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Target assessment for antiparasitic drug discovery

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Drug discovery is a high-risk, expensive and lengthy process taking at least 12 years and costing upwards of US$500 million per drug to reach the clinic. For neglected diseases, the drug discovery process is driven by medical need and guided by pre-defined target product profiles. Assessment and prioritisation of the most promising targets for entry into screening programmes is crucial for maximising the chances of success. Here, we describe criteria used in our drug discovery unit for target assessment and introduce the 'traffic-light' system as a prioritisation and management tool. We hope this brief review will stimulate basic scientists to acquire additional information necessary for drug discovery.

Drugs for neglected tropical diseases

Approximately 1 billion people – a sixth of the world's population – suffer from neglected tropical diseases (NTDs), including the vector-borne parasitic diseases: filariasis, onchocerciasis, schistosomiasis, African sleeping sickness, Chagas disease and leishmaniasis [1]. Unlike the 'big three' infectious diseases [HIV/AIDS, tuberculosis (TB) and malaria], NTDs receive comparatively little international attention [2]. Precise figures for the annual death toll and disease burden, measured as disability-adjusted life years (DALYs) for NTDs are not available. Estimates vary from 137 000 to 534 000 deaths per year and 12.8 to 56.6 million DALYs [2–5]. Many of the existing drugs used to treat NTDs have serious limitations, including: cost, difficulties in administration, poor safety profile and a lack of efficacy (e.g. owing to drug resistance) [6,7]. We understand the biology of these parasites well, yet this knowledge has not been translated into modern therapeutics. In fact, of all new drugs to reach the market in the past 25 years, only 1% was for neglected disease [8]. Together, NTDs account for 5% of the global disease burden; yet, in a typical year, only approximately 0.1% of global investment in research is devoted to drug discovery for such diseases.

Historically, antiparasitic drug discovery has been conducted through low-cost, low-risk strategies by pharmaceutical companies and many such drugs were first developed for other indications. These companies have produced therapeutics by combination therapy using existing drugs, by realizing a new indication for an existing drug or through label extensions from veterinary products [9]. Although there is some merit in this 'piggy-backing' approach, many such drugs have proved too expensive or inappropriate for use in real-life settings to have any substantial health impact [10]. Indeed, only four out of 17 antiparasitic drugs developed from 1974 to 2004 were judged to be entirely suitable for use in resource-poor settings [10,11]. Moreover, evaluating patented compounds or drugs for a non-commercial patient class is perceived by some companies as a serious potential threat to future commercial revenue owing to the possibility of revealing toxicities that were unknown previously [12].

The withdrawal of many large pharmaceutical companies over the past 25 years from anti-parasitic programmes as part of their core strategies, plus the unyielding need for the discovery of new therapeutics against these diseases, means that significant gaps have emerged, particularly in early drug discovery [9]. Fortunately, several recent developments have resulted in credible mechanisms through which validated targets for NTDs can be progressed into and through a drug-discovery pipeline (Box 1). Therefore, the purpose of this review is to illustrate how molecular targets are assessed for entry into a drug-discovery process and to encourage groups to generate the necessary information for target assessment as a matter of course during their research, thereby hopefully producing a plethora of potential anti-parasitic drug targets for the future.

This review will focus on the molecular-target approach to drug discovery. Nevertheless, the authors recognize that *in vitro* screening against whole parasites is also a valuable alternative strategy for antiparasitic drug discovery [13–16].

Goals of a drug-discovery programme

The ultimate goal of a discovery programme is the development of a new therapeutic with substantial benefits over existing therapies. To ensure the requirements of a new drug are established clearly and that they drive the process of discovery and development, a target-product profile (TPP) is established at the beginning of the programme. The TPP is a list that defines and prioritises the key attributes of the intended new agent (Box 2). The full range of attributes needs to be considered, prioritised and agreed on carefully in advance by all stakeholders, including patients, medical personnel, regulatory authorities and policy makers in disease-endemic countries. A TPP evolves and matures as a project advances and thus needs to be reassessed periodically with regard to meeting essential, preferred or minimal acceptable criteria.

To initiate a drug-discovery programme, a pool of putative targets will require assessment. The process thereafter is very much a voyage of discovery and the

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Box 1. Recent developments facilitating drug discovery for tropical diseases[a]

- Whole genome sequencing of several protozoan and helminth parasites, in some cases multiple species (www.sanger.ac.uk/pathogens/)
- Establishment of new public–private partnerships (PPP) and non-profit pharmaceutical companies, such as:
Medicines for Malaria Venture (www.mmv.org)
Drugs for Neglected Diseases initiative (www.dndi.org/)
TB Alliance (www.tballiance.org/)
Institute for One World Health (www.oneworldhealth.org/)
- Injection of funds by philanthropic organisations, such as the Bill and Melinda Gates Foundation and the Wellcome Trust, into PPPs, leading to new neglected disease drug-discovery initiatives expanding out of academic institutions
- In-kind contributions from large pharmaceutical organisations using a no-profit, no-loss model in which scientists from these organisations work within dedicated institutes and in partnership with relevant Public Health bodies

[a] Further details available from [2,6,7,10] and websites listed within Box 1.

chances of ultimate success can be increased by intelligence-based assessment and selection of these targets. This assessment needs to remain in the context of the TPP. By way of example, the TPPs for two contrasting parasitic diseases are shown in Box 2.

Molecular-target assessment

The assessment and prioritisation of targets is well established in commercial drug discovery and this article will illustrate how this process can be applied appropriately to target assessment for entry into a parasitic disease-discovery pipeline. The criteria of key importance against which each target is assessed are shown in Table 1. Each criterion has an associated scoring system depicted in the familiar colours of a traffic light. Once a series of targets have been scored, the traffic-light assignment provides an initial, global view of the pipeline being considered, highlighting the overall most mature targets and any key areas of weakness. In most cases, a red assignment is not a complete 'stop', it simply represents the current status of the target and indicates where further research is required to progress a target towards entry into a pipeline.

Crucial areas for target assessment

Attrition rates for drug discovery are high; in fact, only one in five projects survives through preclinical development and less than one in 10 make it through clinical development; hence, less than one in 50 make it to the clinic [17]. Most projects fail through problems in either biology (selection of targets often revealed to be validated poorly subsequently) or chemistry [failure to identify suitable drug-like lead compounds for optimization, lack of efficacy, toxicity or drug metabolism and pharmacokinetic (DMPK) issues]. It is thus imperative that antimicrobial drug targets are characterised fully and shown to be essential for either growth or (preferably) survival of the pathogen. Wherever possible, this should be demonstrated in the host vertebrate stage(s) rather than the vector stage(s). Ideally, essentiality should be extended to an appropriate

Box 2. Parasitic disease target-product profiles for human African trypanosomiasis and malaria[a]

The target-product profile (TPP) is an important strategic planning and decision-making tool in drug discovery and development [52]. It is used to define the desired features for the end-product, including:
- Therapeutic area
- Spectrum of activity (e.g. active against all species, including drug-resistant isolates)
- Target population (e.g. pregnant women and children)
- Dose, frequency and route of administration (e.g. once a day, oral route)
- Safety and efficacy (better than existing treatments)
- Toxicity (minimal side effects, better than existing treatments)
- Potential for use in drug combinations
- Contraindications (e.g. minimal drug–drug interactions; suitable for use in HIV/AIDS or TB co-infections)
- Low potential of developing parasite resistance
- Stability under tropical conditions (i.e. >2 years shelf life at 40 °C and 75% relative humidity)
- Cost of goods (i.e. equivalent to or cheaper than existing treatments)

A TPP for uncomplicated falciparum malaria includes:

- Oral (ideally once per day for not more than 3 days)
- Low cost of goods (~US$1 per full course of treatment)
- Effective against drug-resistant parasites (e.g. those that have developed resistance to chloroquine or Fansidar)
- Fast acting and curative within 3 days
- Potential for combination with other agents
- Paediatric formulation should be available
- Stable under tropical conditions

A TPP for human African trypanosomiasis (HAT) includes:

- Active against *Trypanosoma gambiense* and *Trypanosoma rhodesiense*
- Active against melarsoprol refractory strains
- Efficacy against early- and late-stage disease desirable
- Formulation (oral against early stage desirable; parenteral against late stage acceptable)
- Curative in 14 days (late stage) or less (early stage)
- Cost less than current treatment for early stage disease (US$100–140)
- Safe during pregnancy
- Stable under tropical conditions

[a] Additional TPPs for neglected diseases are available elsewhere [6].

definitive method for target validation [19,20]. However, essentiality does not mean that the target is different sufficiently from the host to enable selective inhibition, underlining the need for additional chemical validation. The precise techniques used depend on the genetic tools available for any given parasite (e.g. availability of inducible or non-inducible expression vectors, choice of drug-selectable markers) and the genetic and physiological properties of the organism under study (e.g. gene-copy number, ploidy; ease of culture in defined media; susceptibility to drug selection; ease of transfection). In this respect, genetic manipulation of *Leishmania* and *Trypanosoma* [21,22] is generally easier than that of *Plasmodium* [23], although genetic manipulation of the related apicomplexan parasite, *Toxoplasma gondii* [24], can provide invaluable information about probable outcomes in malaria.

At the DNA level, targeted gene deletion by homologous recombination with a gene conferring resistance to a toxic drug or experimental compound will ablate expression of

Table 1. Traffic-light definitions for target assessment

Criterion	Red	Amber	Green
Target validation	No or weak evidence that the target is essential for growth or survival	Either genetic or chemical evidence that target is essential for growth or survival[a]	Genetic and chemical evidence that target is essential for growth or survival[a]
Druggability	No drug-like inhibitors are known and active site of target is not druggable	Drug-like inhibitors are known or active site is druggable potentially	Drug-like inhibitors are known and druggable active site (i.e. clinical precedent within the target family)
Assay feasibility	No *in vitro* assay developed and/or significant problems with reagents (cost or supply)	*In vitro* assay exists, development into plate format feasible but not achieved	Assay ready in plate format and protein supply assured within appropriate timelines
Toxicity	Human homologue present and little or no structural or chemical evidence that selective inhibition is possible	Human homologue present but some structural or chemical evidence that selective inhibition is possible	No human homologue present or human homologue known to be non-essential
Resistance potential	Target has multiple gene copies or isoforms within the same species and is subject to escape from inhibition[b]	Target has isoforms within the same species or might be subject to escape from inhibition[b]	Target has no known isoforms within the same species and is not subject to escape from inhibition[b]
Structural information	No structure of target or closely related homologue	Structure without ligand available and/or poor resolution (>2.3 Å) or opportunity to build a good homology model	Ligand-bound structure of target (or ligand in closely related homologue) available at high resolution (<2.3 Å)

[a]See Table 2 for the relative merits of genetic and chemical validation.
[b]Possible resistance mechanisms include: accumulation of substrate that could reverse inhibition or the target can be deleted, modified readily by point mutation, amplified or by-passed readily.

that particular allele completely. If the organism is diploid, or haploid with more than one genetic locus for the potential target, then multiple rounds of transfection with gene-deletion constructs and selection with multiple drug-selectable markers will be required. Various outcomes of such experiments have been observed and, as discussed later, must be interpreted with caution.

At first sight, the ability to obtain viable organisms in which expression of a target is abolished completely is suggestive that the target is not essential for growth or survival and is therefore probably not a drug target. However, such phenotypes must be demonstrated in the appropriate life-cycle stage and life-cycle environment. Indeed, if the phenotype of such a null mutant can be predicted, it might be possible to obtain viable cells by growth in medium containing an appropriate supplement (e.g. ornithine-decarboxylase deficiency rescued by putrescine [25,26] or thymidylate-synthase deficiency rescued by thymidine [27]). In situations in which chromosomal null mutants cannot be obtained, this negative result is suggestive but not absolute proof of the essential nature of the target. Further evidence can be obtained by 'rescue' of chromosomal null mutants through the expression of another copy of the target (sometimes from a related species), either on an episomal vector or at another chromosomal locus [21–24]. Unfortunately, this reveals no information as to precisely what level of enzyme activity is compatible with growth or survival and therefore what level of inhibition has to be achieved by drug treatment. Inducible or repressible gene-expression system(s), such as the tetracycline-inducible systems for trypanosomes, are useful in this regard [28–30]. In some circumstances, a genetic knockout of an essential target, for example, dihydrofolate reductase (DHFR) [31], might nonetheless yield viable organisms owing to compensatory genetic changes. This further underlines the importance of both chemical and genetic evidence of essentiality.

At the RNA level, expression of a target can be modulated by RNA interference (RNAi) using double-stranded RNA (dsRNA) [32]. Stable expression of dsRNA

Table 2. Strengths and weaknesses of different target-validation methods

Method	Strengths	Weaknesses
Chemical validation	• Addresses the key druggability issues of cell permeability (*in vitro* whole-cell assays); selective toxicity and drug metabolism (*in vivo* animal models); safety and efficacy (clinical) • Identifies non-protein targets • Identifies prodrugs and compounds acting by lethal synthesis	• Highly specific inhibitors not available frequently • Lack of specificity or variable cellular pharmacokinetics might lead to poor structure–activity relationships • Correlation between target inhibition and predicted molecular or biochemical phenotype sometimes difficult to demonstrate *in vitro* or *in vivo*
Genetic validation	• Complete genomes available • Suitable for genes of unknown or uncertain function	• Cannot identify non-gene targets (e.g. haemozoin) • Does not address key druggability issues • Does not identify drugs acting by lethal synthesis
Knockout methods	• Definitive, 'clean' phenotype • Few or no off-target effects	• Laborious (usually requires multiple transfections in diploid organisms) • Null mutants for essential genes require genetic or nutritional rescue • Multicopy genes can be problematic • Compensatory (suppressor) mutations can occur
RNA interference (RNAi)	• Rapid and easy to perform • Suitable for multicopy gene families	• Not possible in many parasite species • No phenotype owing to insufficient silencing • Off-target effects owing to unintentional silencing • 'Escape' mutants with essential genes

using inducible systems is preferable to constitutive expression because transgenic organisms lacking an essential biochemical component generally cannot be selected – only 'escape mutants' can be recovered, making interpretation difficult. However, even with inducible systems, insufficient expression of dsRNA might fail to knockdown target expression to the levels required to reveal a phenotype (Table 2). Thus, a 'negative' result in the absence of careful phenotypic characterization of target expression is almost without value. A 'positive' result, when growth inhibition correlates with decreased target production, can be helpful. However, even positive results can be problematic if RNAi causes 'off-target' effects.

Modulation of target levels by overexpression or knockdown by any of these genetic methods is also useful in mode-of-action studies of lead compounds. Elevated target expression leads to decreased drug sensitivity in whole cells and, conversely, decreased target expression can lead to hypersensitivity.

Druggability

For a target to be ultimately validated by successful clinical trials, it must be both essential to the organism and its function be modulated appropriately, such as inhibition of an enzyme, by compounds capable of achieving therapeutic concentrations on dosing to patients (i.e. drug-like compounds).

Traditionally, the selection of targets for drug discovery has rarely included an assessment of the likelihood of discovering drug-like ligands [17]. This omission has contributed to the failure of screening campaigns because the binding sites of many targets are too large or too small, too polar or lack sufficiently deep binding pockets to potently bind drug-like compounds (Figure 1). Therefore, the concept of target druggability has come to the fore to help identify targets with the greatest chance of success in the hit- and lead-identification process.

Druggability of a target has been described as the presence of protein folds that favour interactions with drug-like chemical compounds. The druggability of a target is not an absolute, rather, more of an assessment of the probability of finding potent drug-like inhibitors. Drug-likeness of a compound, which is a combination of physicochemical properties, DMPK and toxicological profile, determines whether the compound can be formulated and delivered by the required route and, when dosed, affords efficacy with an acceptable safety profile [33]. Several approaches are available to identify potential druggable targets [33,34] (Box 3).

These new paradigms of assessing the suitability of targets for entering hit discovery represent potentially exciting ways of reducing the current high rates of attrition.

Assay feasibility

The traffic-light scoring system for assay feasibility is an assessment of 'readiness' for the process at a given time point. The overall tractability of screening an isolated molecular target *in vitro* is dependent on several other criteria (for ideal requirements, see Box 4).

The guiding principle in all cases is the development of assays that are fit for purpose and are related as directly as possible to the target protein or the pathway concerned. The assessment should be target driven, not format driven, and the process should be governed by quality of the output rather than speed of throughput. This is even more important in the context of enzyme targets in infectious diseases, which often come with little previous drug-discovery precedent and none of the reagents or technologies that accelerate assay development in the standard world of the druggable gene families [i.e. ion channels, kinases and G-protein-coupled receptors (GPCRs)]. A sole focus on homogeneity of assays (performed as simple mix and read protocols) is therefore not appropriate and separation steps can be accommodated if necessary. Sometimes, enzymatic coupling of the target enzyme to yield an assayable end product is required; this requires a judgement on whether time is best spent on deconvolution of hit compounds or on novel reagent-development programmes to avoid enzymatic coupling. In contrast to the isolated target approach, cell-based screening in parasites has been used widely for the identification of cytotoxic agents (phenotypic screening), leading to often complex and time-consuming target-identification studies. Although it offers the advantage of identifying only cell-penetrating agents, it is often limited to medium throughput and small-scale screening and generates a more complex and ambiguous environment for the rational development of structure–activity relationships (SAR) in a compound series (reviewed in [35]). Exploitation of a specific molecular target using whole-organism assays represents a useful compromise between isolated target and phenotypic screening but does require technologies, such as target-linked reporter systems or comparative screening in wild-type and target-deleted organisms, to be available in the organism of interest.

Box 3. Identifying potential druggable targets

- Targets can be selected on the basis of having clinically validated homologues from other species. This assumes that, if one member of a gene family binds drug-like compounds, other members will do so because binding-site architecture is generally conserved among gene-family members.
- Identification of parasite targets that are inhibited by drug-like compounds as opposed to non-drug-like molecules [e.g. inhibition of isocitrate lyase (ICL) with the highly charged 3-nitropropionic acid] does not demonstrate druggability [53].
- Computational definition and characterisation of active sites or binding sites of known druggable proteins can be used to assess potential targets for druggability. Because drugs have defined property parameters, it follows that druggable protein binding-site properties must be complementary with a definable set of characteristics. In addition, because most drugs bind to discrete binding sites, it should be possible to identify druggable binding sites from the abundance of protein structure data available in the Protein Data Bank (PDB) and in the future from structural genomics projects [34].
- A simple model derived using nuclear magnetic resonance (NMR)-based screening data has been used, including terms, such as polar surface area, surface complexity and pocket dimensions, which predicts experimental screening hit rates accurately. The model predicted correctly (94%) the druggability of protein targets not used in the training set of the proteins for which high-affinity, non-covalent, drug-like leads have been reported. Therefore, the model potentially enables the quantitative comparative analyses of protein binding sites of uncharacterized targets derived from genomics research [54].

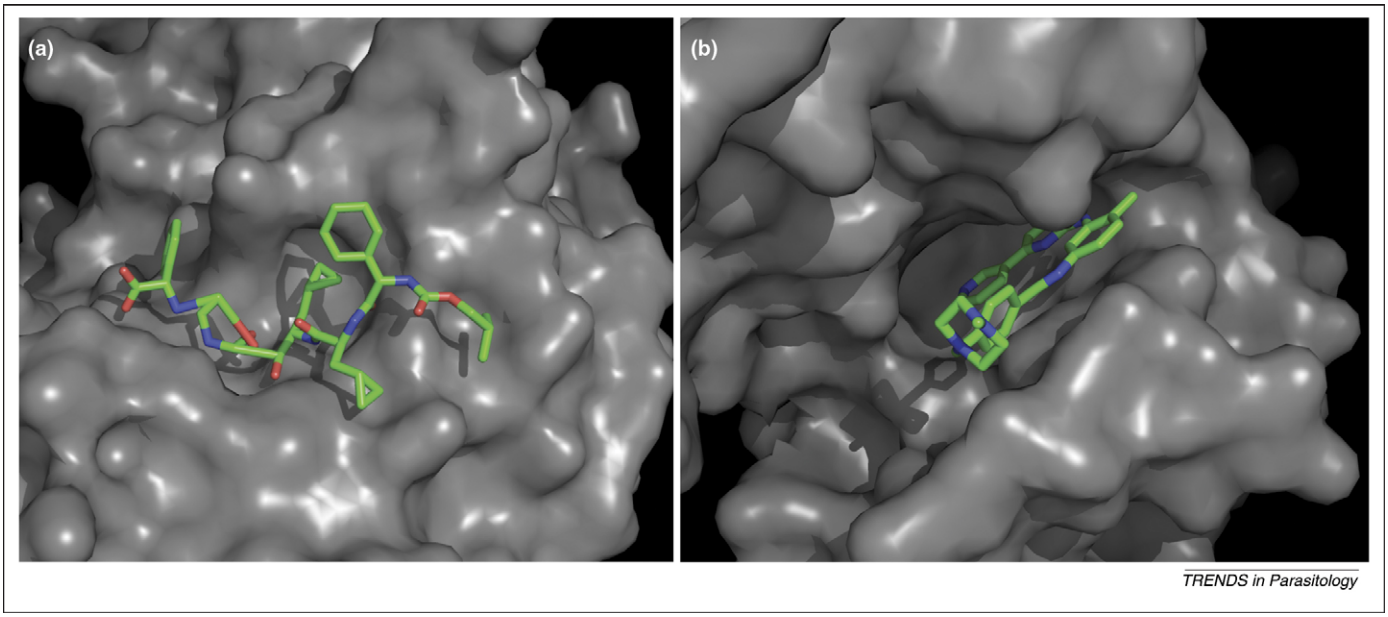

Figure 1. 'Good' and 'bad' pockets for binding of drug-like molecules. **(a)** An example of a poorly druggable protein (hepatitis C serine protease) with a shallow featureless active site (PBD code 2A4R) [57]. **(b)** An example of a highly druggable active site (spleen tyrosine kinase) with a deep pocket enabling an inhibitor (Gleevec) to be buried significantly (PBD code 1XBB) [58].

The decision on assay format is often therefore based on the proposed scale of the compound screen, assay performance and, in the case of cellular screening, how directly linked the read-out is to the target of interest.

Structural information

The structural information available for targets can range from none for a novel target with no close homologues, through limited information from homology models, to full protein–ligand structures derived from NMR or X-ray crystallography. Even when no direct structural information is available, SAR and/or homology models can be used to predict the binding mode of compounds, enabling the design of further analogues to test the model and to help to direct subsequent rounds of chemistry. Although obtaining a structure of a target protein can require considerable effort, the detailed structural information provides advantages over programmes carried out without this knowledge. The use of structure-based drug design has been reviewed extensively [36–39] and some benefits are listed here.

- *In silico* docking can be used to identify alternative compound series for screening and in the optimisation of leads.
- Protein–ligand complexes can validate hits from biochemical screening campaigns by confirming that they bind the target with a defined mode.
- Lead optimisation can be driven by rational design of ideal drug candidates rather than easily achievable chemistry.
- The identification of 'hot spots' of binding from an analysis of the binding mode across compound series, especially fragments, can be used to optimise ligand efficiency.
- Structural information can be used to understand and exploit factors, such as changes in compound-binding mode, conformational changes of the target protein and effects on the ligand owing to binding, to optimise potency or selectivity.
- An understanding of selectivity compared with other targets and how activity against these targets could be tuned.
- An understanding of resistance caused by mutations within the target protein and the design of compounds active against these resistant mutants.

Used intelligently, structural information can reduce the number of compounds needed to be made to identify preclinical candidates; a particularly important requirement in the field of NTDs, owing to the limited resource available.

Toxicity

Target-based toxicity can arise owing to inhibition of human homologues of the parasite-candidate target. In theory, for inhibition of a molecular target that is unique and essential to the pathogen but absent in the human host, this would not be an issue. In practice, not only are unique targets rare but targets might also display coenzyme- or substrate-binding sites common to human homologues. These can be identified through comparison of human and pathogen genomes [40,41] and the human counterpart used in a counterscreen. More commonly, a human homologue is present, although there are often significant differences among the pathogen and the human enzymes. A prime example is dihydrofolate reductase (DFHR), in which structural differences among species enables the development of exquisitely selective inhibitors [42]. The evidence for selectivity is often chemical. Unfortunately, this information is not always available at the stage of selecting a molecular target for a high-throughput screen, although structural data on the molecular target and human homologue can give very useful information as to the possibility of selective inhibition. When an essential human homologue is present with little difference to the parasite enzyme, then toxicity is likely to be a significant

Box 4. Ideal criteria for developing an enzyme assay[a] amenable to screening

Target

- Target protein must be available in batch quantities of sufficient size to support an entire campaign of primary, re-test and potency screening; typically, approximately 1.5-fold the initial number of compounds to be screened equates to the numbers of data points required
- A standard inhibitor(s) must be available against which performance of the assay can be benchmarked; the developed assay is required to return potency values within twofold of an established mean among assay days
- Assay performance: optimised to achieve the following minimum metrics: Z′ score[b] >0.5; intraplate CVs[c] <10%, signal to background >3:1
- Assay format: 384-plate formats are preferred but 96 can often be accommodated
- Assay readout: in a typical screening facility, the following readouts are considered standard: absorbance; variety of fluorescence modalities; radiometric, luminescence. Non-radiometric platforms are preferred for larger-scale screening (>10 000 compounds).

Assay conditions

- Dimethyl sulphoxide (DMSO) tolerance of at least 1% is required to accommodate compound addition
- End-point assay preferable to maximise throughput, with read-time well within time linearity to avoid underestimation of inhibitor potency
- Automated liquid-handling robots require component reagents to be as non-viscous as possible with minimal levels of detergent
- Enzyme load should be well within linearity with respect to enzyme concentration to ensure that initial velocity reduction by inhibitors correlates with the formation of the enzyme–inhibitor complex
- Enzyme load should be within low nanomolar range to ensure sensitivity of compound-inhibitor potency, the lower limit of which is regarded as 10-fold the enzyme concentration for IC_{50} determinations
- Pre-incubation of target with compound can ensure that even slow binders are captured in the hit-finding process
- Small but liquid handler-tolerable levels of detergent in the assay can assist in dissolution of compound aggregates and thereby minimise the identification of non-specific or 'promiscuous' inhibitors
- In the case of enzyme targets, substrate concentration should be at or preferably below K_m; this maximises hit finding for competitive agents; if an uncompetitive agent is sought, the substrate concentration should exceed K_m

[a] See [55] for a useful review of the basics of enzyme kinetics in the context of drug discovery.

[b] The Z′ statistic gives an assessment of the robustness of the assay [56] and is determined using the following equation:

$$Z' = 1 - \frac{3\sigma_H + 3\sigma_L}{\mu_H - \mu_L}$$

where μ_H and μ_L are the mean high (full) and low (background) signals of the assay, respectively, and σ_H and σ_L are the standard deviations. A 'perfect' assay would have a Z′ value of 1.0.

[c] CV, coefficient of variance is an expression of the standard deviation around the mean high signal as a percentage of said mean.

problem. A notable exception is the case of the inhibition of ornithine decarboxylase (ODC) by difluoromethylornithine (DFMO), where selectivity is attributed to more rapid turnover of human ODC and other factors [43,44].

Resistance potential

An organism has many possible mechanisms of generating resistance to a drug, including point mutations, overexpression of the molecular target, gene amplification, reduced uptake of drug, increased efflux, metabolic by-pass and enzymatic inactivation of drug.

However, when attempting to assess a molecular target for drug discovery, it is possible to make some predictions about resistance. Thus, the presence of isoforms of the enzyme within the pathogen leads to possibilities of resistance; if there are several enzymes that appear to carry out the same (or similar) metabolic roles, there is a possibility of one substituting for another. Similarly, resistance can occur by pathways that could bypass the molecular target. An interesting example is the pteridine reductase (PTR1); this enzyme is able to act as a bypass for DHFR in *Leishmania* when DHFR is inhibited [45]. The use of pathogen-genome data can assist in the prediction of the presence of possible bypass mechanisms.

Within the laboratory setting, it is almost always possible to develop pathogens that are resistant to particular agents by culturing the parasites in sublethal concentrations of inhibitors. This can be a useful technique to indicate possible mechanisms of action and modes of resistance, although there are many instances in which mechanisms of resistance seen *in vitro* in the laboratory have not been found during clinical use [46,47]. Within a clinical setting, mechanisms of resistance are often more difficult to deduce owing to the large number of factors that might select for drug resistance.

With any pathogen, resistance is likely to occur eventually, emphasizing the need for a full drug-discovery pipeline to provide alternative drugs. However, one way to slow down the emergence of drug resistance is by the use of combination therapy, such as is seen in the development of recent antimalarial [48–50] and anti-TB therapies [51].

Concluding remarks

The emergence of new alliances among academic and industrial partners that are committed to drug discovery offers exciting new prospects for drug discovery against NTDs. The focus of this discussion has been on the molecular-target approach to drug discovery and the key criteria necessary to commit valuable resources to a drug-discovery campaign. Of all criteria considered here, those of target validation and druggability are thought to be of paramount importance to the probability of success of a drug-discovery programme. It is hoped that this review will encourage basic scientists to generate this information as a matter of course during their research and thereby produce a robust pipeline of potential antiparasitic drug targets for the future.

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