Published in final edited form as:

Future Med Chem. 2011 August ; 3(10): 1279–1288. doi:10.4155/fmc.11.85.

Drug discovery for neglected tropical diseases at the Sandler Center

Stephanie A Robertson^{†,1} and Adam R Renslo²

¹Sandler Center for Drug Discovery, Department of Pharmaceutical Chemistry, University of California, 600 16th Street, S-272, San Francisco, CA 94158, USA

²Small Molecule Discovery Center, Department of Pharmaceutical Chemistry, University of California, 1700 4th Street, Suite 214, San Francisco, CA 94158, USA

Abstract

The Sandler Center's approach to target-based drug discovery for neglected tropical diseases is to focus on parasite targets that are homologous to human targets being actively investigated in the pharmaceutical industry. In this way we attempt to use both the know-how and actual chemical matter from other drug-development efforts to jump start the discovery process for neglected tropical diseases. Our approach is akin to drug repurposing, except that we seek to repurpose leads rather than drugs. Medicinal chemistry can then be applied to optimize the leads specifically for the desired antiparasitic indication.

Drug discovery for neglected tropical diseases

Neglected tropical diseases (NTDs), such as leishmaniasis, filariasis, schistosomiasis, African sleeping sickness and Chagas disease, devastate the lives of approximately one billion people, mainly affecting those who live in poverty in Africa, Asia and the Americas. Despite the fact that current treatments for these diseases are of limited effectiveness and often highly toxic, there has been limited commercial interest in developing improved therapeutics as there is little commercial value in treatments for these poor patient populations. In the years 2000–2009 only 26 new drugs were approved for NTDs, 21 of which were for HIV or malaria [1]. As a result, the burden of drug discovery and development has fallen primarily to academic scientists involved in so-called translational research, a few focused private-sector groups, such as the Drugs for Neglected Disease Initiative and the Institute for One World Health, and public-private partnerships, such as Medicines for Malaria Venture. The Sandler Center for Drug Discovery at the University of California, San Francisco, founded in 2002, is one such academic center that, with grant funding and significant philanthropic aid from the Sandler Foundation, strives to make an impact on the advancement of new therapeutics for treatment of several of these devastating NTDs.

Drug discovery is an inherently risky and complex process. Translating the discovery of a novel therapeutic target into a clinical candidate can take years, requiring the synthesis and evaluation of hundreds of compounds for activity against multiple targets and off-targets,

© 2011 Future Science Ltd

[†]Author for correspondence: Tel.: +1 415 483 5332, Fax: +1 415 514 4507, stephanie.robertson@ucsf.edu.

Financial & competing interests disclosure The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

drug-like properties, and *in vivo* pharmacokinetic and pharmacodynamic behavior. Much of the specialized expertise required for this work is scarce or altogether absent in academe, with real-world expertise in medicinal chemistry, pharmacokinetics and toxicology particularly being lacking. Although academic centers are well situated to explore and understand in depth the complexities of the biology of a novel target, how those complexities manifest themselves in additional hurdles for drug development only become truly apparent once a drug has moved into the clinic. It is at this point that, almost inevitably, difficulties related to unanticipated adverse effects or particular pharmacokinetic/pharmacodynamic requirements inherent to the target are revealed. For academic groups and centers where little to no medicinal chemistry expertise is available, one of the most common strategies in drug discovery is to attempt to identify and repurpose drugs already approved for other indications (see [2-4] for examples of screening with repurposing as a goal). An enormous advantage of this strategy, if an appropriate compound is identified, is the ability to quickly jump into later stage clinical trials in the patient population to assess efficacy. This is best accomplished if the dosing scheme required for the repurposed use is within approved dosing regimens for the drug (dose levels, frequency and duration). Thus, repurposing drugs that are already approved for a different indication avoids the time and costs of the preclinical work leading to development of a clinic-ready drug. However, this strategy risks taking a drug into the clinic for a condition for which it has not been optimized, and often results in lower than ideal efficacy, as well as side effects resulting from on-target effects against the human target.

The Sandler Center has been able to both contemplate a repurposing approach and have the ability to maintain a small medicinal chemistry staff via collaboration with the University of California, San Francisco, Small Molecule Discovery Center. However, even with this resource, the handful of chemists on hand is not sufficient in numbers to efficiently execute a full drug-discovery program. Thus, a strategy that the Sandler Center has incorporated to best utilize limited drug-discovery resources is to build on knowledge already well developed in the pharmaceutical industry. This strategy involves selecting targets already well understood in the pharmaceutical industry, allowing scientists to start with already identified chemical classes to initiate lead optimization programs. Knowledge of the toxicities and selectivity issues inherent in these known classes can also enable scientists to incorporate improvements in selectivity and safety as they optimize leads for anti-parasitic activity. Table 1 highlights the various stages of drug discovery, from screening and **hit** identification, through **lead** optimization, to the investigational new drug (IND)-enabling studies required to gain approval for studies in human subjects, and demonstrates how starting medicinal chemistry efforts at a late 'repurposed' lead stage has the potential to accelerate the path from screening hit to clinical candidate.

Using a highly optimized and potentially clinically validated compound as a starting point for further medicinal chemistry can speed the process from hit identification to reaching the clinic by several years. A clinically validated compound already possesses optimized drug properties, and the series has been highly characterized for selectivity, safety and other critical characteristics. Thus, only a limited set of further modifications may need to be made to optimize anti-parasitic activity, as many of the drug-like properties have already been previously developed within the chemical series. Thus, not only is the lead-optimization time shortened significantly, but the medicinal chemistry resources needed during this time are heavily reduced.

Some examples of chemistry approaches in the Sandler Center that take advantage of the knowledge built around classes of drugs and targets well characterized by the pharmaceutical industry are outlined in the following discussion.

Cysteine protease inhibitors

Many of the parasites responsible for NTDs have cathepsin-like **cysteine proteases** that play a critical role in parasite biology. In *Trypanosoma cruzi*, the etiological agent for Chagas disease, the cysteine protease cruzain, a close homolog of human cathepsin L, is expressed in all lifecycle stages and has been shown to be involved in numerous processes including nutrient processing, immune evasion and differentiation (reviewed in [5]). Early studies on chemical inhibition of cruzain activity in the parasite indicated that parasite death was, at least in part, due to accumulation of the unprocessed protease within the Golgi [6,7]. Eventually, this protein accumulation was found to lead to shock of the Golgi and the endoplasmic reticulum of the parasite and, finally, death of the parasite. These compelling data were generated as the result of a collaboration between University of California, San Francisco, investigators working on this protease and a local bay area company, Khepri Pharmaceuticals (later bought by Arris, eventually merging with Celera), which was, at the time, focused on developing human cathepsin inhibitors to target diseases, such as osteoporosis. Screening a focused library of cysteine protease inhibitors generated at Khepri led to the identification of several compounds of interest [8]. Specifically, the vinylsulfone K11777 (**1**; Figure 1), was found to potently inhibit cruzain in biochemical assays and was also effective *in vitro* and *in vivo* against *T. cruzi* parasites (Table 2).

In this same vein, our recent screening of a much larger, 2100-member, cathepsin inhibitor collection has produced lead compounds for other tropical parasitic diseases [9]. The inhibitors screened here are true leads, in the sense that they were originally synthesized by medicinal chemists for particular targets/indications, and likely also with an eye towards good *in vivo* properties. Among the leads identified, the ketobenzoxazole **2** exhibited selectivity for trypanosome proteases TbCatB and cruzain over the human enzymes cathepsin B and L (Table 2). Compound **2** was found to be effective against cultured blood stage *Trypanosoma brucei* parasites at single digit micromolar concentrations while not significantly affecting Jurkat cells at 10 μ M (Table 2). Also identified in this screen were compounds **3** and **4**, which are representative of a series of structurally related compounds from the library that possess activity against the malaria parasite *Plasmodium falciparum*. Both **3** and **4** are potent reversible inhibitors of falcipain-3, and **3** exhibits greater than 100-fold selectivity for these enzymes over human cathepsin B and L. The compounds are also submicromolar inhibitors of *P. falciparum* growth *in vitro* and thus represent a relatively advanced starting point from which to pursue improved analogs with better *in vitro* and *in vivo* properties.

Of the compounds discussed earlier, K11777 (**1**) has been by far the most studied, its anti-trypanosomal activity having first been identified over a decade ago. The lack of medicinal chemistry capability in the early days of the Sandler Center meant that **1** itself was progressed into IND-enabling studies without further optimization [10]. Given this fact, it is not surprising that the compound has several sub-optimal properties that could complicate further development. The need for one or more back-up candidates is thus apparent and this goal was among the first pursued upon the establishment of medicinal chemistry capabilities within the Sandler Center in recent years via establishment of the University of California, San Francisco, Small Molecule Discovery Center.

Early cathepsin inhibitors, including **1**, act via irreversible covalent modification of the catalytic cysteine residue in the protease active site. Certainly, for chronic indications, such as osteoporosis, inhibition by an irreversible mechanism is considered highly undesirable as it presents risks of reduced selectivity and immunogenic potential. Presumably, concerns regarding these potential risks are the reason that all of the cathepsin inhibitors that have progressed into clinical trials act via reversible (if still covalent) inhibition. Ideally, a clinical

candidate targeting cruzain would also act reversibly, since a prolonged course of treatment is expected to be required (as it is for the current standard of care). With this goal in mind, we have expended significant time and effort to identify reversible-covalent inhibitors of cruzain, testing and synthesizing hundreds of such analogs. Primarily these have been analogs bearing aminoacetonitrile warheads, but heterocyclic ketone warheads have also been examined. While many of these compounds potently inhibit the enzyme *in vitro* and a few halt the proliferation of *T. cruzi* parasites in cells, we have not yet identified reversible inhibitors that confer the same cidal effects observed with **1**. For example, nitrile **5** [11] is an exceptionally potent inhibitor of cruzain in a biochemical assay but, unlike **1**, does not cure macrophage infected with *T. cruzi* parasites.

Optimizing cruzain inhibitors

There are several criteria to consider in developing cysteine protease inhibitors as drug candidates. Standard preferred criteria, including small molecular weight, high selectivity, good oral bioavailability and low toxicity of course apply. Additional insight into this specific class of cysteine proteases can be gleaned from over 15 years of pharmaceutical industry research focused on inhibiting a homologous class of human proteases, the cathepsins. For example, the investigational drug odanacatib is a selective and potent inhibitor of cathepsin K and is currently in Phase III clinical trials as a new treatment for osteoporosis [12]. Odanacatib was specifically designed to overcome a selectivity issue that was revealed when an earlier generation of cathepsin K inhibitors being pursued by Novartis had to be withdrawn from clinical trials due to adverse skin events that were observed in trial subjects [13]. These effects were consistent with what might be expected from the undesired inhibition of cathepsins B and L, which are highly expressed in skin fibroblasts. Researchers at Merck discovered that while the Novartis compound was quite selective for cathepsin K *in vitro*, its basic and lipophilic nature led to a loss of selectivity in cellular assays. This was hypothesized to be due to lysosomotropism (compound accumulation in the acidic lysosome, where cathepsins are concentrated [14]). With this knowledge, the Merck team designed neutral compounds that did not concentrate in the lysosome and that retained high selectivity in cellular assays. Nonbasic compounds, such as Merck's odanacatib and GlaxoSmithKline's relacatib, have shown impressive efficacy in clinical trials without the unwanted skin-related effects.

In the case of targeting the *T. cruzi* protease cruzain, lysosomotropism is also to be avoided since the *T. cruzi* parasite is located in the cytoplasm of host cells, and the protease is expressed not only in the parasite lysosome and the prelysosomal 'reservasome' but also in the flagellar pocket and plasma membrane of both epimastigotes and amastigotes. Furthermore, the likely mechanism of **1** action [6,7] involves targeting unprocessed cruzain (zymogen form) as it is leaving the endoplasmic reticulum. This implies that sequestration of the drug in acidic compartments of the host cell would almost certainly reduce the concentration of drug available to inhibit the desired target. The basic nature of **1** unfortunately suggests the potential for lysosomotropism and we have, therefore, sought to identify back-up candidates that lack this property. Simply replacing the basic piperazine ring in **1** with the much less basic pyridine ring system produced neutral analogs, such as **6** (Figure 1 & Table 2), that had dramatically improved cidal activity against *T. cruzi* parasites despite being less potent inhibitors of cruzain in biochemical assays [15, P Doyle & J Engel. Unpublished Data]. This apparent discrepancy between biochemical and parasite activity can be understood if one postulates that the more potent but lysosomotropic **1** accumulates in acidic compartments while nonbasic analogs, such as **6** do not, and are therefore able to reach higher concentrations at the desired site of action in the endoplasmic reticulum and Golgi. Another intriguing possibility is that analog **6** with its exposed 4-pyridyl ring is capable of also inhibiting *T. cruzi* CYP51, another promising drug target (see later

discussion) and an enzyme that is known to bind small molecules with exposed pyridine rings [16]. In fact, **6** does bind CYP51 with nanomolar affinity *in vitro* (L Podust, Unpublished Data).

In addition to exploring subtle structural modifications as found in vinylsulfone **6**, we also have sought nonpeptidic substructures that might replace the P2 and P3 groups in **1** [17]. In this effort we identified several nonbasic and nonpeptidic inhibitors, including **7**, for which a co-crystal structure bound to cruzain was solved to high resolution (Figure 2) [18]. Nonbasic and presumably nonlysosomotropic compounds, such as **6** and **7**, are anticipated to have reduced potential for off-target effects related to inhibition of lysosomal host enzymes, though this remains to be demonstrated.

Irreversible cruzain inhibitors & target identification

As noted earlier, we have to date been unsuccessful in identifying reversible cruzain inhibitors that confer cidal activity against the *T. cruzi* parasite. This is possibly a function of the unusual mechanism of **1** action in inhibiting cruzain self-processing in the ER and Golgi; existing reversible inhibitors may not effectively impair this self-processing. Of course it may be possible to identify covalent but slowly reversible inhibitors that would confer cidal effects on the *T. cruzi* parasite while still offering reduced potential for immunogenicity and undesired off-target effects. It is also possible that in addition to cruzain, **1** inhibits other hitherto unidentified targets in the parasite and that its cidal effects derive from inhibition of these other targets in addition to cruzain. To better understand the target(s) of **1**, we synthesized the *N*-propargyl analog **8** (Figure 2) and are currently using this chemical probe to identify, in an unbiased fashion, all the covalent and 'saturable' targets of **1** in *T. cruzi*. Preliminary studies with **8** have confirmed its ability to covalently label cruzain, as determined by SDS-PAGE analysis following conjugation to a fluorophore via the propargyl function. Next we plan to use biotin conjugates of **8** to pull-down and identify by mass spectroscopy all the low-abundance targets of **1** that are competitively labeled by **8**. This tool may also provide insight into off-target activities of **1** in human cells.

CYP51 inhibitors

In the 1960s and 1970s drug-discovery programs were initiated to develop treatments for the growing issue of serious fungal infections in humans. Azole-containing compounds were shown to have potent antifungal activity, later identified as being due to interference with the fungal sterol biosynthetic pathway. It was discovered that the target of these azole-containing compounds was lanosterol 14 α -demethylase (cytochrome P450 [CYP] subfamily 51, CYP51), an enzyme on which fungi are highly dependent for production of ergosterol, a critical component of the fungal cellular membrane [19]. Parasites such as *T. cruzi* and *Leishmania donovani* are also dependent on this pathway for sterol biosynthesis and apparently cannot scavenge host cholesterol as a substitute when this pathway is inhibited. Several researchers have shown that a number of inhibitors of fungal CYP51, including the recently developed antifungal drug posaconazole, are potent inhibitors of *T. cruzi* CYP51, and are able to cure mice suffering from acute and chronic Chagas disease (reviewed in [20,21]). Posaconazole is now poised to enter clinical trials to evaluate the effectiveness in treatment of Chagas disease. However, manufacturing costs may limit widespread use of posaconazole, especially in the long-term treatment of the chronic disease. Ravuconazole, another azole antifungal, is similarly poised to enter clinical trials; however, despite potent *in vitro* antiparasitic activity, efficacy in animal models was found to be more limited than posaconazole, presumably due to suboptimal pharmacokinetic properties [21,22]. In fact, the effectiveness of different azoles has been attributed heavily to the pharmacokinetic properties of the azole tested. Since *T. cruzi* is an intracellular parasite, the pharmacokinetic

characteristics of antiparasitic molecules should include a high volume of distribution, indicative of significant tissue distribution.

Structure-based CYP51 inhibitor drug design

Given some of the potential limitations of currently available antifungals in the treatment of Chagas disease, the development of new molecules with potent *T. cruzi* activity, inexpensive manufacturing costs and optimal pharmacokinetic and safety properties may offer improved therapeutic options against this infection. Recent efforts at the Sandler Center and elsewhere have identified potent small-molecule inhibitors of the *T. cruzi* ergosterol pathway including the CYP51, such as pyridine **9** (Figure 3) [23]. Inhibitors of this pathway also show good efficacy in animal models of disease [16,24,25]. Not surprisingly, each of these inhibitors possesses a nitrogen-containing aromatic moiety that is the presumed site of coordination by the heme cofactor at the active site of the CYP51 enzyme. Further optimization of these molecules to improve activity, selectivity and drug-like properties can take lessons from decades of development of this class as antifungal agents. The development of fungal resistance to azoles has been a continual driver for new antifungal molecules. Mechanisms of resistance include both mutations in the target protein as well as upregulation of efflux pumps that remove the drug from fungal intracellular space [26]. In the context of anti-trypansomal activity, it was shown that *T. cruzi* could develop resistance to the azole fluconazole and this also conferred resistance on other azole-based inhibitors [27]. In addition, inhibition at various points in the sterol biosynthetic pathway resulted in upregulation of mRNA and protein expression of CYP51, suggesting that contributions to resistance could be conferred via homeostatic mechanisms [28]. Recently solved structures of *T. cruzi* CYP51 bound with posaconazole or fluconazole have also enabled the development of hypotheses about how mutational hotspots may confer resistance on azole drugs [29]. The authors suggest that interactions between solubilizing groups on the periphery of posaconazole with amino acids at the entrance to the active site tunnel may provide the means by which the organism can, via mutation, circumvent binding of the drug without impacting affinity for the natural substrate. Hypotheses such as these may inform the design of new azole-based inhibitors that would have a reduced potential for the generation of resistance via minimization of contact with such mutational hotspots. It will be critical early on in this program to better define the potential mechanisms of resistance of *T. cruzi* and assess whether minimization of contacts with mutational hotspots are effective and sufficient to limit the risks of development of resistance.

Another key limitation of the earlier antifungal azoles was the occurrence of undesirable side effects. Presumably many of the side effects noted, such as hepatotoxicity, are due to off-target interaction with human CYP450 enzymes, as well as the human CYP51 enzyme [20]. Comparing the long, hydrophobic sterol-binding pocket of *T. cruzi* CYP51 to structures of human CYP51 can provide insights into modifications that would provide selectivity for parasite versus human enzyme. In addition, concomitant screening of human CYP51 and other key human CYP enzymes will be critical in optimizing activities of inhibitors towards parasite enzymes and away from human analogs.

Early azole antifungals were further limited by poor pharmacokinetic properties. Structural analysis of complexes of small molecules with CYP51 will aid understanding of the binding modes and provide insights into regions of the inhibitor that may be amenable to modifications aimed at improving pharmacokinetic properties (e.g., the introduction of solubilizing groups).

Finally, in addition to a structure-based approach to designing optimized CYP51 inhibitors, the Sandler Center is engaged in applying a strategy of unbiased fragment screening to

identify small-molecule fragments that bind the enzyme in new and perhaps unexpected ways. Because the orientation of heme-binding warheads is relatively straightforward to model computationally [23] we expect it may be possible to design viable chemical linkages between such warheads and fragments that are found to bind on the periphery of the heme cofactor site. Alternatively, the fragment screen could yield new heme-binding heterocycles that possess chemical handles from which to grow more potent and selective inhibitors.

Kinase targets

In the late 1980s and early 1990s, researchers uncovered the critical role protein kinase-signaling cascades play in cancer, heightening interest in protein kinases as potential drug targets. There were, however, clear hurdles to targeting protein kinases with small molecules. Conceptually, targeting the substrate-binding pocket could provide some specificity towards a given kinase, but these binding sites are generally shallow and difficult to target with small molecules. Conversely, the ATP-binding pocket in kinases is small and highly amenable to targeting with small molecules, but it is highly conserved across families of protein kinases (not to mention other purine-dependent proteins) and may, therefore, be difficult to target selectively. In addition, cellular ATP concentrations are high, risking reduced efficacy of ATP-competitive inhibitors. In practice, chemical design and structural data eventually revealed the means by which the ATP site of kinases could be targeted with reasonable selectivity [30], and this has led to several classes of ATP-competitive protein kinase inhibitors being approved for clinical use in recent years, primarily in the oncology area.

The Sandler Center is applying both genetic and chemical biological approaches to identify potential kinase targets in trypanosome parasites. Gene silencing by RNA interference is now a well-established approach to probe gene function. We have applied RNAi to identify kinases in *T. brucei* parasites that appear to be essential for parasite viability [Nishino M, Choy JW, Gushwa NN *et al.* Quantitative chemical proteomics with a 'clickable' hypothemycin probe reveals new therapeutic targets in *Trypanosoma brucei*. Manuscript in Preparation]. In parallel, a chemical biological approach was employed using novel small-molecule probes that covalently modify reactive residues present in a subset of the trypanosome kinome [Nishino M, Choy JW, Gushwa NN *et al.* Quantitative chemical proteomics with a 'clickable' hypothemycin probe reveals new therapeutic targets in *Trypanosoma brucei*. Manuscript in Preparation]. Interestingly, we found that the same kinases shown to be important by RNAi techniques were also labeled in live parasites by the active site probes; consistent with the RNAi data, the probes themselves were shown to be trypanocidal. This cross-validation by genetic and small-molecule approaches gives us greater confidence in pursuing these specific kinases as targets for intervention in trypanosomal disease. We have performed both whole-cell and biochemical screens of kinase-targeted small-molecule libraries in the search for more drug-like inhibitor probes of these kinases (manuscript in preparation). Importantly, our activity-based probes can potentially be used to confirm mode of action during the further optimization of screening hits targeting these kinases.

US FDA/clinical screening library

As discussed earlier, the fastest way to the clinic can often be through repurposing drugs already approved for other indications. The potential use of the antifungal posaconazole in the treatment of Chagas disease is just one such example of this repurposing strategy. Thus, an additional strategy that the Sandler Center is moving towards is to screen all suitable FDA-approved drugs as potential advanced leads. To facilitate this strategy, the Small Molecule Discovery Center in collaboration with the Sandler Center is currently in the

process of expanding the bioactive library to include a more complete set of FDA approved drugs. However, drugs developed for other indications often have characteristics that prevent them from easily being repurposed for poor populations in rural settings. Some of these characteristics include high manufacturing costs that result in drugs that are unaffordable to the target population and routes of administration such as intravenous injection which are difficult to perform outside of a sterile hospital environment. Thus, we are expanding our approach to encompass not only FDA-approved drugs, but also compounds that have passed Phase I human clinical trials but failed to reach market for various reasons other than overt safety issues, and are in the process of generating a list of compounds that fulfill this criterion to enable expansion of libraries to include this set of potential drugs. If such compounds do have appropriate characteristics, they may be repurposed directly, or be utilized as a highly advanced lead with advanced drug-like properties. Since many of these compounds will have good drug-like properties, little optimization may be needed, and data concerning activity at human targets would enable us to make data-driven decisions about modifications that might be utilized to improve characteristics specific to the disease and patient population. The recent work using the protein farnesyltransferase inhibitor tipifarnib as an advanced lead for the development of a drug for treatment of Chagas disease is an excellent example of this strategy [24,25].

This approach of using clinical and approved compounds as leads would be greatly accelerated if pharmaceutical companies would provide access to structural congeners of the clinical or approved compounds, along with associated ADME/pharmacokinetic data. The disclosure of such information should have little if any negative commercial consequences and many are now arguing that companies should be providing such focused libraries and data [31,32,33]. The donation GlaxoSmithKline has made to patent pools with a focus on NTDs is a positive step in this direction. However, these patent pools and data currently have limited potential since they only provide access to patents and data generated for compounds that have been shown by GlaxoSmithKline to have activity against certain parasites, such as malaria, and thus exclude many of the drug classes that may be compelling for use in a lead-repurposing strategy.

Future perspective

Drug-discovery research in NTDs is fortunately not nearly as neglected as it once was. The appearance of discovery and development organizations and public-private partnerships such as the Drugs for Neglected Diseases *initiative* and the Medicines for Malaria Venture has provided a pathway to the clinic for promising molecules emerging from industrial and academic drug-discovery centers. The contraction of the global pharmaceutical industry in recent years has arguably been a positive factor for work on NTDs, as experienced drug-discovery scientists have migrated from the private sector into academia as a result. Quite unimaginable 5 years ago, there exists now a large pool of experienced and talented medicinal chemists that can be readily recruited to work on drug leads for NTDs. Most important, however, has been the support of philanthropy for both the discovery and development of drugs for treatment of NTDs. Given the long timelines inherent in drug development, it is perhaps too soon to take a full account of the ultimate success of this philanthropic support. The next 5 years are sure to see more new chemical entities targeting treatment of NTDs progressing through human clinical trials. An important driver of early-stage discovery will be continued basic research in parasite biology, including the nature of host-parasite interactions. The identification of parasitic targets for which a wealth of information is available from pharmaceutical industry experience will continue to have great potential in accelerating the rate of development of therapeutics for use in the treatment of NTDs. In addition, new targets that are unique to the parasite or to the host-parasite interaction will be the discoveries with the greatest potential to produce major advances in

drug efficacy and safety. Incentives that result in the engagement of the pharmaceutical industry in the development of drugs against these novel targets should be a major goal of government and philanthropic funding agencies to enhance the rapid, successful development of well-designed therapeutics against both well-understood and unique target classes.

Acknowledgments

The authors would like to thank the Sandler Foundation for their generous support of the work described in this article. Aspects of the work described were also supported by funding granted by the National Institute of Allergy and Infectious Diseases, US Department of Health and Human Services, National Institutes of Health.

Bibliography

1. Cohen J, Dibner MS, Wilson A. Development of and access to products for neglected diseases. PLoS One. 2010; 5(5):e10610. Gives an excellent overview on the current status of neglected diseases drug development. [PubMed: 20485552]
2. Engel JC, Ang KK, Chen S, Arkin MR, McKerrow JH, Doyle PS. Image-based high-throughput drug screening targeting the intracellular stage of *Trypanosoma cruzi*, the agent of Chagas' disease. Antimicrob Agents Chemother. 2010; 54(8):3326–3334. [PubMed: 20547819]
3. Gut J, Ang KK, Legac J, Arkin MR, Rosenthal PJ, McKerrow JH. An image-based assay for high throughput screening of *Giardia lamblia*. J Microbiol Methods. 2011; 84(3):398–405. [PubMed: 21262276]
4. Mackey ZB, Baca AM, Mallari JP, et al. Discovery of trypanocidal compounds by whole cell HTS of *Trypanosoma brucei*. Chem Biol Drug Des. 2006; 67(5):355–363. [PubMed: 16784460]
5. Sajid, M.; Robertson, SA.; Brinen, LS.; McKerrow, JH. CRUZAIN: the path from target validation to the clinic. In: Robinson, RW.; Dalton, DP., editors. Cysteine Proteases of Pathogenic Organisms. Vol. 100. Landes Biosciences and Springer; NY, USA: 2011.
6. Engel JC, Torres C, Hsieh I, Doyle PS, McKerrow JH. Upregulation of the secretory pathway in cysteine protease inhibitor-resistant *Trypanosoma cruzi*. J Cell Sci. 2000; 113(Pt 8):1345–1354. [PubMed: 10725218]
7. Engel JC, Doyle PS, Palmer J, Hsieh I, Bainton DF, McKerrow JH. Cysteine protease inhibitors alter Golgi complex ultrastructure and function in *Trypanosoma cruzi*. J Cell Sci. 1998; 111(Pt 5): 597–606. [PubMed: 9454733]
8. Engel JC, Doyle PS, Hsieh I, McKerrow JH. Cysteine protease inhibitors cure an experimental *Trypanosoma cruzi* infection. J Exp Med. 1998; 188(4):725–734. [PubMed: 9705954]
9. Ang KK, Ratnam J, Gut J, et al. Mining a cathepsin inhibitor library for new antiparasitic drug leads. PLoS Negl Trop Dis. 2011; 5(5):e1023. [PubMed: 21572521]
10. McKerrow JH, Doyle PS, Engel JC, et al. Two approaches to discovering and developing new drugs for Chagas disease. Mem Inst Oswaldo Cruz. 2009; 104(Suppl. 1):263–269. [PubMed: 19753483]
11. Beaulieu C, Isabel E, Fortier A, et al. Identification of potent and reversible cruzipain inhibitors for the treatment of Chagas disease. Bioorg Med Chem Lett. 2010; 20(24):7444–7449. [PubMed: 21041084]
12. Gauthier JY, Chauret N, Cromlish W, et al. The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K. Bioorg Med Chem Lett. 2008; 18(3):923–928. Nice example summarizing the strategies towards optimization of a drug, including improvements based on clinical findings from previous cathepsin K inhibitors. [PubMed: 18226527]
13. Desmarais S, Black WC, Oballa R, et al. Effect of cathepsin k inhibitor basicity on *in vivo* off-target activities. Mol Pharmacol. 2008; 73(1):147–156. [PubMed: 17940194]
14. Black WC, Percival MD. The consequences of lysosomotropism on the design of selective cathepsin K inhibitors. Chembiochem. 2006; 7(10):1525–1535. [PubMed: 16921579]

15. Jaishankar P, Hansell E, Zhao DM, Doyle PS, McKerrow JH, Renslo AR. Potency and selectivity of P2/P3-modified inhibitors of cysteine proteases from trypanosomes. *Bioorg Med Chem Lett*. 2008; 18(2):624–628. [PubMed: 18055204]
16. Doyle PS, Chen CK, Johnston JB, et al. A nonazole CYP51 inhibitor cures Chagas' disease in a mouse model of acute infection. *Antimicrob Agents Chemother*. 2010; 54(6):2480–2488. [PubMed: 20385875]
17. Bryant C, Kerr ID, Debnath M, et al. Novel non-peptidic vinylsulfones targeting the S2 and S3 subsites of parasite cysteine proteases. *Bioorg Med Chem Lett*. 2009; 19(21):6218–6221. [PubMed: 19773167]
18. Brak K, Kerr ID, Barrett KT, et al. Nonpeptidic tetrafluorophenoxymethyl ketone cruzain inhibitors as promising new leads for Chagas disease chemotherapy. *J Med Chem*. 2010; 53(4):1763–1773. [PubMed: 20088534]
19. Heeres J, Meerpoel L, Lewi P. Conazoles. *Molecules*. 2010; 15(6):4129–4188. [PubMed: 20657432]
20. Buckner FS. Sterol 14-demethylase inhibitors for *Trypanosoma cruzi* infections. *Adv Exp Med Biol*. 2008; 625:61–80. Detailed review on the development of this class of inhibitor for Chagas. [PubMed: 18365659]
21. Urbina JA. Ergosterol biosynthesis and drug development for Chagas disease. *Mem Inst Oswaldo Cruz*. 2009; 104(Suppl. 1):311–318. Excellent detailed review on the development of this class of inhibitor for Chagas. [PubMed: 19753490]
22. Diniz Lde F, Caldas IS, Guedes PM, et al. Effects of ravuconazole treatment on parasite load and immune response in dogs experimentally infected with *Trypanosoma cruzi*. *Antimicrob Agents Chemother*. 2010; 54(7):2979–2986. [PubMed: 20404124]
23. Chen CK, Doyle PS, Yermalitskaya LV, et al. *Trypanosoma cruzi* CYP51 inhibitor derived from a *Mycobacterium tuberculosis* screen hit. *PLoS Negl Trop Dis*. 2009; 3(2):e372. [PubMed: 19190730]
24. Huckle O, Gelb MH, Verlinde CL, Buckner FS. The protein farnesyltransferase inhibitor Tipifarnib as a new lead for the development of drugs against Chagas disease. *J Med Chem*. 2005; 48(17):5415–5418. [PubMed: 16107140]
25. Kraus JM, Tatipaka HB, McGuffin SA, et al. Second generation analogues of the cancer drug clinical candidate tipifarnib for anti-Chagas disease drug discovery. *J Med Chem*. 2010; 53(10):3887–3898. [PubMed: 20429511]
26. Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S. Molecular basis of resistance to azole antifungals. *Trends Mol Med*. 2002; 8(2):76–81. [PubMed: 11815273]
27. Buckner FS, Wilson AJ, White TC, Van Voorhis WC. Induction of resistance to azole drugs in *Trypanosoma cruzi*. *Antimicrob Agents Chemother*. 1998; 42(12):3245–3250. [PubMed: 9835521]
28. Hankins EG, Gillespie JR, Aikenhead K, Buckner FS. Upregulation of sterol C14-demethylase expression in *Trypanosoma cruzi* treated with sterol biosynthesis inhibitors. *Mol Biochem Parasitol*. 2005; 144(1):68–75. [PubMed: 16165233]
29. Chen CK, Leung SS, Guilbert C, Jacobson MP, McKerrow JH, Podust LM. Structural characterization of CYP51 from *Trypanosoma cruzi* and *Trypanosoma brucei* bound to the antifungal drugs posaconazole and fluconazole. *PLoS Negl Trop Dis*. 2010; 4(4):e651. [PubMed: 20386598]
30. Noble ME, Endicott JA, Johnson LN. Protein kinase inhibitors: insights into drug design from structure. *Science*. 2004; 303(5665):1800–1805. [PubMed: 15031492]
31. Gold ER, Kaplan W, Orbinski J, Harland-Logan S, N-Marandi S. Are patents impeding medical care and innovation? *PLoS Med*. 2010; 7(1):e1000208. Examines whether and how patents are impeding health care and innovation. [PubMed: 20052274]
32. Lexchin J. One step forward, one step sideways? Expanding research capacity for neglected diseases. *BMC Int Health Hum Rights*. 2010; 10:20. Thought-provoking analysis of the current barriers in neglected tropical disease drug development and possible approaches to address to these challenges. [PubMed: 20630063]

- 33•. Edwards AM, Bountra C, Kerr DJ, Willson TM. Open access chemical and clinical probes to support drug discovery. Nat Chem Biol. 2009; 5:436. Provides compelling arguments on how to improve drug discovery and development efficiency by utilizing open-access industry-academia partnerships. [PubMed: 19536100]

Key Terms

Hit	Small molecule with confirmed activity against a target or pathway. Hits are usually identified by screening naive compound libraries (i.e., with unenumerated biological activity).
Lead	Small molecule that has been optimized via chemical synthesis for <i>in vitro</i> potency, selectivity, and the potential for good <i>in vivo</i> properties including both activity and drug-like properties such as pharmacokinetics. A lead will usually possess efficacy in an animal disease model, whereas a hit may not.
Cysteine proteases	Large class of enzymes that possess a catalytic cysteine thiol function that promotes cleavage of peptide amide bonds. Examples of parasite cysteine proteases described in the text include TbCatB (<i>Trypanosoma brucei</i> cathepsin B-like protease), cruzain and falcipain-3. Human analogs are cathepsin B and cathepsin L.
CYP51	Also known as sterol 14 α -demethylase, a heme-containing enzyme involved in the biosynthesis of membrane sterols. In the parasite <i>Trypanosoma cruzi</i> this enzyme is essential for the biosynthesis of ergosterol. The analogous enzyme in fungi is a clinically validated drug target.

Executive summary

- Many drug targets for the treatment of human disease have homologs in the parasites responsible for neglected tropical diseases (NTDs). Information the pharmaceutical industry has generated on strategies and safety issues around these target classes can be invaluable in facilitating drug development for these NTDs.
- Irreversible inhibitors of the cathepsin-like protease cruzain are effective against *Trypanosoma cruzi* parasites in culture and in animal models of infection. Optimization strategies for cruzain inhibitors have benefited significantly from the knowledge generated during cathepsin drug development by the pharma industry.
- We have recently identified chemical analogs of the cysteine protease inhibitor K11777 that are more potent and eliminate some of its potential development liabilities.
- The enzyme CYP51 is a promising new drug target in *T. cruzi* with possible lessons to be learned from current experience with azole antifungals.
- We have recently identified essential kinases in *Trypanosoma brucei* using both RNAi and chemical biological approaches.
- Screening of FDA-approved and clinical drugs, as well as lead libraries from pharmaceutical companies, can serve to generate high-quality leads for drug discovery on NTDs.

Figure 1. Protease inhibitor drug leads for *Trypanosoma cruzi* (1, 5 & 6), *Trypanosoma brucei* (2) and *Plasmodium falciparum* (3 & 4), identified at the Sandler Center

Figure 2. Crystal structure of cruzain in complex with the nonpeptidic and nonbasic vinylsulfone analog 7, solved to a resolution of 1.75 Å by Sandler Center scientists (PDB ID 3HD3) Shown is the orientation for bound 7 in the cruzain active site (courtesy of Iain Kerr). Vinylsulfone 8 is a chemical probe designed to identify covalent targets of K11777 (1) and related vinylsulfone-based compounds.

Figure 3. Novel inhibitor of TcCYP51

Table 1

Drug-discovery timelines and resources: traditional versus repurposing leads.

Stage	Overall goal	Traditional leads strategy		Repurposing leads strategy	
		Typical time	Staff	Typical time	Staff
Hit identification and validation	Identify chemotypes with biological activity that can be modulated	3–6 months	Two biologists One chemist	3–6 months	Two biologists One chemist
Hit to lead	Identify one or more chemical series that have reasonable and tunable activity, and <i>in vitro</i> ADME, safety and selectivity	9–12 months	Three to four biologists Four chemists		
Lead optimization	Optimize the <i>in vivo</i> properties of the lead series, identify and characterize one to three potential clinical candidates	18–24 months	Six plus biologists Eight chemists	6–12 months	Six plus biologists One to three chemists
Investigational new drug-enabling studies	Complete US FDA requirements to initiate clinical trials on a new chemical entity	12 months	Contract research laboratories	12 months	Contract research laboratories

Protease inhibition and protease growth inhibition data for selected lead compounds identified by screening a cathepsin inhibitor library.

Table 2

Compound	k_{inact}/K_i (s ⁻¹ M ⁻¹)	IC ₅₀ (μM)		K _i (μM)		GI ₅₀ (μM)		MTC (μM)	
		Cruz.	TbCatB	FP-3	Cat L	Cat B	<i>Plasmodium falciparum</i>		<i>Trypanosoma brucei</i>
1	118,000	0.004	4				NT	7.0	8
2		0.030	0.070		1.1	0.32	NT	4.2	
3		NA	NA	0.016	2.2	18	0.84	NT	NT
4		NA	NA	0.0024	0.032	6.7	0.87	NT	NT
5		<0.006	>100	0.011				2.5	>10
6	26,000								1

Cat: Cathepsin; Cruz: Cruzain; FP-3: Falcipain-3; GI₅₀: Concentration required to inhibit parasite growth by 50%; MTC: Minimal trypanocidal concentration (the drug concentration that completely clears parasite from *Trypanosoma cruzi* infected macrophage cells); NA: Not significantly active; NT: Not tested; TbCatB: *Trypanosoma brucei* cathepsin B-like protease.