)	Chagas disease	Macromolecules	T. brucei gambiense	Second stage human African trypanosomiasis, in combination with eflornithine	Priotto <i>et al.</i> , 2009; Hall <i>et al.</i> , 2012

**Table 5. Repurposed drugs.** Examples of repositioned drugs against trypanosomatid caused diseases including the actual treatment, protein target and the new drug indication.

Fexinidazole (7)	Broad-spectrum antimicrobial agent	DNA	T. brucei gambiense T. cruzi	Phase III clinical trials for African trypanosomiasis Clinical trials for Chagas disease	Raether & Seidenath, 1983; Deeks, 2019 Bahia <i>et al.</i> , 2012; Francisco <i>et al.</i> , 2016
HoC					
Amphotericin B (12)	Antifungal agent	Ergosterol	Leishmania spp.	Visceral leishmaniasis	Meyerhoff, 1999; Roberts <i>et al.</i> , 2003
				×	
Paromomycin (11)	Antibiotic (bacteria, protozoa and cestodes)	Cytoplasmic and mitochondrial protein synthesis	Leishmania spp.	Visceral and cutaneous leishmaniasis	Davidson <i>et al.</i> , 2009; Jhingran <i>et al.</i> , 2009; Jain & Jain, 2013
			NUS		
Miltefosine (10)	Cutaneous cancer	Lipid biosynthesis	Leishmania spp.	Visceral and cutaneous leishmaniasis	Sindermann <i>et al.</i> , 2004; Dorlo <i>et al.</i> , 2012; Ortega <i>et al.</i> ,
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0			2017; Pinto- Martinez <i>et al.</i> , 2018

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**Fig. 1. Chemical structures.** Detailed structures of approved drugs to treat Chagas disease (1 and 2), human African trypanosomiasis (3–7) and leishmaniasis (8–12).



**Fig. 2. Common virtual screening techniques.** Different VS approaches based on the available information about the protein targets and/or ligands. Receptor-based VS requires experimentally determined 3D structures or good-quality homology models that, in the case of trypanosomatid, are scarce. In the case of ligand-based VS, only small molecules (substrates, inhibitors, etc.) that interact with the target protein are needed.

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**Fig. 3. Membrane transporters as drug targets.** Ligand- and receptor-based VS was applied in this example to identify inhibitors of the *T. cruzi* polyamine permease (Reigada *et al.*, 2017). Retinol acetate was first reported as a leishmanicidal compound that reduce the intracellular concentration of polyamines (Mukhopadhyay & Madhubala, 1994). Through a similarity VS using a database of FDA approved drugs and retinol acetate as a reference molecule, a group of candidate drugs was identified. After a second step of receptor-based VS (molecular docking) followed by *in vitro* assays, it was demonstrated that the retinoid isotretinoin is a polyamine transport inhibitor with a strong anti-*T. cruzi* activity (A). Some advantages of membrane transport processes are the only way to obtain essential metabolites (i.e. polyamines in *T. cruzi*); the presence of extracellular spans in the transporter facilitate the accessibility of the drugs; some inhibitors are incorporated to the cell presenting additional intracellular targets such as enzymes or nucleic acids.