Modern Approaches for the Discovery of Anti-Infectious Drugs for the Treatment of Neglected Diseases

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Abstract: Neglected diseases comprise a number of infectious diseases historically endemic to low- and middle-income countries, though recently they have spread to high-income countries due to human migrations. In the past, pharmaceutical companies have shown hesitant to invest in these health conditions, due to the limited return on investment. As a result, the role of the academic sector and non-for-profit organizations in the discovery of new drugs for neglected diseases has been particularly relevant.

Here, we review recent applications of modern drug discovery technologies in the field of neglected diseases, including high-throughput screening, *in silico* screening and computer-aided drug design. The suitability and perspectives of each approach is discussed depending on the context, along with the technology and translational gaps influencing them.

Keywords: Drug repurposing, Drug repositioning, In silico screening, Virtual Screening, High-throughput Screening, Computer-Aided Drug Discovery, Neglected diseases, Infectious diseases

1. INTRODUCTION

Neglected tropical diseases (NTD) encompass a diversity of communicable diseases (at present, 20, according to World Health Organization - WHO) that prevail in tropical and subtropical conditions; they particularly affect populations living in poverty, without appropriate sanitation and in close contact with infectious vectors [1]. NTD can be in general considered high-morbidity but low-mortality infections; accordingly, their impact may be best understood in terms of disability-adjusted life years (DALYs), which puts them among the top disabling conditions [2]. It should be noted that, whereas almost all NTD are infectious in nature, snakebite envenoming has recently been included in this category by WHO.

In the last years a positive trend has been perceived in the field of NTD (in some cases with more emphasis than in others); the "neglected" status appears to be slowly but steadily reversing [3-5]. Nevertheless, around 90% of the limited investment in research and development on NTD still comes from public organizations or philanthropic donors [6]. Such landscape implies that the development of novel diagnostic and therapeutic solutions for NTD is not a strictly biological or medical matter, but should always take into consideration economic and social aspects.

Consequently, drug discovery and development in the context of NTD must become as much rational as possible, and not only take into consideration improved efficacy and/or safety of the emerging drug candidates, but also other relevant aspects such as cost or ease of administration and administration logistics [7-8].

But what do we mean when we say "rationality" within the drug discovery community? There, rationality usually integrates different dimensions: efficiency (including cost- and time-efficiency) and knowledge (in the drug discovery field, understanding the underlying mechanisms that explain a biological response is much appreciated). In general, the rationality also involves a (quantitative) cost-benefit analysis and cultural concerns (e.g. environment-friendly green chemistry synthesis procedures are most welcome, and so are reduction and refinement practices in relation with the use of *in vivo* models to screen for drugs or to characterize the pharmacological and safety profile of a drug candidate).

Here, we will review modern approaches towards the discovery of new drugs for the treatment of NTD, namely high-throughput screening (HTS), virtual screening (VS), rational drug design and systematic drug repositioning. All of them involve different degrees of rationality and technological requirements, that will be opportunely discussed. We will thus concentrate on rational methods that can make a contribution at hit discovery, lead identification and lead optimization phases; previous stages of the drug discovery process (basic research involving target identification and validation) are considered out of the scope of this review. In each case, we will present recent applications in the field of NTD (preferably, from 2014 onwards). In the case of reports of HTS assays, we have focused on articles that include, at least, pilot screens. In the case of computer-guided methods, we have focused on drug discovery campaigns including experimental validation of the emerging hits.

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2. HIT IDENTIFICATION

The accessible chemical space is growing exponentially. Back in 2012, the number of compounds indexed in the Chemical Abstract Service accounted to about 70 million. Presently, that number sums more than 130 million, which speaks of an astonishing average expansion of 12 million new chemical entities per year during the last 5 years. According to the current numbers in PubChem Compound (now more than 93 million entries) and ZINC databases (over 35 million entries), a considerable proportion of the accessible chemical space comprises small, druglike molecules. This represents an encouraging picture, since it speaks of an impressive chemical diversity to seek for new bioactive scaffolds which in turn will undergo lead-finding and lead optimization programs. However, it also poses a practical question: how can we explore such a vast chemical space in an efficient manner?

During the hit identification phase of the drug discovery process compound screening assays are developed. A hit molecule is a compound which has the desired activity in a compound screen and acts as a chemical starting point for drug discovery projects [9]; importantly, activity must be confirmed upon retesting [10]. The potency of a hit will usually be in the high nM to low μM range.

Independently of the chosen screening system, the screening campaign will likely result in not one but many hits, i.e. a hit series. The drug discovery team must then decide which compounds are the best to work on. The list of compounds taken forward should include a broad spectrum of chemical classes and demonstrate concentration-dependent behavior in the primary assay [9, 10]. Furthermore, the effects of the compounds in a secondary assay for the target of choice, if available, should be examined. Reversible compounds are often favored.

A diversity of screening paradigms exists to identify hit molecules, which are separately described in the following subsections.

2.1. High-throughput screening and focused libraries

HTS couples automation and miniaturization to allow the *in vitro* screening of tens or hundreds of thousands of compounds per day [11]. While originally focused on biochemical screens against relatively simple systems (e.g. an isolated enzyme), HTS has experienced a remarkable evolution by expanding to cell-based screens [12-14].

Increased emphasis placed by regulatory agencies on knowing the mechanism of action of new drugs encourages pursuing clearly defined molecular targets [15]; cell-based screening potentially faces a costly and lengthy target deconvolution process and displays higher variability [8, 15]. However, there are also advantages to phenotypic screening: the results in cell-based systems may provide early information on a number of pharmaceutically relevant processes besides drug-target interaction (e.g. cell uptake, drug metabolism) [16, 17]. Cell-based screens can include multiple targets in a single screen and reveal valuable and unforeseen drug targets [8, 18, 19]. Cell-based HTS can be of particular interest in the field of anti-infectious drug development, where target-focused discovery has not been as productive as phenotypic screening [8, 9, 19-21] and multi-target agents could display reduced probability of drug resistance issues [22, 23].

False positives and false negatives are a persistent issue when implementing HTS studies [24]. Besides using adequate counterscreens, one effective strategy to minimize these errors involves getting rid of frequent hitters in HTS campaigns: the pan-assay interference compounds (PAINS) [10, 25]. PAINS are compounds that display apparent bioactivity across unrelated biological targets and testing methods, either due to promiscuity or assay interference [25]. However, such early pruning approach has been questioned, when automated, due to the possibility of discarding potentially valuable compounds containing privileged scaffolds [26].

A breakthrough in the field has been the implementation of quantitative HTS (qHTS): modern screening capacity allows the determination of concentration responses as the initial screening result [19]. For instance, the seminal study by Miller et al. resorted to microfluidics [27] to generate, for each screened compound and in only 3 seconds per compound, a high-resolution dose–response profile containing around 10,000 data points (compared with the usual 7 to 10 data points), allowing determination of the IC_{50}. It is expected that qHTS approach will greatly reduce the number of false positives and false negatives in HTS campaigns [24, 28].

Where does the rationality of HTS lies? First (and obvious), in time efficiency: automation allows high-throughput and liberates qualified staff from routine tasks. Second, in miniaturization: small samples of the screened compounds and assay reactants are required. At last, HTS is in good agreement with bioethical principles that demand the use of *in silico* and/or *in vitro* assays before advancing to animal models. Unfortunately, the reductionist approach (biochemical or cell-based screens) are not always appropriate when complex disorders (e.g. depression, schizophrenia) are addressed. It may be observed that, aside from the intrinsic efficiency of HTS, it is a "brute force" approximation if used to explore the chemical space in a random manner. In contrast, the rationality of the approach is greatly boosted when HTS is used to search focused libraries, i.e. relatively small libraries of molecules that are likely to have a pursued activity based on knowledge of the target protein and literature precedents for the chemical classes likely to have activity at the drug target [10, 29].

It should be noted, though, that HTS is with no doubt the most technologically demanding approach among those reviewed in the present article. It requires complex and costly technological platforms which are also expensive from an operative point of view. There are relatively few HTS facilities within academia and most of them are concentrated at high-income countries. Researchers from most of the countries affected by NTD would not access to these technologies if not through scientific collaborations or public-private partnerships. The gap is even wider in some particular cases, e.g. when sophisticated imaging resources are required.

HTS has been unevenly applied in the field of NTD, with many applications in some cases and none in others. The most prominent efforts are observed in the fields of Dengue, trypanosomatid-caused infections, schistosomiasis and other helminth-caused conditions, a fact that suggests that, even within the NTD area, some diseases are more neglected than others regarding application of modern drug discovery strategies.

Back in 2014, Basavannacharya and Vasudevan reported a fluorescence-based HTS assay capable of identifying inhibitors against the helicase activity of the Dengue virus non-structural protein 3 [30]. The assay uses a duplex RNA substrate containing a fluorophore on the 5′ end and a quencher on the 3′ end of one of the strands. It was adapted to 384-well plates, with an average Z' factor of 0.65 and signal to noise ratio of 6. The assay was validated with a small library of 1,600 compounds and suramin was identified as inhibitor, with a *Ki* of high nM order. A high-quality high-throughput lead-finding campaign to discover Dengue virus RNA polymerase was conducted by Smith et al. in 2015 [31]. For that purpose, the authors developed two biochemical assays (a fluorescence-coupled assay using a modified nucleotide analogue and an orthogonal label free assay using the four native nucleotides and measuring, via liquid chromatography–mass spectrometry, the pyrophosphate liberated during nucleotide incorporation). The authors also used a cellular renilla luciferase–based replicon assay to monitor cell activity of the inhibitors. The screen was performed on different Novartis compound collections (totaling more than 250,000 compounds). The hits from the primary screen (fluorescence-coupled assay) were confirmed and, after proper counterscreens, removal of frequent hitters and confirmation of concentration-dependent response, the surviving candidates were submitted to the orthogonal screen. Only 300 compounds reached the cell-based assays, Forty-two of which displayed an EC_{50} below 30 μM. Five of them, regarded as high-priority hits, display a cytotoxic concentration (CC_{50}) above 30 μM. Also in 2015, Stolp et al. reported a cell-based platform to monitor Dengue virus pre-membrane (prM) protein processing [32]. The assay relies on an engineered two-tag scaffold; it discriminates between a single cell-surface tag when prM is cleaved and two tags when it is not, as detected by flow cytometry through fluorescent-coupled antibodies. The assay was miniaturized into a 96-well plate format and multiplexed with the HIV-1 envelope boundary. Interestingly, the staining procedure was further calibrated to avoid washes, in an attempt to streamline sample preparation and decrease the cost of antibodies for large-scale screens. The assay achieved an average Z' value of 0.74. A pilot screen against 1,280 compounds was performed, leading to the identification of an active compound (thiostrepton, IC_{50} = 4.94 μM). In addition to HTS campaigns targeting the Dengue etiologic agent, this approach has also been applied to find novel compounds to be potentially used in vector control [33, 34]. Very recently, Bilsland et al developed a yeast-based, high-throughput screening system whereby essential yeast genes are replaced with their filarial or human counterparts [35]. This platform could be useful to identify new hits as potential starting points for the development of new treatments against lymphatic filariasis. The yeast strains are labeled with different fluorescent proteins to allow the simultaneous monitoring of strains with parasite or human genes in competition. Therefore, it is possible to investigate the hit selectivity directly in the primary assay (identifying compounds that inhibit the parasite target without affecting its human ortholog). The authors constructed yeast strains expressing eight different *Brugia malayi* drug targets and seven of their human counterparts. Medium-throughput drug screens were performed using the Malaria Box collection (400 compounds) and nine filarial specific inhibitors were found, five of which confirmed activity against *Brugia pahangi* using *in vitro* assays.

In the field of schistosomiasis, Chant et al. developed a miniaturized screen of a *Schistosoma mansoni* serotonergic G protein-coupled receptor [36]. The assay relies on a permutated form of firefly luciferase incorporating a cAMP-binding domain from protein kinase A; cAMP-binding induces a conformational change in the enzyme that enhances the luminescent signal. HEK293 cells transiently transfected with either the human 5HT7 receptor or the schistosome receptor are used in the screening, which thus allows initial assessment of selectivity during the primary assay. The assay was used to screen a small commercial focused G protein-coupled receptor focused library and characterize the extent of pharmacological conservation between the parasite and humans. The primary screen identified Twenty-five compounds as potential antagonists of the parasite serotonin receptor. Two validation experiments were then performed to discard false positives. Twenty-three compounds were kept for subsequent validation; only a small proportion of these compounds displayed inhibition at both the human and parasite targets.

Leung et al. developed a whole-organism ultraHTS (uHTS) screening campaign on the 364K compound collection from the NIH Molecular Libraries Small Molecule Repository (MLSMR) in a 1536-well format [37]. They used a *Caenorhabditis elegans* strain expressing a GFP reporter, which encodes a Glutathione S-transferase strongly activated by SKN-1, a master regulator of detoxification genes. They obtained an average Z factor of 0.74 and signal to noise ratio of 34. Cheminformatic filters were applied to remove known PAINS and promiscuous compounds, and the surviving 1,381 hits were retested at six concentrations, resulting in 364 confirmed hits that were again retested at 10 doses leading to only 128 compounds. The authors then used a heat-shock protein assay as a counterscreen to assess the specificity of the effects observed in the primary assay and disregard off-target hits. Fifty-six hits were kept for further SAR studies.

In the field of trypanosomatid-caused infections, Diaz et al. performed a high-throughput screen (average Z' of 0.78 and mean signal to background > 5) testing 42,444 kinase focused inhibitors from the GlaxoSmithKline screening collection against *Trypanosoma brucei* cell cultures, with and counter-screened against human hepatocarcinoma (HepG2) cells for initial assessment of selectivity [38]. The collection included the Published Kinase Inhibitor Set, consisting in 369 kinase inhibitor compounds with inhibitory data available against 224 human kinases. The authors identified 797 sub-μM inhibitors that are at least 100-fold selective over HepG2 cells. The 797 hits were grouped into

59 clusters (plus 53 singletons), intended to prompt new studies of mechanism of action and further pursuit for drug optimization. The authors prioritized the compounds by application of a composite score that included, among other considerations, potency, rate of action, cidality and CNS bioavailiability. Three compounds advanced to pharmacokinetic assessment and one of the demonstrated parasitological cure of a murine bloodstream infection of *T. brucei rhodesiense*. Retrospective analysis of the hits revealed a correlation between the inhibitory activity against a particular subset of human kinases and the *T. brucei* ortholog (which was not observed against other trypanosomatids or *Plasmodium falciparum*) [39]. Faria et al. reported the use of SYBR Green–based whole-cell assay (which had been intensively used in antimalarial drug discovery) to be applied in HTS campaigns to discovery new treatments for African trypanosomiasis [40]. To validate their method, they performed a pilot screen on a kinase inhibitor-focused commercial library of 4,000 compounds and compared their results with the ones obtained with the resazurin assay. The same compounds had previously been counter-screened against several human cell lines; confirmatory counter-screen against THP-1 cells was performed. The resazurin assay screen displayed higher hit rate, and the hits had higher activity in comparison with the activity found in the SYBR Green assay screen, which seems to be more sensitive to fast-killing compounds. Secventy-two confirmed hits were analyzed for chemical clustering, yielding 13 clusters, 11 of which consisted of novel scaffolds with previously unknown antitrypanosomal activity. Zimmermann et al. reported a fluorescent-based HTS assay to identify inhibitors of *T. brucei* RNA editing ligase (unlike traditional assays that use radioactive substrates coupled with gel analysis and are thus incompatible with HTS) [41]. They performed a pilot screen on the commercial LOPAC library (Z' = 0.74; signal to baseline ratio = 2.3); they retrieved Twenty-two confirmed hits; a subset of 6 confirmed concentration-response behavior. A second pilot screen on the Maybridge library with a slightly modified protocol resulted in improved Z' (0.88) and signal to baseline ratio of 3.6. Interestingly, the assay is readily adaptable for other polynucleotide ligases. In the field of Leishmaniasis, Nühs et al. have reported the development of a novel *Leishmania donovani* screening cascade for HTS (which includes a single point axenic assay, followed by confirmation, potency assessment, counterscreen against mammalian cells and an intramacrophague assay) [42]. Importantly, the assay is high predictive of leishmanicidal activity on the intracellular, clinically-relevant parasite stage. In order to assess the utility of the screening cascade, a diversity-oriented synthesis library of 9,907 compounds was analyzed; an average robust Z factor of 0.88 was observed and a signal to background ratio of 18.7. The screen yielded two novel antileishmanial chemotypes. Whereas most of the previously reviewed studies explored synthetic libraries, Annang et al. used a HTS platform to explore a subset of around 6K microbial extracts from the MEDINA Natural Products Library [43]. The platform included the β-D-galactosidase transgenic *Trypanosma cruzi* assay and the reazurin-based *T. brucei* and *L. donovani* assays as primary screens, and a secondary intracellular amastigote screen in the case of *L. donovani*. Very recently, Benítez et al. reported a screening assay against *T. brucei, T. cruzi* and *L. infantum* trypanothione synthetase [44]. The screening conditions were carefully adjusted to the enzyme kinetic parameters and intracellular concentration of substrates corresponding to each trypanosomatid species, and to avoid assay interference. It yielded Z's ≥0.85 and a signal background coefficient of 3.5. A pilot screen on a 144-compound library was conducted, finding several novel chemical scaffolds as low μM and selective inhibitors. Sykes and Avery developed a high content image-based assay to estimate the effect of compound treatment on the clinically relevant *T. cruzi* amastigotes in 3T3 fibroblasts [45]. The effect of compounds on host cells can also be determined in the same well, as an initial indicator of cytotoxicity. The assay has been used to identify active compounds from an in-house library of compounds with either known biological activity or that are FDA-approved, and separately, from the Malaria Box collection. Active compounds were also screened against trypomastigotes, using a reazurin-based assay. Twelve compounds with reconfirmed solid sample activity, with IC50 values of less than 10 μM and high selectivity indices to *T. cruzi* amastigotes over 3T3 host cells were identified.

Other HTS campaigns related to NTD and drug repurposing will be discussed in the Drug Repurposing section.

2.2. Virtual screening

VS or *in silico* screening comprises the application of computational models or algorithms to explore large collections (typically, from tens of thousands to millions of compounds) to obtain a ranked list and prioritize which drug candidates will be subjected to experimental testing. VS comprises a wide diversity of computational techniques, from the very simple 2D similarity screening to the complex structure-based approximations. Likewise HTS, VS can be considered a bioethical approach, since *in silico* computations or simulations are applied to define which drug candidates are more likely to display positive results *in vitro* and/or *in vivo*. As pointed out by Huges et al. [10] VS can also provide the starting structures for a focused screen without the need to use expensive large library screens, as weel as to look for novel patent space around existing compound structures. In addition to focusing of libraries, VS can be closely coordinated with HTS for hit expansion purposes. i.e. quickly identifying new active compounds based on HTS confirmed hits, without further random screening [46].

An important difference between VS and HTS is that VS requires considerably more accessible technology, with many resources being completely publicly available, from specialized software to online chemical repositories/databases. Most of the VS methods can be applied in a personal computer (of course, the more computer power the more throughput). The advent of low cost parallel computing and public computing grids has made possible to compute even complex task within small

academia groups. In other words, the technological gap in the field of cheminformatics and bioinformatics, though existent, is probably the lowest in the drug discovery field, a significant advantage in the field of NTD. Consistently with such benefit, we will see that the number of recent NTD-focused VS campaigns greatly exceeds that of HTS campaigns. What is more, many of the VS applications have been performed by researchers from low- and medium-income countries, in many cases from countries where the targeted disease is endemic. As we have already observed in the case of HTS campaigns, we will observe that VS campaigns are unevenly distributed across NTDs. In other words, modern approaches towards hit identification have been applied more profusely for some neglected conditions than for others.

The dependence of VS performance on certain compound classes, (in other words, its limited scaffold hopping), is one of its potential disadvantage of VS (in particular, when using some ligand-based methods, e.g. similarity searches). The potency distribution of VS hits must also be considered. Retrospective studies demonstrate that VS tends to retrieve hits which are active in the low μM range, whereas hits in the sub- μM range represent a minority [47, 48]. Consequently, most of the VS hits should be subjected to molecular optimization programs to obtain active compounds in the desired activity range.

A large quantity of VS reports focused on Dengue has recently been published. All throughout this subsection, we will limit to those studies containing some level of experimental validation to the computational predictions. However, many more VS studies that do not satisfy this condition can be found in the literature. A complex and very interesting VS protocol to discover dual target inhibitors against the host c-Src kinase and the viral NS5 RNA polymerase has been performed by Vincetti et al [49]; it is expected that such multi-target compounds will be associated to diminished drug resistance issues. Starting from a library of known Src active scaffolds obtained from ChEMBL and BindingDB, and from an internal collection of kinase inhibitor, the authors performed parallel structure-based searches against the allosteric pocket of NS5 polymerase. From the resulting hits, scaffolds for chemical synthesis were selected by analyzing the most recurrent scaffolds, synthetic accessibility, docking scores and binding modes. The idea of obtaining cheap-to-produce scaffolds is fundamental here: it is necessary to guarantee accessibility to affected populations in case that the selected candidates move forward to approved drug status. Three scaffolds were selected and a virtual library of synthetically accessible derivatives was designed using the software SmiLib, resulting in about 10,000 virtual compounds that were also docked. A series of purines emerged as the most interesting candidates able to inhibit virus replication at low μM concentrations with no significant toxicity to the host cell. Among the identified antivirals, one compound resulted 10 times more potent than ribavirin and showed a better selectivity index, representing the first-in-class allosteric inhibitor capable of targeting both the virus NS5−NS3 interaction and two host kinases. Li et al performed a cascade VS campaign to identify inhibitors against NS2B-NS3 protease [50]. From the X-ray crystal structure of the target, the authors inferred a pharmacophore model and applied it in a flexible search of a 5 million compound library obtained from several commercial vendors. The hits were subsequently filtered by rigid and flexible docking. Fourteen hits were submitted to experimental validation; one of them verified to be effective in both the *in vitro* protease inhibition test and an infectivity assay. The same target was addressed by Brecher et al., who performed a structure-based screen to identify allosteric inhibitors within the National Cancer Institute diversity set II [51]. Among twenty-nine hits, three compounds inhibited the Dengue virus 2 protease, with IC_{50} values within the low μM range (Figure 1). Virus titer reduction assays revealed that one of the confirmed hits is a broad spectrum flavivirus protease inhibitor, and can significantly reduce titers of Dengue virus 2, Zika virus, West Nile virus and Yellow fever virus in low μM concentrations. Very recently, Leal et al. used structure-based VS to identify novel inhibitors targeting the β-OG hydrophobic binding site of the Dengue virus envelope glycoprotein [52]. They screened 110K molecules from the Maybridge database. They used different criteria aside from docking results (e.g. scaffold diversity, synthetic tractability) to select 23 compounds which were assayed *in vitro*. 5 hits displayed antiviral activity at low μM concentrations.

Figure 1: Structure of the three selected inhibitors of the Dengue protease 2 with IC_{50} in low μM range (Brecher et al, 2017).

In the field of schistosomiasis, Kannan et al. resorted to homology modeling for structure-based VS to identify inhibitors of *S. mansoni* histone deacetylase 8 [53]. seventy-five compounds (mostly hydroxamates and sulfonamide-thiazole derivatives) were selected from the ZINC database, and eight of them confirmed activity *in vitro.* Remarkably, solving the crystal structure of the targetwith two of the virtual screening hits confirmed the predicted binding mode. Melo-Filho et al. developed a series of QSAR models to identify novel inhibitors of *S. mansoni* thioredoxin glutathione reductase [54]. They combined models obtained through the HQSAR, CoMFA and CoMSIA approximations; the individual models were built from a series of thrity-five oxadiazoles-2-oxides. They used a consensus approach to screen the Hit2Lead library from the ChemBridge database. 10 of the hits were acquired and tested on both shistosomula and adult worms. Two of the hits displayed activity against both systems at low μM concentrations. Another VS campaign has been performed by the same group pursuing the same target [55]. In this case, they built QSAR classifiers using a far more diverse dataset containing 2,854 active compounds against the enzyme, and an equivalent number of inactive compounds. The authors also resorted to consensus QSAR; they screened around 150K compounds from a diversity of ChemBridge libraries. After removing PAINs and compounds with unacceptable pharmaceutical and

pharmacokinetic properties, 29 compounds were cherry-picked and screened against shistosomula and adult worms, with two of them showing activity at low μM concentrations (Figure 2).

Figure 2: Compounds active for schistomula and adult worms found (Neves et al., 2016). **A**) 2-[2-(3-methyl-4-nitro-5-isoxazolyl)-vinyl]pyridine (EC_{50}=3.23μM) and **B)** 2-(benzylsulfonyl)-1,3-benzothiazole (EC_{50}= 2.62μM).

Other recent VS applications related to helminth-caused diseases include the work from Zheng et al., who choose *Ascaris suum* ACR-16 nicotinic acetylcholine receptor as drug target and, after homology modeling, performed a virtual screen on the lead-like subset of commercially available compounds in the ZINC Database [56]. They discovered four acetylcholine inhibitors that behave as negative allosteric modulators, according to electrophysiological recording from ACR-16 receptors expressed in *Xenopus* oocytes. France and coworkers used a combination of ligand- and structure-based methods for the *in silic*o prediction of DPY-31 (a nematode-specific metalloprotease) inhibitors [57]. For that purpose, they prepared a custom virtual library to molecules in the PDB that bind to enzymes homologous to DPY-31. Several μM inhibitors of DPY-31 from *Brugia malayi* were identified.

Beyond a shadow of doubt, trypanosomatid-caused conditions represent the NTD where VS applications are most abundant. For instance, Parameswaran et al. performed a comparative modeling of *L. donovani* Lip3 lipase using a lipase from *Rhizomucor miehei* in its inhibitor bound conformation [58]. The homology model was used to screen the National Cancer Institute diversity set II. The top ten hits were selected as queries for similarity-based VS, retaining molecules from ZINC with a Tanimoto coefficient above 0.6. The docking process was repeated with these similar molecules (around 20K) on both the *Leishmania* Lip3 and the human ortholog, monoglyceride lipase. This similarity-based screening (coupled with docking) was intended to understand the structure–activity relationships and to identify molecules with better free energy of binding than hits from the initial screen. From the 10 top-ranked compounds, 4 were acquired and assayed against *Leishmania*, with good results, though their ability to inhibit the target was not confirmed experimentally. Singh et al. explored *L. donovani* L-asparaginase as a potential new target for antileishmanial drugs [59]. They modeled the target using *Escherichia coli* orthologs. The resulting model was used to screen 23 million compounds from ZINC database. To reduce such number to a manageable one, they retained only those compounds showing 90% similarities in structural and physicochemical parameters to the natural substrate of the targeted enzyme, L-asparagine. In this way they obtained around 11K candidates that were submitted to structure-based screening. The top 100 screened compounds were selected and the 20 compounds with the best toxicity profiles were kept. The final step involved free energies calculations and comparative binding to the human type 3 L-asparaginase, resulting in only five final hits. After molecular dynamics simulations, one of the hits confirmed antileishmanial activity, though no mechanism validation was performed. Ochoa et al. have implemented an interesting approach (the *Relaxed Complex Scheme*) to incorporate target flexibility in structure-based VS applications for new leishmanicidal drugs [60]: they used an ensemble of protein conformations that encompass the protein's conformational space. The Relaxed Complex Scheme may be used when there are different experimental structures of the target protein (as commonly occurs for proteins bound to different ligands) or when VS screens use structures from different organisms (e.g. a set of related pathogens). The authors retrieved seventy *Leishmania spp.* proteins from the Protein Data bank; they kept fifty-three high-resolution unique proteins and screened a collection of 600K drug-like molecules from the ZINC database against this set of potential targets. The ten compounds with the most negative trajectory-averaged conformational scores against the *L. major* dihydroorotate dehydrogenase were prioritized for in vitro validation. Four hits showed activity against *L. panamensis* intracellular amastigotes. It is important to underline that all the computational protocols were adapted for the World Community Grid. Agnihotri et al. used a series of structure-based tools to screen the Maybridge screening collection (around 54K compounds) against *L. donovani* γ-glutamylcysteine synthetase [61]. VS was carried out in a hierarchical manner involving several steps, starting with a preliminary docking exercise with subsequent refining and reranking on the basis of diverse docking algorithms, molecular dynamics simulations and visual examination. Experimental testing of five hits against the enzyme confirmed the predicted inhibitory activity (IC_{50} values below 100 μM) and low to moderate inhibitory activity against *L. donovani* promastigotes. The Aggregation Advisor tools were used to discard nonspecific inhibition due to colloidal aggregation in four out of five cases. Mansuri et al. performed a structure-based screening against *L. donovani* ascorbate peroxidase, a redox enzyme that regulates the trypanothione cascade [62]. After homology modeling of the target, they screened a drug library gathering compound libraries from diverse suppliers plus natural compounds from ZINC. After applying PAINS and ADMET filters, they selected twenty-six compounds which were tested on promastigote cultures, with twelve of them confirming antileishmanial activity. Six compounds were safe on BALB/c macrophages and were effective against intracellular amastigotes. Three of them inhibited recombinant ascorbate peroxidase noncompetitively and demonstrated partial reversion of resistance in an amphotericin B (AmB)-resistant strain. After purification, X-ray structure elucidation and functional characterization of *L. amazonensis* nucleoside diphosphate kinase, Mishra et al. implemented a structure-based VS (including both docking and molecular dynamics simulations) on the Maybridge library [63]. Six hits were assayed against the enzyme; five of them confirmed the predicted activity, but only one of the inhibited Leishmania proliferation (the authors attributed the lack of effect of the inactive compounds to permeability issues); they also predicted that three of the compounds were similar to known aggregators, which could cause nonspecific

inhibition of proteins. Prokopczyk et al. performed a structure-based VS to discover new inhibitors against *T. cruzi* glyceraldehyde-3-phosphate dehydrogenase [64]. They studied a subset of about 3 million drug-like compounds from the ZINC database. Key interactions in the active site via hydrogen bonding (Thr167 and Ser247) were incorporated in the docking protocol using Glide extra precision module as a constraint in the docking process. 25 compounds were selected for biochemical assays by isothermal titration calorimetry; three of them showed Michaelis-Menten constants in the low μM range. The most promising hit was tested against *T. cruzi* trypomastigotes and mouse spleen cells, displaying similar activity and selectivity to those of the reference drug benznidazol. Demir et al. used the Relax Complex Scheme to discover novel *T. brucei* RNA-Editing Terminal Uridylyl Transferase inhibitors [65]. In an initial VS round, they screened the National Cancer Institute diversity set 2, using the static crystal structure of the target and the three most populated cluster centroids of the molecular dynamics simulations. Twenty-four compounds were selected to be tested in an assay against *T. brucei* circulating form; three of them displayed EC_{50} values below or equal to 4 μM. A second VS round was performed on the National Cancer Institute Plated compounds. Sixty compounds were selected for experimental testing in this second screen, from which forty compounds were experimentally assayed. Twelve hits showed EC_{50} values below or equal to 4 μM and, remarkably, three of them in the low nM range, quite a rare positive result in the VS field. A similarity search was performed around these three inhibitors with nM potency (hit expansion stage). Ten of the resulting similar hits were tested and half of them showed EC_{50} values below or equal to 4 μM; two of these hits were active in the low nM range. All in all, twenty novel inhibitors were selected in this complex VS campaign. Herrmann et al. screened a 700-natural product database from a commercial supplier against *T. brucei* glyceraldehyde-3-phosphate dehydrogenase [66]. The screen involved a pharmacophore-based VS and subsequent molecular docking of the identified hits. Thirteen compounds were predicted to possess significant affinity towards the enzyme and therefore tested in an *in vitro* enzyme assay. Nine of these *in silico* hits showed significant inhibitory activity at 50 μM and moderate in vitro activity against *T. brucei*. Almeida et al. developed a structure-based VS approach to identify novel *T. cruzi* prolyl oligopeptidase inhibitors [67]. They explored a library of about 6K compounds including compounds from different protease inhibitor sources from different commercial suppliers. Thirteen hits proved to inhibit the enzyme at sub-μM or μM concentrations.

Other VS campaigns with a focus on computer-based drug repurposing have been included in the correspondent (Drug Repurposing) section.

3. FROM A HIT TO A PRECLINICAL CANDIDATE AND COMPUTER-AIDED DRUG DESIGN

After identifying one or more hits, the hit-to-lead stage begins. At this level, Medicinal Chemists will try to produce more potent and selective compounds with adequate biopharmaceutical and pharmacokinetic properties to examine their efficacy in available *in vivo* models of the target disease [10]. Remember that, whereas most HTS and VS campaigns deliver hits with potency in the μM order, most of the existing drugs are active in the low nM order. Accordingly, it is expected that the hit-to-lead and beyond stages improve potency by at least two orders of magnitude. Note that, however, some counter-examples to potency-driven discovery (e.g. low affinity ligands) exist in specific therapeutic fields [68-70], though potency-driven research is probably the best option in the field of anti-infective drugs. From the 1990s onwards, the search of more potent derivatives of an active scaffold has been balanced with early detection of potential bioavailability and toxicity issues.

Typically, the work at the hit-to-lead stage consists in iterative and systematic SAR investigations around each core compound structure. The actual drug design process starts here. Drug design is inherently related to finding molecular novelty, i.e. novel chemical entities. Screening approaches usually explore the known chemical universe in search of new active motifs. The novelty in *in silico* or wet screening is not always in the chemistry of the emerging hits, but in uncovering an unknown, hidden association between known chemicals and a given biological activity or molecular target. Although *de novo* drug design (from scratch) is possible, hit and lead optimization programs are possibly the most frequent applications of drug design. This stage of development will often give rise to the discovery of new binding pockets on the target proteins.

As an example of a classical hit optimization program in the field of NTD, the reader is referred to the work of Peddibhotla et al [71]. From a HTS campaign focused on inhibitors of the SKN‑1 pathway in nematodes, the authors identified a vanillamine derivative with an IC50 of 4.5 μM, which after subsequent Medicinal Chemistry optimization led to the discovery of ML358 (IC50 = 0.24 μM). This compound is a selective inhibitor of the nematode SKN-1 detoxification pathway and is inactive against the human homologue of SKN-1. It also sensitizes the worms to ivermectin and levamisole, two broad-spectrum anti-helmitics. In another example, Jacques et al. applied structure-based VS to discover novel inhibitors against *S. mansoni* NAD+ catabolizing enzymes. Further structure−activity relationship studies have allowed a 3-log gain in potency, accompanied by a largely enhanced selectivity for the parasitic enzyme over the human homologue [72].

If the structure of the intended target has been solved, it is possible to explore in a rational manner and without the need of trial and error learning, interactions with regions of the target that have not been exploited with previously known ligands. For instance, building on the results of a HTS campaign of a subset of the Pfizer corporate collection against *L. donovani* N-myristoyltransferase, Hutton et al. performed the structure-guided fusion of two of the emerging series of inhibitors (piperidinylindoles and

aminoacylpyrrolidines) [73]. Enzyme inhibition was increased 40-fold through hybridization of two distinct binding modes, resulting in novel, potent inhibitors with good selectivity over the human ortholog. Another beautiful example of structure-based design can be found in the study of Yokokawa et al., who from an X-ray-based fragment screen of the Novartis fragment collection discovered Dengue virus RNA-dependent RNA polymerase inhibitors [74]. A biphenyl acetic acid fragment hit was optimized resulting in a 1000-fold enhanced potency *in vitro* and acquired anti-dengue activity against clinically relevant serotypes (Figure 3).

Figure 3: An optimized biphenyl acetic acid active against different serotypes of dengue virus with IC_{50} and EC_{50} < 10μM, by Yokokawa *et al*.

Among the computational approximations to rational drug design, docking, molecular dynamics and structure-based pharmacophores are the first choices to guide optimization. If information on known ligands is available, ligand-based approximations are also viable.

Once that the initial goals of the hit-to-lead stage have been met, the final drug discovery phase of lead optimization begins, where favorable properties in lead compounds are maintained while improving on deficiencies in the lead structure [10]. At this point, animal models of the disease are often used to guide the process.

4. DRUG REPURPOSING

The term drug repurposing involves finding novel therapeutic indications for existing drugs, including approved, discontinued and abandoned drugs, as well as clinical candidates. It is important to underline that this approach focuses on late-stage chemical matter (drugs that are or have been approved, and drugs that are or have undergone clinical trials) [75]. The appeal of drug repurposing can be easily recognized: it avoids expensive and time-consuming pharmacokinetic and toxicological profiling typically required for *de novo* drugs (Figure 4) [76], which translates into major savings in economical and time investments. Likewise, the stability, large-scale synthesis and manufacturing issues of a repurposed drug are already known [77], though dosing and formulation modification could be required for the new indication. Drugs that have not achieved approved status due to safety issues might be rescued if the cost-benefit analysis justifies their administration in a new therapeutic area, or if the adverse reactions found when investigating the originally pursued indication are not relevant in a different drug administration schedule or in a different population. For instance, a drug abandoned at late-stage drug development due to chronic toxicity might be repurposed for the treatment of some infectious diseases where short-term treatments are required.

The most significant challenges faced by drug repurposing initiatives are probably of commercial, regulatory or patentability nature [77-79]. However, such considerations are less likely to impact on drug discovery projects targeting NTD, where the main driving force is usually not of commercial nature. Accordingly, most of the later or ongoing clinical trials focused on neglected conditions are based on the repurposing approach [8, 75, 81].

Initially, successful drug repurposing stories emerged from serendipitous observations or rational exploitation of drug side effects. Lately, however, more systematic approximations (including VS) have been actively investigated as tools for identifying candidates for drug repurposing [76, 82, 83]. An important consideration is that, if real drug repurposing is being pursued, the repurposed drug should be used for the second indication without any further modification of the compound at hand. In other words, no hit-to-lead or further optimization programs are allowed when implementing drug repurposing. This is a non-trivial point if one recalls that most of the HTS and VS hits usually display scarcely or moderately potencies (frequently within the low μM order) while free drug levels in bio-fluids, within therapeutic settings, are usually below that concentration. Also, the advantages of drug repurposing (avoiding pharmacokinetic assessment; known safety) will only be fully exploited when the doses required for the new indication are compatible (i.e. equal or lower) with those used for the already approved medical use/s [84].

It is worth distinguishing drug repurposing from other closely related strategies. For example, *target repurposing* is being increasingly used in anti-infective drug discovery. Here, the idea is to exploit a target of the infectious agent with established homologs in other species (i.e. other pathogens or the host itself) [75]. Chemical compounds targeting the host protein (or the established target from other pathogen) are then used as starting point to develop compounds that inhibit the ortholog in the species of interest. Target repurposing often requires Medicinal Chemistry optimization, though some examples that have been repurposed across pathogen species with no modifications can be mentioned. For instance, the CYP51 azole inhibitor posaconazole (an antifungal) has reached clinical trials as potential new treatment for Chagas disease, with limited results [85]. In another example, Gillan et al. have screened known heat shock protein 90 inhibitors previously investigated as antitumor candidates, against filarial nematodes [86].

A very similar approximation is *target class repurposing*. Here, the specific target in the infectious agent may not be known, though the guest is known to express essential targets within a homologous target class or to perform cellular functions homologous to those carried out by a certain target class; the approach is similar to *lead repurposing*, which focuses on early-stage chemical matter [75]. The already discussed articles on assays of collections

of kinase inhibitors (including human kinases inhibitors) in the area of NTD can be mentioned as good examples [38, 39].

In the field of systematic drug repurposing, Johnston et al. have reported a screen of around 2,600 drugs from the approved human drug-pharmacopeia (CRX library) in a *Wolbachia* cell-based assay [87]. Sixty-nine orally available hits from different therapeutic classes (prominently, antibiotics and drugs for CNS conditions) were identified. Fifteen of them were assayed in a *Litomodoides sigmodontis* mouse model, in which four antibiotics from the tetracycline, fluoroquinolone and rifamycin classes resulted active. Rausch et al. screened a library of 1,000 FDA approved drugs plus 1,000 additional bioactive compounds in Zika virus infection models [88]. 19 hits were found, including several approved drugs. The antibiotic ningnanmycin also proved active against other flavivirus, including West Mile, dengue and chikungunya viruses. Similarly, Bulman et al. screened a library of over 2,000 FDA-approved compounds and found that auranofin, a gold-containing compound used for the treatment of rheumatoid arthritis, inhibits adult *Brugia* motility [89]. The compound proved effective at an *in vivo* gerbil model and thioredoxin reductase was found to be its likely molecular target. Cowan and Keiser screened the approved oncology drug set from the National Cancer Institute's Developmental Therapeutic Program for antischistosomal activity (*S. mansoni* larval and adult stages) [90]. Eleven compounds displayed activity at both stages with IC_{50} between 10 and 50 μM; five of them lost activity in the presence of serum albumin, indicating a high level of binding to plasma proteins. Six compounds were studied *in vivo*, with two kinase inhibitors showing reduced worm burden at single oral doses of 400 mg/kg. Panic et al. examined 1,600 FDA-approved drugs against *S. mansoni* schistosomula [91]. After confirming activity against adult worms and examining pharmacokinetic and toxicological data, eleven compounds advanced to *in vivo* studies, with doramectin and clofazimine reducing the worm burden (400 mg/kg). Kaiser et al. tested a set of 100 registered drugs (quite biased for anti-infectious agents and psychoactive compounds) against a panel of *in vitro* assays to be profiled for their antiprotozoal activities [92]. Among the hits, we may mention azole antifungals (*T. cruzi*), rifamycin and auranofin (*T. brucei*), clofazimine and tripanavir (*Leishmania*) and tricyclic antidepressants (*Plasmodium*).

In the field of VS-based drug repurposing, we might highlight the efforts of Bellera and coworkers towards the identification of novel cruzipain inhibitors with trypanocidal activity though ligand-based approximations [93-95]. Their initial efforts identified de activity of bromocriptine and levothyroxine against *T. cruzi* [93, 94]. Later, they refined their selection criteria by combining ligand based approaches and molecular docking and complementing the computational filters with analysis of pharmacokinetic data of the drug candidates (particularly, steady states plasma levels obtained for the original therapeutic indication), and additional benefits or contraindications for the patient with chronic Chagas disease [95]. In this later study, they selected clofazimine and benidipine (Figure 5) for *in vivo* testing in an acute model of Chagas, with positive results at relatively low doses. Later, they observed some positive effects in a chronic model, as well [96]. The same group has identified polyamine analogs with confirmed activity at biochemical and cell-based assays [97], among them triclabendazol and the antidepressant paroxetine. On the other hand, Reigada et al. resorted to structure-based VS to identify new trypanocidal drugs acting through inhibition of putrescine uptake; the anti-acne drug isotretinoin was selected, with IC_{50} values in the low μM range [98].

A possibly interesting observation emerging from the previously reviewed drug repurposing initiatives is that, time and again, drugs from a small number of therapeutic categories show potential against NTD. Among them, we may mention anti-infective drugs and central nervous system drugs. This seems in agreement with previous studies which suggest systematic connections between drug therapeutic classes [99].

Figure 4: A) Traditional process involved in drug development. B) Phase of the process where HTS, Virtual Screening and Bioinformatics are involved. C) Phases where the optimizations techniques appear and the hit to lead stage begins. D) The repositioned drug may avoid phase I in the drug discovery process.

It should be noted that Phase I trials may still be required to establish maximum tolerated doses for a repurposed candidate if the dosing required to reach relevant levels is far in excess of the standard doses used in the initial indication [100, 101].

CONCLUSIONS

The neglected status of NTD seems to be slowly reverting during the last decades. A considerable number of modern applications of drug discovery approaches in the field of NTD can be found in literature. Nevertheless, several considerations result from the analysis of the recent publications on the subject.

First, regarding drug discovery initiatives, not all neglected conditions seem to be equally neglected. Whereas significant drug discovery efforts have been dedicated to some of the NTD included in WHO list, no applications of modern drug discovery strategies have been found in other cases.

Second, different approaches are associated to different technological gaps, a significant point if one has in mind the endemic nature of many NTD and the economic limitations linked to the search of new treatments for these conditions. In particular, HTS represents a technology demanding approach which, in general, is only accessible for scientist in low- and mid-income countries through scientific collaborations with academic groups from high-income countries or international pharmaceutical companies. At the other end of the spectrum, computational approaches are widely accessible due to a multiplicity of factors, from availability of free computational resources (software,

databases) to the advent of low-cost parallel computing and public computing grids. This is reflected in the fact that a considerable proportion of the computer-aided drug discovery studies focused on NTD have been performed by academic groups from the affected countries. Although of course contributions from international organizations and developed countries should never be disregarded, it would also be desirable for the affected countries (whenever possible) to explore novel therapeutic solutions at the local level (i.e. autonomously). In that sense, their efforts should be concentrated on cost- and translationally-efficient approximations.

Third, it is interesting to note that many screening campaigns focused on neglected conditions have relied on cell-based assays, which is consistent with the fact that target-driven methods have provided relatively poor results in the field of infectious diseases and that phenotypic screening could provide hits acting through multiple mechanisms and thus less prone to appearance of resistance issues.

At last, drug repurposing appears as a translationally efficient approach to the discovery of innovative medications, potentially representing a considerable shortcut in the drug development cycle. This is especially true for drugs being repurposed at similar or lower dosage compared to the maximum dose that has already been approved by regulatory agencies for the previous indication/s. Consistently, most of the novel NTD therapies that have reached clinical trials (and, in some cases, survived them!) have relied on this strategy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] World Health Organization. Neglected tropical diseases. http://www.who.int/neglected_diseases/diseases/en/ (Accessed July 31, **2017**).

[2] Hotez, P.J. In: *The Causes and Impacts of Neglected Tropical and Zoonotic Diseases: Opportunities for Integrated Intervention Strategies*; Institute of Medicine (US) Forum on Microbial Threats; National Academic Press (US). Washington DC, **2011**.

[3] van Lieshout, L.; Yazdanbakhsh, M. Landscape of neglected tropical diseases: getting it right. *Lancet Infect. Dis.*, **2013**, 13, 469-470.

[4] Pedrique, B.; Strub-Wourgaft, N.; Some, C.; Olliaro, P.; Troullier, P.; Ford, N; Pécoul, B.; Bradol, J.H. The drug and vaccine landscape for neglected diseases (2000-11): a systematic assessment. *Lancet Glob. Health*, **2013**, *1*, e371-e379.

[5] Utzinger, J.; Raso, G.; Brooker, S.; De SAvigny, D.; Tanner, M.; Ornbjerg, N.; Singer, B.H.; N´goran, E.K. Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. *Parasitology*, **2009**, *136*, 1859-1874.

[6] Moran, M.; Guzman, J.; Ropars, A.L.; McDonald, A.; Jameson, N.; Ornune, B.; Ryan, S.; Wu, L. Neglected disease research and development: how much are we really spending? *PLoS Med.*, **2009**, *6*, e1000030.

[7] Duschak VG. Targets and patented drugs for chemotherapy of chagas disease in the last 15 years-period. *Recent Pat. Antiinfect. Drug Discov.*, **2016**, *11*, 74-113.

[8] Sbaraglini, M.L.; Vanrell, M.C.; Bellera, C.L.; Benaim, G.; Carrillo, C.; Talevi, A.; Romano P.S. Neglected tropical protozoan diseases: drug repositioning as a rational option. *Curr. Top. Med. Chem.*, **2016**, *16*, 2201-2222.

[9] Katsuno, K.; Burrows, J.N.; Duncan, K.; Hooft van Huijsduijnen, R.; Kaneko, T.; Kita, K.; Mowbray, C.E.; Schmatz, D.; Warner, P.; Slingsby, B.T. Hit and lead criteria in drug discovery for infectious diseases of the developing world. *Nat. Rev. Drug Discov.*, **2015**, *14*, 751-758.

[10] Hughes, J.P.; Rees, S.; Kalindkian, S.B.; Philpott, K.L. Principles of early drug discovery. *Br. J. Pharmacol.*, **2011**, *162*, 1239-1249.

[11] Mayr, L.M.; Bojanic, D. Novel trends in high-throughput screening. *Curr. Opin. Pharmacol*., **2009**, *9*, 580-588.

[12] Fox, J.T.; Myung, K. Cell-based high-thoughput screens for the discovery of chemotherapeutic agents. *Oncotarget*., **2012**, *3*, 581-585.

[13] An, W.F.; Tolliday, N.J. Introduction: cell-based assays for high-throughput screening. *Methods Mol. Biol.*, **2009**, *486*, 1-12.

[14] An, W.F.; Tolliday, N.J. Cell-based assays for high-throughput screening. *Mol. Biotechnol.*, **2010**, *45*, 180-186.

[15] Pereira, D.A.; Williams, J.A. Origin and evolution of high throughput screening. *Br. J. Pharmacol.*, **2007**, *152*, 53-61.

[16] Büttner, F.H. Cell-based asssay for high-thoughput screening. *Expert Opin. Drug Discov.*, **2006**, *1*, 373-378.

[17] Talevi, A.; Bruno-Blanch, L.E. In: *Antiepileptic Drug Discovery. Novel Approaches*. Talevi, A.; Rocha, L., Eds.; Springer. New York, **2016**.

[18] Wetsby, M.; Nakayama, G.R.; Butler, S.L.; Blair, W.S. Cell-based and biochemical screening approaches for the discovery of novel HIV-1 inhibitors. *Antiviral Res.*, **2005**, *67*, 121-140.

[19] Moutsaysos, I.K.; Parker, C.N. Recent advances in quantitative high throughput and high content data analysis. *Expert Opin. Drug Discov.*, **2016**, *11*, 415-423.

[20] Silver, L.L. Challenges of antibacterial discovery. *Clin. Microbiol. Rev*., **2011**, *24*, 71-109.

[21] Payne, D. J.; Gwynn, M.N.; Holmes, D.J.; Pompliano, D.L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov*., **2007**, *6*, 29–40.

[22] Silver, L.L. Multi-targeting by monotherapeutic antibacterials. *Nat. Rev*., **2007**, *6*, 41-55.

[23] Silver, L.L.; Bostian K.A. Discovery and development of newantibiotics: the problem of antibiotic resistance. *Antimicrob. Agents. Chemother*., **1993**, *37*, 377-383.

[24] Bibette, J. Gaining confidence in high-throughput screening. *Proc. Natl. Acad. Sci. USA*, **2012**, *109*, 649-650.

[25] Dahlin, J.L.; Nissink, J.W.M.; Strasser, J.M.; Frances, S.; Higgins, L.; Zhou, H.; Zhang, Z.; Walters, M.A. PAINS in the assay: chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. *J. Med. Chem.*, **2015**, *58*, 2091-2113.

[26] Senger, M.R.; Fraga, C.A.; Dantas, R.F.; Silva, F.P. Filtering promiscuous compounds in early drug discovery: is it a good idea? *Drug Discov. Today*, **2016**, *21*, 868-872.

[27] Miller O.J.; El Harrak, A.; Mangeat, T.; Baret, J.C:; Frenz, L.; El Debs, B.; Mayor, E.; Samuels, M.L.; Rooney, E.K.; Dieu, P.; Galvan, P.; Galvan, M.; Link, D.R.; Griffiths, A.D. High-resolution dose–response screening using droplet-based microfluidics. *Proc. Natl. Acad. Sci. USA*, **2011,** *109*, 378–383.

[28] Shockley, K.R. Quantitative high-throughput screening data analysis: challenges and recent advances. *Drug Discov. Today*, **2015**, *20*, 296-300.

[29] Harris, C.J.; Hill, R.D.; Sheppard, D.W.; Slater, M.J.; Stouten, P.F.W. The design and application of target-focused compound libraries. *Comb. Chem. High Throughput Screen.*, **2011**, *14*, 521-531.

[30] Basavannacharya, C.; Vasudevan, S.G. Suramin inhibits helicase activity of NS3 protein of dengue virus in a fluorescence-based high throughput assay format. *Biochem. Biophys. Res. Comunn.*, **2014**, *453*, 539-544.

[31] Smith, T.M.; Lim, S.P.; Yue, K.; Busby, S.A.; Arora, R.; She, C.C.; Wright, S.K.; Nutiu, R.; Niyomrattanakit, P.; Wan, K.F.; Beer, D.; Shi, P.Y.; Benson, T.E. Identifying initiation and elongation inhibitors of dengue virus RNA polymerase in a high-throughput lead-finding campaign. *J. Biomol. Screen.*, **2015**, *20*, 153-163.

[32] Stolp, Z.D.; Smurthwaite, C.A.; Reed, C.; Williams, W.; Dharmawan, A.; Djaballah, H.; Wolkowicz, R. A multiplexed cell-based assay for the identification of modulators of pre-membrane processing as a target against Dengue virus. *J. Biomol. Screen.*, **2015**, *20*, 616-626.

[33] Engdahl, C.; Knutsson, S.; Ekström, F.; Linusson, A. Discovery of selective inhibitors targeting acetylcholinesterase 1 from disease-transmitting mosquitoes. *J. Med. Chem.*, **2016**, *59*, 9409-9421.

[34] Raphemot, R.; Rouhier, M.F.; Swale, D.R.; Days, E.; Weaver, C.D.; Lovell, K.M.; Konkel, L.C.; Engers, D.W.; Bollinger, S.R.; Hopkins, C.; Piermarini, P.M.; Denton, J.S. Discovery and characterization of a potent and selective inhibitor of Aedes aegypti inward rectifier potassium channels. *PLoS One*, **2014**, *9*, e110772.

[35] Bilsland, E.; Bean, D.M.; Devaney, E.; Oliver, S.G. Yeast-based high-throughput screens to Identify novel compounds active against *Brugia malayi*. *PLoS Negl. Trop. Dis.*, **2016**, 10, e0004401.

[36] Chan, J.D.; McCorvy, J.D.; Acharya, S.; Johns, M.E.; Day, T.A.; Roth, B.L.; Meachant, J.S. A miniaturized screen of a *Schistosoma mansoni* serotonergic G protein-coupled receptor identifies novel classes of parasite-selective inhibitors. *PLoS Pathog*., **2016**, 12, e1005651.

[37] Leung, C.K.; Wang, Y.; Malany, S.; Deonarine, A.; Nguyen, K.; Vasile, S.; Choe, K.P. An ultra high-throughput, whole-animal screen for small molecule modulators of a specific genetic pathway in *Caenorhabditis elegans*. *PLoS One*, **2013**, *8*, e62166.

[38] Diaz, R; Luengo-Arratta, S.A.; Seixas, J.D.; Amata, E.; Devine, W.; Cordon-Obras, C.; Rojas-Barros, D.I.; Jimenez, E.; Ortega, F.; Crouch, S.; Colmenarejo, G.; Fiandor, J.M.; Martin, J.J.; Berlanga, M.; Gonzalez, S.; Manzano, P.; Navarro, M.; Pollastri, M.P. Identification and characterization of hundreds of potent and selective inhibitors of *Trypanosoma brucei* growth from a kinase-targeted library screening campaign. *PLoS Negl. Trop. Dis.*, **2014**, *8*, e3253.

[39] Amata, E.; Xi, H.; Colmenareji, G.; González-Diaz, R.; Cordon-Obras, C.; Berlanga, M.; Manzano, P.; Erath, J.; Roncal, N.E.; Lee, P.J.; Leed, S.E.; Rodriguez, A.; Scotti, R.J.; Navarro, M.; Pollastri, M.P. Identification of "preferred" human kinase inhibitors for Sleeping Sickness lead discovery. Are some kinases better than others for inhibitor repurposing? *ACS Infect. Dis.*, **2016**, *2*, 180-186.

[40] Faria, J.; Moraes, C.B.; Song, R.; Pascoalino, B.S.; Siquiera-Neto, J.L.; Cruz, D.J.; Parkinson, T.; Ioset, J.R.; Cordeiro-da-Silva, A.; Freitas-Junior, L.H. Drug discovery for human African trypanosomiasis: identification of novel scaffolds by the newly developed HTS SYBR Green assay for *Trypanosoma brucei*. *J. Biomol. Screen.*, **2015**, *20*, 70-81.

[41] Zimmermann, S.; Hall, L.; Riley, S.; Sørensen. J.; Amaro, R.E.; Schnaufer, A. A novel high-throughput activity assay for the Trypanosoma brucei editosome enzyme REL1 and other RNA ligases. *Nucleic Acids Res.*, **2016**, *44*, e24.

[42] Nühs, A.; De Rycker, M.; Manthri, S.; Comer, E.; Scherer, C.A.; Schreiber, S.L.; Ioset, J.R.; Gray, D.W. Development and validation of a novel Leishmania donovani screening cascade for high- throughput screening using a novel axenic assay with high predictivity of leishmanicidal intracellular activity. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e0004094.

[43] Annang, F.; Pérez-Moreno, G.; García-Hernández, R.; Cordon-Obras, C.; Tormo, J.T.; Rodríguez, L.; de Pedro, N.; Gómez-Pérez, V.; Valente, M.; Reyes, F.; Genilloud, O.; Vicente, F.; Castanys, S.; Ruiz-Pérez, L.M.; Navarro, M.; Gamarro, F.; Gonmzález-Pacanowska, D. High-throughput screening platform for natural product-based drug discovery against 3 neglected tropical diseases: human African trypanosomiasis, leishmaniasis, and Chagas disease. *J. Biomol. Screen.*, **2015**, *20*, 82-91

[44] Benítez, 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.; Flohé, L.; Comini, M.A. Identification of novel chemical scaffolds inhibiting trypanothione synthetase from pathogenic trypanosomatids. *PLoS Negl. Trop. Dis.*, **2016**, 10, e0004617.

[45] Sykes, M.L.; Avery, V.M. Development and application of a sensitive, phenotypic, high-throughput image-based assay to identify compound activity against *Trypanosoma cruzi* amastigotes. *Int. J. Parasitol. Drugs Drug Resist.*, **2015**, *5*, 215-228.

[46] Stahura, F.L.; Bajorath, J. Virtual screening methods that complement HTS. *Comb. Chem. High. Throughput Screen*., **2004**, *7*, 259-268.

[47] Zhu, T.; Cao, S.; Su, P.C.; Patel, R.; Shah, D.; Chokshi, H.B.; Szukala, R.; Johnson, M.E.; Hevener, K. E. Hit identification and optimization in virtual screening: practical recommendations based on a critical literature analysis. *J. Med. Chem.*, **2013**, *56*, 6560-6572.

[48] Ripphausen, P.; Nisius, B.; Peltason, L.; Bajorath, J. *Quo vadis*, virtual screening? A comprehensive survey of prospective applications. *J. Med. Chem.*, *53*, 8461-8467.

[49] Vincetti, P.; Caporuscio, F.; Kaptein, S.; Gioiello, A.; Mancino, V.; Suzuki, Y.; Yamamoto, N.; Crespan, E.; Lossani, A.; Maga, G.; Rastelli, G.; Castagnolo, D.; Neyts, J.; Leyssen, P.; Constantino, G.; Radi, M. Discovery of multitarget antivirals acting on both the Dengue virus NS5-NS3 interaction and the host Src/Fyn kinase. *J. Med. Chem.*, **2015**, *58*, 4964-4975.

[50] Li, L.; Basavannacharya, C.; Chan, K.W.; Shang, L.; Vasudevan, S.G.; Yin, Z. Structure-guided discovery of a novel non-peptide inhibitor of dengue virus NS2B-NS3 protease. *Chem. Biol. Drug Design*, **2015**, *86*, 255-264.

[51] Brecher, M.; Li, Z.; Liu, B.; Zhang, J.; Koetzner, C.A.; Alifarag, A.; Jones, S.A.; Lin, Q.; Kramer, L.D.; Li, H. A conformational switch high-throughput screening assay and allosteric inhibition of the flavivirus NS2B-NS3 protease. *PLoS Pathog.*, **2017**, *13*, e1006411.

[52] Leal, E.S.; Aucar, M.G.; Cebhard, L.G.; Iglesias, N.G.; Pascual, M.J.; Casal, J.J.; Gamarnik, A.V.; Cavasotto, C.N., Bollini, M. Discovery of novel dengue virus entry inhibitors via a structure-based approach. *Bioorg. Med. Chem. Lett.*, **2017**, *27*, 3851-3855.

[53] Kannan, S.; Melesina, J.; Hauser, A.T.; Chakrabarti, A.; Heimburg, T.; Schmidtkunz, K.; Walter, A.; Marek, M.; Pierce, R.J.; Romier, C.; Jung, M.; Sippl, W. Discovery of inhibitors of *Schistosoma mansoni* HDAC8 by combining homology modeling, virtual screening, and in vitro validation. *J. Chem. Inf. Model.*, **2014**, *54*, 3005-3019.

[54] Melo-Filho, C.C.; Dantas, R.F.; Braga, R.C.; Neves, B.J.; Senger, M.R.; Valente, W.C.; Rezende-Neto, J.M.; Chaves, W.T.; Muratov, E.N.; Paveley, R.A.; Furnham, N.; Kamentsky, L.; Carpenter, A.E.; Silva-Junior, F.P.; Andrade, C.H. QSAR-driven discovery of novel chemical scaffolds active against *Schistosoma mansoni*. *J. Chem. Inf. Model.*, **2016**, 56, 1357-1372.

[55] Neves, B.J.; Dantas, R.F.; Senger, M.R.; Melo-Filho, C.C.; Valente, W.C.G.; de Almeida, A.C.M.; Rezende-Neto, J.M.; Muratov, E.; Kamentsky, L.; Carpenter, A.N.; Braga, R.C.; Silva-Junior, F.P.; Andrade, C.H. Discovery of new anti-schistosomal hits by integration of QSAR based virtual screening and high content screening. *J. Med. Chem.*, **2016**, *59*, 7075-7088.

[56] Zheng, F.; Robertson, A.P.; Abongwa, M.; Yu, E.W.; Martin, R.J. The *Ascaris suum* nicotinic receptor, ACR-16, as a drug target: Four novel negative allosteric modulators from virtual screening. *Int. J. Parasitol. Drugs Drug Resist.*, **2016**, *6*, 60-73.

[57] France, D.J.; Stepek, G.; Houston, D.R.; Williams, L.; McCormack, G.; Walkinshaw, M.D.; Page, A.P. Identification and activity of inhibitors of the essential nematode-specific metalloprotease DPY-31. *Bioorg. Med. Chem. Lett.*, **2015**, *25*, 5752-5755.

[58] Parameswaran, S.; Saudagar, P.; Dubey, V.K.; Patra, S. Discovery of novel anti-leishmanial agents targeting LdLip3 lipase. *J. Mol. Graph. Model.*, **2014**, 49, 68-79.

[59] Singh, J.; Srivastava, A.; Jha, P.; Sinha, K.K.; Kundu, B. L-asparaginase as a new molecular target against leishmaniasis: insights into the mechanism of action and structure-based inhibitor design. *Mol. Biosyst.*, **2015**, *11*, 1887-1896.

[60] Ochoa, R.; Watowich, S.J.; Flórez, A.; Mesa, C.V.; Robledo, S.M.; Muskus, C. Drug search for leishmaniasis: a virtual screening approach by grid computing. *J. Comput. Aided Mol. Des.*, **2016**, *30*, 541-552.

[61] Agnihotri, P.; Mishra, A.K.; Mishra, S.; Srihi, V.K.; Sahasrabuddhe, A.A.; Pratap, J.V.; Ochoa, R.; Watowich, S.J.; Flórez, A.; Mesa, C.V.; Robledo, S.M.; Muskus, C. Identification

of novel inhibitors of *Leishmania donovani* γ-Glutamylcysteine synthetase using structure-based virtual screening, docking, molecular dynamics simulation, and in vitro studies. *J. Chem. Inf. Model.*, **2017**, *57*, 815-825.

[62] Mansuri, R.; Kumar, A.; Rana, S.; Panthi, B.; Ansari, M.Y.; Das, S.; Dikhit, M.R.; Sahoo, G.C.; Das, P. In vitro evaluation of antileishmanial activity of computationally screened compounds against ascorbate peroxidase to combat amphotericin B drug resistance. *Antimicrob. Agents. Chemother.*, **2017**, *61*, e02429-16.

[63] Mishra, A.K.; Singh, N.; Agnihotri, P.; Mishra, S.; singh, S.P.; Kolli, B.K.; Chang, K.P.; Sahasrabuddhe, A.A.; Siddigi, M.I.; Pratap, J.V. Discovery of novel inhibitors for Leishmania nucleoside diphosphatase kinase (NDK) based on its structural and functional characterization. *J. Comput. Aided Mol. Des.*, **2017**, *31*, 547-562.

[64] Prokopcyk, I.M.; Ribeiro, J.F.; Sartori, G.R.; Sesti-Costa, R.; Silva, J.S.; Freitas, R.F.; Leitão, A.; Montanari, C.A. Integration of methods in cheminformatics and biocalorimetry for the design of trypanosomatid enzyme inhibitors. *Future Med. Chem.*, **2014**, 17-33.

[65] Demir, O.; Labaied, M.; Merritt, C.; Stuart, K.; Amaro, R.E. Computer-aided discovery of *Trypanosoma brucei* RNA-editing terminal uridylyl transferase 2 inhibitors. *Chem. Biol. Drug. Des.*, **2014**, *84*, 131-139.

[66] Herrmann, F.C.; Lenz, M.; Jose, J.; Kaiser, M.; Brun, R.; Schmidt, T.J. In silico identification and in vitro activity of novel natural inhibitors of *Trypanosoma brucei g*lyceraldehyde-3-phosphate-dehydrogenase. *Molecules*, **2015**, *20*, 16154-16169.

[67] de Almeida, H.; Leroux, V.; Motta, F.N.; Grellier, P.; Maigret, B.; Santana, J.M.; Bastos, I.M. Identification of novel *Trypanosoma cruzi* prolyl oligopeptidase inhibitors by structure-based virtual screening. *J. Comput. Aided. Mol. Des.*, **2016**, *30*, 1165-1174.

[68] Talevi, A. Computational approaches for innovative antiepileptic drug discovery. *Expert Opin. Drug Discov.*, **2016**, *11*, 1001-1016.

[69] Lipton, S.A. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat. Rev. Drug Discov.*,. **2006**, *5*,160–170.

[70] Rundfeldt, C.; Löscher, W. The pharmacology of imepitoin: the first partial benzodiazepine receptor agonist developed for the treatment of epilepsy. *CNS Drugs*. **2013**, *28*, 29–43.

[71] Peddibhotla, S.; Fontaine, P.; Leung, C.K.; Maloney, P.; Hershberger, P.M.; Wang, Y.; Bousquet, M.S.; Luesch, H.; Mangravita-Novo, A.; Pinkerton, A.B.; Smith, L.H.; Malany, S.; Choe, K. Discovery of ML358, a selective small molecule inhibitor of the SKN-1 Pathway involved in drug detoxification and resistance in nematodes. *ACS Chem. Biol.k*, **2015**, *10*, 1871-1879.

[72] Jacques, S.A.; Kuhn, I.; Koniev, O.; Schuber, F.; Lund, F.E.; Wagner, A.; Muller-Steffner, H.; Kellenberger, E. Discovery of potent inhibitors of *Schistosoma mansoni* NAD+ catabolizing enzyme. *J. Med. Chem.*, **2015**, *58*, 3582-3592.

[73] 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.; Tate, E.W. Structure-based design of potent and selective Leishmania N-myristoyltransferase inhibitors. *J. Med. Chem.*, **2014**, *57*, 8664-8670.

[74] Yokokawa, F.; Nilar, S.; Noble, C.G.; Lim, S.P.; Rao, R.; Tania, S.; Wang, G.; Lee, G.; Hunziker, J.; Karuna, R.; Manjunatha, U.; Shi, P.Y.; Smith, P.W. Discovery of potent non-nucleoside inhibitors of dengue viral RNA-dependent RNA polymerase from a fragment hit using structure-based drug design. *J. Med. Chem.*, **2016**, *59*, 3935-3952.

[75] Klug, D.M.; Gelb, M.H.; Pollastri, M.P. Repurposing strategies for tropical disease drug discovery. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 2569-2576.

[76] Anighoro, A.; Bajorath, J.; Rastelli, G. Polypharmacology: challenges and opportunities in drug discovery. *J. Med. Chem.*, **2014**, *57*, 7874-7887.

[77] Novac, N. Challenges and opportunities of drug repositioning. *Trends Pharmacol. Sci.*, **2013**, *34*, 267-272.

[78] Shineman, D.W.; Alam, J.; Anderson, M.; Black, S.E.; Carman, A.J.; Cummings, J.L.; Dacks, P.A.; Dudley, J.T.; Frail, D.E.; Green, A.; Lane, R.F.; Lappin, D.; Simuni, T.; Stefanacci, R.G.; Sherer, T.; Fillit, H.M. Overcoming obstacles to repurposing for neurodegenerative disease. *Ann. Clin. Transl. Neurol.*, **2014**, *1*, 512-518.

[79] Bloom, B.E. Creating new economic incentives for repurposing generic drugs for unsolved diseases using social finance. *Assay Drug Dev. Technol.*, **2015**, *13*, 606-611.

[80] Witkowski, X. Intellectual property and other legal aspects of drug repurposing. *Drug Discov. Today Ther. Stratet.*, **2011**, *8*, 139-143.

[81] Ferreira, L.G.; Andricopulo, A.D. Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective. *Drug Discov. Today*, *21*, 1699-1710.

[82] Bellera, C.L.; Di Ianni, M.E.; Sbaraglini, M.L.; Castro, E.A.; Bruno-Blanch, L.E.; Talevi, A. In: *Frontiers in Computational Chemistry Volume 1*. Ul-Haq, Z.; Madura, J.D., Eds.; Bentham, Sharjah, **2014**.

[83] Jin, G.; Wong, S.T.C. Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipelines. *Drug Discov. Today*, **2014**, *19*, 637-644.

[84] Oprea, T.I.; Overington, J. Computational and practical aspects of drug repositioning. *Assay Drug Dev. Technol.*, **2015**, *13*, 299-306.

[85] Chatelain, E. Chagas disease drug discovery: toward a new era. *J. Biomol. Screen.*, **2015**, *20*, 22-35.

[86] Gillan, V.; O´Neill, K.; Maitland, K.; Sverdrup, F.M.; Devaney, E. A repurposing strategy for Hsp90 inhibitors demonstrates their potency against filarialnematodes. *PLoS Negl. Trop. Dis.*, **2014**, *8*, e2699.

[87] Johnston, K.L.; Ford, L.; Umareddy, I.; Townson, S.; Specht, S.; Pfarr, K.; Hoerauf, A.; Altmeyer, R.; Taylor, M.J. Repurposing of approved drugs from the human pharmacopoeia to target *Wolbachia* endosymbionts of onchocerciasis and lymphatic filariasis. *Int. J. Parasitol Drugs Drug Resist.*, **2014***; 4*, 278-286.

[88] Rausch, K.; Hackett, B.A.; Weinbren, N.L.; Reeder, S.M.; Sadovsky, Y.; Hunter, C.A.; Schultz, D.C.; Coyne, C.B.; Cherry, S. Screening bioactives reveals nanchangmycin as a broad spectrum antiviral active against Zika virus. *Cell. Rep.*, **2017**, *18*, 804-815.

[89] Bulman, C.A.; Bidlow, C.M.; Lustigman, S.; Cho-Ngwa, F.; Williams, D.; Rascón, A.A. Jr., Tricoche, B.; Samje, M.; Bell, A.; Suzuki, B.; Lim, K.C.; Supakorndej, N.; Supakorndej; P.; Wolfe, A. R.; Knudsen, G.M.; Chen, S.; Wilson, C.; Ang, K.H.; Arkin, M.; Gut, J.; Franklin, C.; Marcellino, C.; McKerrow, J.H.; Debnath, A.; Sakanari, J. A. Repurposing auranofin as a lead candidate for treatment of lymphatic filariasis and onchocerciasis. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e003534.

[90] Cowan, N.; Keiser, J. Repurposing of anticancer drugs: in vitro and in vivo activities against *Schistosoma mansoni*. *Parasit. Vectors*, **2015**, *8*, 417.

[91] Panic, G.; Vargas, M.; Scandale, I.; Keiser, J.; Cowan, N.; Keiser, J. Activity profile of an FDA-approved compound library against *Schistosoma mansoni*. *PLoS Negl. Trop. Dis.*, **2015**, *9*, e0003962.

[92] Kaiser, M.; Mäser, P.; Tadoori, L.P.; Ioset, J.R.; Brun, R. Antiprotozoal activity profiling of approved drugs: a starting point toward drug repositioning. PLoS One, **2015**, *10*, e0135556.

[93] Bellera, C.L.; Balcazar, D.E.; Alberca, L.; Labriola, C.A.; Talevi, A.; Carrillo, C. Application of computer-aided drug repurposing in the search of new cruzipain inhibitors: Discovery of amiodarone and bromocriptine inhibitory effects. *J. Chem. Inf. Model.*, **2013**, *53*, 2402-2408.

[94] Bellera, C.L.; Balcazar, D.E.; Alberca, L.; Labriola, C.A.; Talevi, A.; Carrillo, C. Identification of levothyroxine antichagasic activity through computer-aided drug repurposing. *Sci. World. J.*, **2014**, *2014*, 279618.

[95] Bellera, C.L.; Balcazar, D.E.; Vanrell, M.C.; Casassa, A.F.; Palestro, P.H.; Gavernet, L.; Labriola, C.A.; Gálvez, J.; Bruno-Blanch, L.E.; Carrillo, C.; Talevi, A. Computer-guided drug repurposing: identification of trypanocidal activity of clofazimine, benidipine and saquinavir. *Eur. J. Med. Chem.*, **2015**, *93*, 338-348.

[96] Sbaraglini, M.L.; Bellera, C.L.; Fraccaroli, L.; Larocca, L.; Carrillo, C.; Talevi, A.; Alba Soto, C.D. Novel cruzipain inhibitors for the chemotherapy of chronic Chagas disease. *Int. J. Antimicrob. Agents*, **2016**, *48*, 91-95.

[97] Alberca. L.N.; Sbaraglini, M.L.; Balcazar, D.; Fraccaroli, L.; Carrillo, C.; Medeiros, A.; Benitez, D.; Comini, M.; Talevi, A. Discovery of novel polyamine analogs with anti-protozoal activity by computer guided drug repositioning. *J. Comput. Aided Mol. Des.*, **2016**, *30*, 305-321.

[98] Reigada, C.; Valera-Vera, E.A.; Sayé, M.; Errasti, A.E.; Avila, C.C.; Miranda, M.R.; Pereira, C.A. Trypanocidal effect of isotretinoin through the inhibition of polyamine and amino acid transporters in *Trypanosoma cruzi*. *PLoS Negl. Trop. Dis.*, **2017**, *11*, e0005472.

[99] Wu, L.; Ai, N.; Liu, Y.; Wang, Y.; Fan, X. Relating anatomical therapeutic indications by the ensemble similarity of drug sets. *J. Chem. Inf. Model.*, **2013**, *53*, 2154-2160.

[100] Pantziarka, P.; Bouche, G.; Meheus, L.; Sukhatme, V.; Sukhatme, V. P.; Vikas, P. The Repurposing Drugs in Oncology (ReDO) Project. *Ecancermedicalscience*, **2014**, *8*, 442.

[101] Oprea, T. I.; Mestres, J. Drug repurposing: far beyond new targets for old drugs. *AAPS J.*, **2012**, *14*, 759-763.