OPPORTUNITIES AND CHALLENGES IN ANTIPARASITIC DRUG DISCOVERY

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Abstract | New antiparasitic drugs are urgently needed to treat and control diseases such as malaria, leishmaniasis, sleeping sickness and filariasis, which affect millions of people each year. However, because the majority of those infected live in countries in which the prospects of any financial return on investment are too low to support market-driven drug discovery and development, alternative approaches are needed. In this article, challenges and opportunities for antiparasitic drug discovery are considered, highlighting some of the progress that has been made in recent years, partly through scientific advances, but also by more effective partnership between the public and private sectors.

PROTOZOA
Single-celled eukaryotic organisms with nuclei that show some characteristics usually associated with animals, most notably mobility and heterotrophy. A few are important parasites.

HELMINTH
A multicellular organism, generally longer than it is wide or deep, commonly called a worm. There are three major groups causing parasitic diseases in humans: nematodes, flukes, and tapeworms.

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Parasitic diseases continue to take an enormous toll on human health, particularly in tropical regions. The major burden is caused by the PROTOZOA and HELMINTHS listed in TABLE 1. The drugs used to treat these diseases are far from ideal, and many of them were introduced decades ago. Problems associated with some of the commonly used drugs are noted in the TABLE 1. As many authors have emphasized, market forces are insufficient to drive the discovery and development of new drugs for these diseases. Of more than 1,300 new drugs introduced for all indications between 1975 and 1999, only 13 were for TROPICAL DISEASES such as those listed in TABLE 1[1]. In 2000, only about 0.1% of global investment in health research was devoted to drug discovery for selected tropical diseases (malaria, leishmaniasis and trypanosomiasis) and tuberculosis, which together contribute about 5% of the global disease burden[2,3].

Several welcome developments during the past few years have given new impetus to antiparasitic drug discovery. These include the publicly-funded sequencing of the genomes of several of the parasites in TABLES 1,2, and the establishment of new public–private partnerships (PPPs) whose focus is specifically on tropical diseases[4,5], counteracting, at least to some extent, the withdrawal of many large pharmaceutical companies from direct involvement in antiparasitic drug discovery. The injection of new funds into the area of antiparasite research, particularly by the Bill and Melinda Gates Foundation, is also having a significant impact. Some PPPs currently involved in antiparasite drug discovery are the Medicines for Malaria Venture (MMV), the Drugs for Neglected Diseases initiative (DNDi) and the Institute for One World Health (IOWH)[4]. These PPPs combine a pharmaceutical industry-derived approach to drug discovery and development with the disease-specific knowledge and experience of public healthcare organizations.

In light of these opportunities, we discuss some challenges to the discovery of new antiparasitic drugs, defined as the work leading up to the definition of a drug development candidate (FIG. 1). Drug discovery is an iterative process which, depending on the strategy used, typically comprises several discrete stages: target identification and validation; assay development; screening (whole cell or molecular target-based) to identify hits (BOX 1); procurement/synthesis and assessment of analogues to develop structure–activity relationships (SAR) and identify leads; iterative medicinal chemistry to optimize leads; and preclinical development prior to clinical evaluation. We describe different approaches to antiparasitic drug discovery, discuss the

Table 1 | **Major tropical parasitic diseases — toll and treatment***

Disease (parasite responsible)	Population at risk (millions)	Deaths in 2002 (thousands)	DALYs‡ 2002 (millions)	Some widely used or recently introduced drugs or drug combinations (year first used)§	Disadvantages
Malaria (*Plasmodium* spp., particularly *P. falciparum*, responsible for most fatalities, and *P. vivax*)	>2,100	1,272	46.4	Chloroquine (1945); sulphadoxine/pyrimethamine (1961); mefloquine (1984); artemisinins (1994); artemether/lumefantrine (1999); atovaquone/proguanil (1999); chlorproguanil/dapsone (2003)	Drug resistance is widespread to chloroquine and sulphadoxine/pyrimethamine, growing to mefloquine, and a threat to other components. Adverse effects in certain patients are well described for chloroquine, mefloquine and the proguanils. Cost is an issue for other drugs or combinations. Availability of artemisinins (from plant sources) is problematic.
Leishmaniasis (*Leishmania* spp., particularly *L. donovani* causing visceral disease)	350	51	2.1	Pentamidine (1939); pentavalent antimonials (1950); liposomal amphotericin B (1990); miltefosine (2002)	Efficacy loss/drug resistance to pentamidine and antimonials. Cost high for liposomal amphotericin B. Adverse effects well described for other drugs. Miltefosine is contraindicated in women of child-bearing age.
African trypanosomiasis (*Trypanosoma brucei gambiense*, *T.b. rhodesiense*)	>60	48	1.5	Suramin (1920); pentamidine (1939); melarsoprol (1949); eflornithine (1991)	Risk of severe adverse effects with all drugs. Suramin and pentamidine not effective in late-stage disease, eflornithine expensive and only effective against *T. gambiense*.
Chagas' disease (*T. cruzi*)	120	14	0.7	Nifurtimox (1970); benznidazole (1974)	Long treatment courses and adverse effects limit compliance; not effective in late-stage disease.
Schistosomiasis (*Schistosoma mansoni*, *S. haematobium*, *S. japonica*)	600	15	1.7	Oxamniquine (1967); praziquantel (1975)	Oxamniquine only effective against *S. mansoni*. Praziquantel does not kill immature worms; possible resistance reported.
Lymphatic filariases (*Brugia malayi*, *Wuchereria bancrofti*)	1,000	0	5.8	Diethylcarbamazine (DEC) (1949); ivermectin (1989); albendazole/DEC; albendazole/ivermectin	Diethylcarbamazine cannot be used in *O. volvulus*-endemic areas (risk of adverse effects). Albendazole only used in combination therapy. Ivermectin does not eliminate adult worms.
Onchocerciasis (*Onchocerca volvulus*)	120	0	0.5	Ivermectin (1989)	See above.

*Data selected and summarized from the more comprehensive tables in REFS. 4,49,52. ‡Disability-adjusted life years (sum of years of life lost and years lost through disability[52]). §Approximate dates, usually for registration, taken from REF. 49 except for eflornithine and miltefosine.

promise of high-throughput screening (HTS) on new molecular targets and emphasize the importance of lead optimization. Finally, we mention the contributions that different types of partnership can make to the discovery process.

Challenges in antiparasitic drug discovery

Drug discovery for parasitic diseases is not intrinsically more costly or technically demanding than for other indications. Generally, for infectious (including parasitic) diseases, preclinical models tend to be more predictive, and clinical trials less complex and costly, than for non-infectious, chronic disorders. It has been estimated that the cost of bringing a new antimalarial to market is about US$300 million, compared with the cost for a new drug averaged over all indications of at least US$500 million. The risk of failure in Phase II clinical trials is estimated to be 50% for a new antimalarial, which is lower than the corresponding risk for a non-infectious disease[4,6].

Antiparasitic drug discovery is not primarily driven by the commercial need to introduce novel compounds. Historically, many antiparasite drugs were first developed for other indications. This opportunistic approach of capitalizing on knowledge gained from work on non-parasitic indications has been very successful, as described in the next section, and has clear advantages in terms of cost reduction. However, the approach does not favour the introduction of chemically novel agents and might be reaching a point of diminishing returns, particularly as a result of widespread resistance to certain drug classes. It does not fully exploit new knowledge of parasite genome sequences, leading to the view that "the next big challenge in tropical diseases is determining the best way to translate the insights obtained from genomics into new, robust chemical leads that can form the basis of innovative drug discovery"[4].

A second major challenge in this arena is that multiple organizations with vastly differing cultures and underlying objectives need to work together. As a result of the abandonment of in-house discovery research for antiparasitics by many large pharmaceutical companies, a key factor in drug development for neglected diseases has been the formation of effective partnerships for 'virtual drug discovery' [4,5,7]. Much progress in recent years has been made by effectively re-engaging the private biopharmaceutical industry in the effort. Public support for early drug discovery feeds into the drug development projects undertaken by industry in partnership with public-health organizations. Increasing the role in such partnerships of researchers, public-health

TROPICAL DISEASES
Information on the parasitic diseases discussed here (see Table 1), their pathology, treatment, and incidence can be found on the WHO/TDR web site (http://www.who.int/tdr/) and by linking to the section titled Disease Watch (www.who.int/tdr/dw/default.html). The Disease Watch pages include links to articles originally published in *Nature Reviews Microbiology*.

Table 2 | **Parasite genomes: information sources and status of some genome projects.**

Organism	Genome studies status	Website
General		Sanger Institute: http://www.sanger.ac.uk/Projects/Protozoa/, http://www.genedb.org/ The Institute for Genomic Research (TIGR): http://www.tigr.org/tdb/parasites/, http://www.tigr.org/tdb/tgi/
P. falciparum *P. yoelii* *P. vivax* *P. berghei*	Sequence complete Sequence complete Sequencing underway	*P. falciparum* GeneDB: http://www.genedb.org/genedb/malaria/index.jsp Plasmodium genome database: http://plasmodb.org/
L. major	Sequence complete	*Leishmania* major GeneDB: http://www.genedb.org/genedb/leish/index.jsp
T. b. brucei *T. b. gambiense*, *rhodesiense*	Sequence complete Partial sequencing underway	*T. brucei* GeneDB: http://www.genedb.org/genedb/tryp/index.jsp *Trypanosoma brucei* Genome Network: http://parsun1.path.cam.ac.uk/
T. cruzi	Nearing completion	*T. cruzi* GeneDB: http://www.genedb.org/genedb/tcruzi/index.jsp *T. cruzi* Genome Network: http://www.dbbm.fiocruz.br/genome/tcruzi/tcruzi.html
S. mansoni	Sequence complete	*Schistosoma mansoni* GeneDB: http://www.genedb.org/genedb/smansoni/index.jsp *Schistosoma* Genome Network: http://www.nhm.ac.uk/hosted_sites/schisto/
B. malayi *W. bancrofti* *O. volvulus*	Sequence complete Underway Nearing completion	Filarial Genome Network (FilGenNet mirror sites): http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html TIGR *Brugia malayi* genome project: http://www.tigr.org/tdb/e2k1/bma1/ Nematode.net: Genome Sequencing Center: http://www.nematode.net/Species.Summaries/Wuchereria.bancrofti/index.php TIGR *O. volvulus* gene index: http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=o_volvulus

and industry leaders in the disease-endemic countries remains a challenge. Some examples of partnerships of different types are discussed below.

Finally, for the 'neglected' diseases, drug discovery is principally field-driven — that is, designed to meet the needs of disease-control programmes in the field. This generally means an emphasis on low cost of goods, short treatment regimes and the ability to use the drug safely in the absence of close medical supervision. It is therefore vitally important to define, in conjunction with those responsible for control programmes in the affected countries, desired product profiles based on what is required for use in resource-poor settings (TABLE 3). Optimizing lead compounds so that they have the characteristics required to meet product profiles is the rate-limiting factor in preclinical drug development.

Approaches to drug discovery

Different basic approaches to drug discovery for tropical diseases, as reviewed recently for malaria[8] or tuberculosis[9], can be classed as short-to-medium term (based on exploiting existing compounds or compound classes) or long-term (requiring discovery of new chemical classes). The approaches we describe are illustrated by examples, with emphasis on the diseases in TABLE 1 other than malaria. They aim at the discovery of pure chemical entities: the possible advantages of, and problems associated with, encouraging the wider use of traditional medicines, particularly for malaria, have been discussed elsewhere[10,11].

Combinations of existing drugs. Combinations of existing drugs (TABLE 1), such as eflornithine and melarsoprol for African trypanosomiasis[12], or praziquantel and oxamniquine for schistosomiasis[13], offer possibilities of synergy, reduced toxicity, shorter treatment regimens and slowing the development of resistance. In particular, extensive use of drug combinations is being made in malaria therapy[8], and the rationale for this has been recently reviewed[14]. Wherever possible, fixed-dose combinations are being developed to increase patient compliance, particularly using artemisinin-like compounds as one of the components. Combinations of an antimalarial drug with a RESISTANCE REVERSER are also being considered[8].

New indications for existing drugs. An attractive short-term strategy offering major savings in development time and expense involves extending the indications of drugs that were first developed for other indications. Historically, this 'piggy-back' approach has been very successful; many antiparasite drugs first entered development for other indications (see case histories in TABLE 4). For example, DB289, which was initially used to treat *Pneumocystis* pneumonia, is now in clinical trials as a potential oral treatment for malaria and early-stage African trypanosomiasis[15,16]. A downside of this strategy can be the reluctance of pharmaceutical companies to allow their products to be tested in a non-commercial patient class and so risk uncovering toxicities that might blight the economic potential of their drugs. For example, several companies have

RESISTANCE REVERSER
A compound that will alter a cell's properties to make it sensitive to a certain drug to which it has developed resistance.

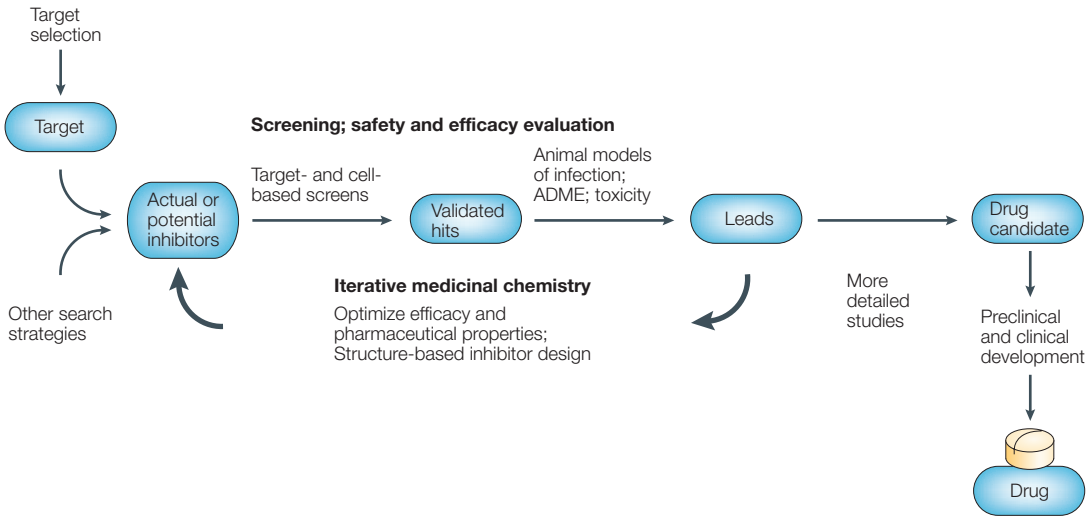

Figure 1 | **Schematic illustrating the stages in drug discovery.** Drug discovery is an iterative process involving discrete stages. This often begins with basic exploratory biology and biochemistry to identify molecular targets. In other cases compounds are tested, without knowledge of the target, for activity against the whole parasite. Compounds (actual or potential inhibitors) are assayed for activity against the target, if known, and for activity against the whole parasite. (Inhibitors of the target are often used to validate the target.) Compounds active against the whole parasite are defined as hits (see BOX 1) that can be considered for further testing in animal models of the disease. Other tests that monitor the compounds' pharmacokinetic properties are also initiated at this stage. Compounds that are active in the animal models and considered to be 'druggable' are defined as leads (see BOX 1). Lead compounds generally require optimization for efficacy and good pharmaceutical properties. Note the importance of medicinal chemistry in both identifying an appropriate lead molecule and in the more time-consuming iterative process (thick arrows) of lead optimization. Early pharmacokinetic studies are also emphasized in this diagram. The process of optimization for pharmaceutical properties (adsorption, distribution, metabolism and excretion (ADME)) and lack of overt drug toxicity, as well as for efficacy against the target organism, is crucial. Once a compound reaches the stage at which it can be considered for testing in human patients, it is defined as a drug candidate. From there it enters the preclinical and then clinical studies of a typical drug development pathway. Adapted from REF. 4.

been reluctant to permit clinical trials of antifungal triazoles against Chagas' disease, in spite of their demonstrated activity against *Trypanosoma cruzi* in animal models[17].

Improvements to known drugs and compound classes. In the medium-term, analogues of existing antiparasitics (TABLE 5) might prove effective. For malaria, novel analogues of pyrimethamine are being specifically designed to overcome drug resistance resulting from mutations in dihydrofolate reductase[18], whereas analogues of amodiaquine with potentially reduced toxicity are being investigated[19]. Ferroquine contains a quinoline nucleus similar to chloroquine but with a novel ferrocenic group in its side chain. It has excellent activity against malaria parasites, including those resistant to chloroquine[20]. In the anthelmintic area, moxidectin, an analogue of ivermectin, is being pursued for the treatment of lymphatic filariasis and onchocerciasis[21]. This compound, already licensed as a veterinary product, has very different pharmacokinetic properties from ivermectin, and this difference is expected to result in improved efficacy against onchocerciasis and other FILARIAL infections.

Focused sample collections. An alternative, and arguably a more productive, approach to screening large libraries of compounds against whole parasites (see below) is to screen focused sample collections. Here the emphasis is on identifying compounds with either defined biological effects against related parasites, or biochemical activity against isoenzymes or receptors related to known molecular targets of other organisms. This strategy is particularly important in the search for new antifilarials and schistosomicides, for which screening capacity is limited by the supply of relevant test helminths. For example, in laboratories funded by the United Nations Children's Fund/United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), it has only been possible to evaluate some 1,000 samples per year against *Onchocerca* and SCHISTOSOMES. To improve the quality of the test compounds, efforts are being made to source samples with existing anthelmintic properties — for example, from crop protection and animal-health companies.

Work on parasite genome sequences coupled with biochemical investigations has pinpointed enzymes such as protein farnesyl transferases, cysteine proteases, histone deacetylases and fatty-acyl synthases (TABLE 6) as potential drug targets for malaria and TRYPANOSOMATID diseases. Investigators working in both academia and the pharmaceutical industry have established compound collections focused on inhibitors of such enzyme classes. Opportunities to access these compounds and evaluate them for their antiparasitic activity need to be exploited, as the

FILARIAL WORMS
Long, hair-like nematodes of which the adults (macrofilariae) live in the blood or tissues of vertebrates. In some species, the larvae (microfilariae) may be found in the blood. Examples of diseases caused by filarial worms include lymphatic filariasis and onchocerciasis.

SCHISTOSOME
A group of flukes of the genus *Schistosoma*, many of which are parasitic in the blood of humans and other mammals.

TRYPANOSOMATID PARASITES
A group of flagellated protozoal parasites of the order *Trypanosomatidae*, transmitted to the vertebrate bloodstream, lymph, and spinal fluid by certain insects and often causing diseases such as African trypanosomiasis, Chagas' disease, and leishmaniasis.

Box 1 | **Criteria for antiparasite hits, leads, and drug candidates**

Hit

At the start of a screening campaign, compound that is:

- Active *in vitro* against whole protozoa with IC_{50} of ≤1 μg per ml* (for protozoa), or inhibiting mobility of helminths *in vitro* to, for example, ≥ 75% at 10 μg per ml*
- Selective (at least tenfold more active against parasite than against a mammalian cell line, such as MRC-5*)

Lead

At the start of a screening campaign, compound that is:

- Active *in vivo* against parasites at a dose ≤ 100 mg per kg*
- Not overtly toxic in animals at efficacious dose
- Active *in vitro* against relevant parasite types (for example, drug-resistant parasites — see product profiles)
- Chemically tractable (analogues can be obtained)

During the campaign, criteria are made more stringent. A candidate for lead optimization (see text) should be:

- Active *in vitro* with activity approaching that of standard drugs
- Active *in vivo* against parasites in the relevant small animal model (for example, chronic or late-stage disease), when delivered by a relevant route (preferably oral) in an acceptable formulation at a reasonable dose (<< 100 mg per kg)*
- Show good selectivity when tested against several mammalian cell lines

Drug development candidate

Compound that has emerged from a lead optimization process (see text) and looks likely to fulfil at least the essential criteria in the desired product profile (TABLE 3). It should:

- Be active *in vivo* with activity comparable to or exceeding that of standard drugs in the most relevant animal models
- Be effective against desired range of parasites (for example, drug-resistant parasites and different species)
- Pass early toxicity/mutagenicity (for example, Ames Test) criteria
- Have an acceptable metabolic profile *in vitro* and *in vivo* (preferably with no major species differences)
- Have an acceptable pharmacokinetic profile
- Be amenable to cost-effective scale-up
- Preferably have a mode of action that is well understood

Clinical development candidate

Drug development candidate for which additional criteria have been met in studies of detailed pharmacology, pharmacokinetics/absorption, distribution, metabolism and excretion, mutagenicity and toxicity, formulation, scale-up for production, cost of goods and so on.

*Illustrative values only, based on experience of the screening network laboratories mentioned in the Acknowledgements. Actual values depend on the particular parasite, assay and compound type under study.

compounds can be a valuable source of new leads. As an example, MMV has in its portfolio a lead-optimization project based on inhibitors of protein farnesyl transferase that originated with compounds from Yale University and from a cancer chemotherapy programme at Bristol-Myers Squibb[22].

De novo discovery: whole parasite assays

Longer-term strategies aim to discover novel active substances unrelated to known drugs. In the biopharmaceutical industry, molecular target identification and HTS dominate much of the early drug discovery process. However, for the parasitic diseases there has been, and still is, a valuable alternative approach based on screening and analysing compounds for their activity against whole parasites. Screening diverse compound collections on whole parasites *in vitro* has been steadily declining during the past two decades but is now undergoing a renaissance, due mainly to assay improvements. This is particularly true for test systems using *Plasmodium falciparum, T. brucei*, *T. cruzi* and *Leishmania* species. Screening now often relies on the use of parasites transfected with reporter genes, such as those encoding green fluorescent protein, β-lactamase, or β-galactosidase, to enable easy, rapid detection of antiparasitic activity. Some progress has also been made in adapting protozoal screens to work in 384-well plates, especially with *P. falciparum*.

One recent example in which the capacity to test large numbers of compounds against whole parasites has been developed is at the Harvard University Institute of Chemistry and Cell Biology, Initiative for Chemical Genetics (ICCB-ICG), where whole parasites are used in an HTS format to assess tens of thousands of samples (see Further information). The Belgian company Tibotec, working with support from TDR, also developed assays based on whole parasites in 384-well plates in order to analyse relatively large numbers of compounds. For example, in one project, Tibotec analysed 10,000 compounds (purchased from a commercial compound supplier) for activity against four parasites (*P. falciparum, T. brucei, L. donovani* and *T. cruzi*) and a mammalian cell line (to assess cytotoxicity). Numerous active compounds were detected and further investigated by the TDR network of drug discovery laboratories. The hits were subjected to more detailed analysis, involving accurate IC50 determinations against whole parasites, measurement of general cytotoxicity and, for the most promising compounds, *in vivo* assessment in animal models of the relevant parasitic infection. This led to the identification of one compound as a novel lead active against malaria. Further optimization via analogue synthesis is now required to try to identify a credible development candidate. These data illustrate the high rate of attrition in lead identification and the need to screen large compound collections against whole cells in order to have a reasonable chance of success.

The quality of the compound libraries being assessed is a key factor in determining the success rate of screening (see also below). Screening libraries of natural products has special advantages for parasitic diseases, as well as other infectious diseases and cancer. Natural products are attractive because their structural diversity is remarkable and, for the parasitic diseases in particular, medicinal plants can be potential sources of novel pharmacophores[23]. For example, the antimalarial artemisinins were first isolated from a traditional Chinese medicine[24]. Other natural products whose chemistry is currently being explored in focused drug discovery programmes include manzamines[25], chalcones[26] and borrelidins[27], all of which have antimalarial activity in animal models. Large-scale screening of natural products against other parasites has been less exploited. The possibilities (and problems) of this

Table 3 | **Examples of points to be considered for product profiles of antiparasite drugs***

Disease indication	Essential properties	Desirable properties
Malaria (uncomplicated, *Plasmodium falciparum*, especially in infants and pregnant women)	Effective against drug-resistant parasites Oral formulation Short course (once daily for 3 days or less) Cost per treatment less than US$1	Potential partner for combination therapy Active against *P. vivax* Potential for use in unconscious patients Potential for use in cerebral malaria Can be used for prophylaxis Fast-acting to relieve symptoms Transmission-blocking
Leishmaniasis (visceral/mucocutaneous forms)	Effective against drug-resistant parasites Short course (14 days or less for oral formulation, less for parenteral) Cost less than current treatment (US$200–400)	Oral formulation
African trypanosomiasis (early and late stages of *Trypanosoma gambiense* and *T. rhodesiense* infections)	Short treatment course (14 days or less) Effective against drug-resistant parasites Oral formulation for early stage Parenteral formulation for late stage Cost less than current treatments	Cost significantly less than current treatment for early stage (US$100–140)
Chagas' disease (chronic stage)	Effective in chronic disease (against intracellular parasites in heart and gut) Oral formulation Short course (once daily for 1 month or less)	Cost per treatment significantly less than US$100
Schistosomiasis	Effective against all schistosome species Oral formulation Short course (once daily for 3 days or less) Cost less than current treatments	Active against all parasite stages in humans Active in single oral dose
Lymphatic filariases and onchocerciasis	Eliminates or sterilizes adult worms Suitable for community treatment (safe for children and pregnant women, acceptable treatment regimen, for example, once a year) Low cost	Oral formulation Active against other helminths

*Adapted from REFS. 4,38,49.

approach are illustrated by PX-6518, a glycosylated saponin whose antileishmanial activity was detected in a screen of some 10,000 plant extracts, but whose development was stopped due to toxicity concerns[28]. This case illustrates the difficulties in pursuing natural products, which are often chemically complex and provide few opportunities for rapidly investigating SAR by synthesising or procuring analogues. Converting the original natural product to a metabolically robust, orally bioavailable drug can be extremely challenging, with very long time lines. Because many natural products are produced as biological defence mechanisms, cytotoxicity is also a common problem. Many plant products are produced both at specific times in the growing cycle and in different parts of the organism, and this can lead to difficulties in sourcing sufficient material for study. Nevertheless, because of the amazing diversity of plant species and the heavy reliance on herbal remedies in tropical/subtropical disease-endemic countries, pursuing natural products as antiparasitics remains an attractive proposition.

De novo discovery: molecular targets and HTS

Although HTS against molecular targets has become the preferred mode of early drug discovery for much of the biopharmaceutical industry, it is has only recently been used to any wide extent in the search for new drugs for the neglected parasitic diseases. This strategy is expected to increase in importance as the genome sequences of the relevant parasites become available, and as HTS facilities and compound libraries become more accessible to research groups in academia.

Target identification and validation. Access to parasite genome sequences offers exciting opportunities for drug discovery based on the identification and validation of new molecular drug targets. The numbers (hundreds of thousands) of compounds that can be screened in a typical HTS campaign based on a molecular target far exceed the throughput possible using assays based on whole-cell parasite viability. This is particularly true for screening against helminths, for which throughput in whole-parasite assays is at least ten times less than for protozoa, which therefore makes the identification and validation of molecular targets for helminths an especially important and valuable approach for *de novo* drug discovery.

Nevertheless, one should not overestimate the number of suitable drug targets that parasite genomes could encode. The *Plasmodium* genome contains about 5,000 genes, of which Yeh *et al.*[29] estimated about 200 (4%) might encode suitable drug targets, using a computational algorithm that identified enzymes that catalyse 'chokepoint' reactions (those that uniquely either consume a specific substrate or produce a specific product). Among these were about 30 that were not significantly similar to any human enzyme. This compares favourably with Hopkins and

Table 4 | **Some case histories in antiparasite drug discovery**

Compound	Chemical name or class	Indication	Status	Brief development history	Year
Eflornithine[53]	Difluoromethyl-ornithine, inhibitor of ornithine decarboxylase	African trypanosomiasis	Registered drug component	Anticancer activity described Antitrypanosomal activity in mice Registered as injectable treatment for trypanosomiasis Supply threatened on commercial grounds Agreement with producer to guarantee supply for 5 years Phase II clinical trials of oral formulation	1970s 1980 1991 1998 2001 On-going
Fosmido-mycin[54]	Phosphonic acid derivative, inhibitor of DOXP* reductoisomerase	Malaria	In Phase II clinical trials, combined with clindamycin	Investigated as antibacterial agent Target identified in *P. falciparum* genome, antiparasite efficacy shown in animals Phase II clinical trials Phase II clinical trials of combination	1980s 1999 2002 On-going
OZ 277[47] Artemisone[15]	Synthetic peroxide; semi-synthetic peroxide	Malaria	In separate Phase II clinical trials	*Artemisia* extracts used for fever treatment Artemisinins characterized as endoperoxides Purified artemisinins clinically effective Fully synthetic active peroxides described Antimalarial screening of many analogues Phase II clinical trials	Ancient 1972 1979 1992 1985–2003 2005
Miltefosine[55]	Phosphocholine analogue	Visceral leishmaniasis	Registered, in Phase IV clinical studies	Anticancer activity in animals described Active against *Leishmania in vitro* and animal models Selected for clinical development Active in Phase III clinical trials Registered in India	1987 1987 1995 1999 2002
DB289[15,16]	Bisamidine	Malaria, African trypanosomiasis	In Phase II clinical trials	Anti-*Pneumocystis carinii* activity described Activity against parasites described Selected for clinical development against African trypanosomiasis Active in Phase II clinical trials against malaria and African trypanosomiasis	1996 1998 2000 2004

*1-Deoxy-D-xylulose 5-phosphate reductoisomerase.

Groom's estimate[30] that less than 1,500 (5%) of the ~30,000 genes in the human genome are suitable drug targets, using quite different criteria.

Experience with the genome-based discovery of new antibacterials also suggests that enthusiasm for this approach should be based on a realistic view of its limitations. None of the 18 new antibacterials now in clinical trials were discovered through a genomics programme[31,32]. Numerous potential targets have been identified and explored, but the limiting factor in developing new antibacterials is clearly not the characterization of compounds that are active against new targets. Rather, the limiting factor is the conversion of such compounds into drug candidates that are optimized not only for activity but also for other desirable pharmaceutical and physicochemical properties[33–35].

Ideally, targets selected for a screening campaign should be genetically and/or chemically validated, biochemically and structurally characterized, open to selective inhibition without a tendency for the parasite to develop resistance, and technically amenable to screening large numbers of compounds (BOX 2). Some parasite-specific points should be noted. First, parasite species that differ from the human pathogen are commonly used in validating hits and searching for leads (TABLE 7). For example, *P. berghei* is widely used in animal models of malaria. This requires consideration of homologous targets from different parasite species, as discussed in the next section. Second, development of resistance (for example, to dihydrofolate reductase) is well documented and the resistance potential of parasites to new chemical leads should therefore be investigated early in development. Third, kinetic factors can strongly affect target suitability: for example, inhibition of trypanosomal ornithine decarboxylase might only be successful because the turnover rates of the human and parasite enzymes differ such that the parasite is unable to regenerate enzyme fast enough to survive its irreversible blockade[36]. This example highlights the importance of a detailed knowledge of parasite biochemistry in target selection.

Potential parasite drug targets are being validated using chemical (TABLE 6) or genetic methods (for example, gene-expression profiling following drug treatment, RNA interference (RNAi) or genetic knockout techniques) for most of the protozoa in TABLE 1. About 20 potential targets with known inhibitors have been identified for *P. falciparum*[29], fewer for other parasites (TABLE 6). *In vitro* culture and manipulation of the helminths in TABLE 1 is technically more demanding than for protozoa. The use of the readily available, free-living NEMATODE *Caenorhabditis elegans* as a model organism[37], coupled with RNAi methodology, has been recommended for systematic identification of new targets in *Onchocerca* and *Brugia* species[38].

HTS and compound libraries. Examples of the relatively few HTS campaigns that have been conducted using parasite enzymes include lactate dehydrogenase, peptide deformylase, glyceraldehyde-3-phosphate dehydrogenase, enoyl-ACP reductase (Fab I) and trypanothione reductase. Not all of these campaigns have yielded hits worth pursuing and several of these targets have since

NEMATODE
A group of organisms also known as roundworms. They reproduce by laying eggs, or larvae which hatch from their eggs inside the body of the female worm. They are among the most common multicellular parasites of humans and include the filarial worms.

Table 5 | **Some enzyme or receptor targets of antiparasite drugs**

Targets*	Biochemical pathway	Parasite	Drug	Comments	References
Dihydrofolate reductase	Folate biosynthesis	*Plasmodium falciparum*	Pyrimethamine, cycloguanil	In MMV portfolio; new inhibitors are being designed from structural data on wild-type and mutant enzymes	18,56
Dihydropteroate synthase	Folate biosynthesis	*P. falciparum*	Sulphones/sulphonamides, for example, dapsone, sulphamethoxazole	Resistance requires use in combination with other drugs	57
Cytochrome *b*	Electron transport	*P. falciparum*	Atovaquone (marketed in combination with proguanil as Malarone)	Resistance at target site requires combination therapy with proguanil for treatment/prophylaxis	58–61
1-Deoxy-D-xylulose 5-phosphate reductoisomerase	Non-mevalonate isoprenoid biosynthesis	*P. falciparum*	Fosmidomycin	Cure rate insufficient for use as a single agent but effective in combination with clindamycin	54,62,63
Ornithine decarboxylase‡	Polyamine biosynthesis	*Trypanosoma brucei gambiense*	Difluoromethylornithine	Treatment of early- and late-stage *T. b. gambiense*, but not *T. b. rhodesiense*, infections	64
Sterol C-14 α-demethylase	Sterol biosynthesis	*T. cruzi*	Antifungal triazoles — for example, posaconazole	Await investigation in Chagas' patients	65,66
Farnesyl pyrophosphate synthase‡	Polyisoprene biosynthesis	*P. falciparum*, KINETOPLASTIDS	Bisphosphonates used for bone resorption, for example, risedronate	TDR portfolio. Current drugs unlikely to be clinically effective against protozoans	67
Nicotinic acetyl-choline receptors	Neurotransmission	Nematodes	Levamisole	Does not kill adult worms; used for gut nematodes and not tissue dwellers	68
Tubulin	Cytoskeleton component	Nematodes	Albendazole	Used in combination with other drugs against filariasis	69
Glutamate-gated chloride channels	Neurotransmission	Nematodes	Ivermectin	Does not kill adult worms	70,71

*Updated information is available from sites listed at http://www.who.int/tdr/kh/res_link.html#genomes. ‡The targets have been adapted to a high- or medium-throughput screen. MMV, Medicines for Malaria Venture; TDR, the UNICEF/UNDP/World Bank/WHO/Special Programme for Research and Training in Tropical Diseases.

been downgraded — for example, lactate dehydrogenase (TABLE 6). As more parasite molecular targets are identified from genomics programmes, the use of HTS campaigns is expected to increase. The choice of which compound collections to screen is crucial. If the target is related to one already being pursued by pharmaceutical companies for other indications — for example, protein kinases relevant to oncology — then it would be feasible and desirable to conduct the campaign using a small, focused compound collection (perhaps 500–1,000 compounds) based around chemical scaffolds known to provide inhibitors of such protein classes. However, in situations in which the target has not been well studied, then recourse must be made to screening large, diverse compound or natural product libraries, often numbering in excess of 100,000 samples. The advent of commercial suppliers of large compound collections now enables academic institutions to organize screening campaigns using either focused or diverse collections. In all cases chemical libraries should undergo rigorous triaging to ensure 'drug-likeness' and to eliminate compounds likely to be generally toxic, mutagenic, highly reactive, unstable or intractable to chemical modification. A surprising number of non-drug-like molecules still permeate many libraries, due to the difficulty of writing general formulae that will ensure their detection and removal without eliminating many other useful compounds. For example, MICHAEL ACCEPTORS are often present and in general these can be expected to be susceptible to nucleophilic attack, thereby rendering them nonspecific in their biological actions. However, this class contains some notable exceptions such as the vinylsulphones. Here at least one member of this potent series of parasite cysteine protease inhibitors has good target specificity and is being developed under the auspices of IOWH for treatment of Chagas' disease[39] (TABLE 6). In addition, because of their common use as synthetic intermediates, most libraries also contain aromatic or heterocyclic nitro compounds. These compound types are undesirable for screening against parasites, as they often show good activity, particularly against protozoa, due to bio-reduction and formation of reactive free radicals. However, such properties are also commonly associated with mutagenicity, and on balance it seems reasonable to exclude these compounds from screening libraries.

The creation of chemical libraries has been greatly furthered by advances in new technologies relating to combinatorial and parallel synthesis[40]. Such libraries can be based on proprietary or non-proprietary compounds. Companies might have patents, or be seeking patent protection, on proprietary compounds in their libraries. This issue is important because several pharmaceutical companies are now allowing academia-driven HTS campaigns to be conducted against parasite proteins using their sample collections. Difficulties can arise when the chemical structures of the hits need to be released for further study if such compounds

KINETOPLASTIDS
A group of flagellated protozoa, including the trypanosomatids (see below), that are distinguished by the presence of a kinetoplast, a DNA-containing granule located within the single mitochondrion and associated with the flagellar bases.

MICHAEL ACCEPTOR
A compound containing an activated carbon-carbon double bond susceptible to nucleophilic attack.

Table 6 | **Some additional parasite molecular targets under study by PPPs, TDR or in selected other collaborations***

Target	Biochemical pathway	Parasite	Some inhibitors with antiparasite activity	Comments	References
Dihydro-orotate dehydrogenase‡	Pyrimidine biosynthesis	*Plasmodium falciparum*	Potent and specific amides active against enzyme; activity on parasites awaited	NIH funded. MMV project in abeyance	72§,¶
2C-Methyl-D-erythritol 2,4-cyclodiphosphate (MECP) synthase	Non-mevalonate pathway of isoprenoid biosynthesis	*P. falciparum*	None yet disclosed	Pathway validated by antimalarial activity of fosmidomycin	73
Cysteine proteases (falcipain) Cysteine proteases (cruzain)‡	Haemoglobin degradation?	*P. falciparum* *T. cruzi*	Vinylsulphones	In MMV and IOWH portfolios	39,74§,‖
Protein farnesyltransferase (PFT)	Protein farnesylation	*P. falciparum*; Kinetoplastids	Human PFT inhibitors	In MMV and DNDi portfolios	75,76§,#
Type II enoyl-acyl carrier protein reductase (Fab I)‡	Fatty-acid biosynthesis	*P. falciparum*, *Trypanosoma*	Triclosan	In MMV portfolio	77–79
Peptide deformylase (PDF)	Protein biosynthesis	*P. falciparum*	Hydroxamates	In MMV portfolio, but concerns that enzyme may not be essential to parasite	80§
Hexose transporter	Glucose uptake	*P. falciparum*	Glucose analogues		81
Trypanothione reductase‡	Defence against chemical/oxidant stress	Kinetoplastids	Tricyclics	DNDi, TDR portfolios	82,83
Glyceraldehyde-3-phosphate dehydrogenase§	Glycolysis	*P. falciparum*	No specific inhibitors disclosed	MMV portfolio 2003	84§
Amino-acyl tRNA synthetase‡	Protein synthesis	*Filariae, Wolbachia*	No specific inhibitors disclosed	University of Michigan/NIH	85¶
Lactate dehydrogenase‡	Energy metabolism	*P. falciparum*	Gossypol, azoles	MMV discontinued interest in 2003 for failure to identify an orally active compound	86,87§
PfSub 1‡	Erythrocyte invasion	*P. falciparum*	No specific inhibitors disclosed	TDR portfolio	88
Cyclin-dependent kinase PfCDK1‡	Nuclear division	*P. falciparum*	No specific inhibitors disclosed, modulators of related kinases identified	TDR portfolio	89,90
7,8-Ddihydro-6-hydroxymethylpterin pyrophosphokinase‡	Folate biosynthesis	*P. falciparum*	No specific inhibitors disclosed	TDR portfolio	91

*Updated information is available from sites listed at http://www.who.int/tdr/kh/res_link.html#genomes. For further putative molecular targets in kinetoplastids, see REF. 92; for targets in plasmodia, see REF. 29. ‡The targets have been adapted to a high- or medium-throughput screen. §See the MMV website http://www.mmv.org/pages/page_main.htm. ‖See the IOWH web site http://www.oneworldhealth.org/. ¶See the NIH web site http://crisp.cit.nih.gov. See the DNDi website http://www.dndi.org/. DNDi, Drugs for Neglected Diseases initiative; IOWH, Institute for One World Health; MMV, Medicines for Malaria Venture; NIH, National Institutes of Health; PPP, public–private partnership; TDR, the UNICEF/UNDP/World Bank/WHO/Special Programme for Research and Training in Tropical Diseases.

belong to commercially sensitive chemical series. Such problems generally do not arise when the compounds in the library have been purchased from commercial suppliers and are considered non-proprietary. It will still be advisable, however, to run patent searches on compounds that are being considered as the basis of a lead-optimization programme, to gain information on the chemical class as well as to note any claims that could interfere with future commercial development.

Moving from hits to leads to drug candidates

The progression from 'hit' to 'lead' to 'drug candidate' (FIG. 1; BOX 1) follows the same general pattern for the discovery of antiparasitics as for other drugs. Compounds are selected for improved efficacy and pharmaceutical properties by studies of analogues and iterative medicinal chemistry. The structure of the target molecule, if known, can be very helpful in directing medicinal chemistry efforts[18]. An advantage for the discovery of new antiparasitic drugs is the existence of good, highly predictive *in vitro* and *in vivo* assays for activity, which often use the same parasitic organism that infects the human patient. However, protocols are not standardized and it is often not straightforward to compare results from different laboratories. Techniques are always evolving and are also being adapted to achieve higher throughput (for example, by the use of reporter genes to allow fluorescence-based assays to be used instead of microscopy). A recent paper has reviewed the models used for antimalarial

Table 7 | **Commonly used *in vitro* and animal models in antiparasite drug discovery**

Parasite	Main primary *in vitro* models	Main primary animal models	Secondary animal models	References
Plasmodium falciparum *P. vivax* *P. ovale* *P. malariae*	*P. falciparum* (drug-sensitive and resistant strains)	*P. berghei*/mouse *P. chabaudi*/mouse *P. vinckei*/mouse	*P. yoelii* or other resistant strains/mouse	41
Leishmania donovani *L. major* *L. braziliensis* Over 20 species and sub-species	*L. donovani* *L. major*	*L. donovani*/mouse *L. donovani*/hamster	Resistant strains/mouse or hamster	93,94
Trypanosoma gambiense, *T. rhodesiense*	*T. brucei brucei* *T. b. rhodesiense* *T. b. gambiense*	*T. brucei* spp./mouse (acute infection model)	*T. brucei* spp/ mouse (chronic infection)	95,96
T. cruzi	*T. cruzi*	*T. cruzi*/mouse (acute infection)	*T. cruzi*/mouse (chronic infection)	97–99
Schistosoma mansoni *S. haematobium* *S. japonica*	*S. mansoni*	*S. mansoni*/hamster *S. mansoni*/mouse	Resistant strains	100 101 102 103,104
Brugia malayi *Wuchereria bancrofti*	*O. gutturosa* (adult worms)	*O. lienalis*/mouse (microfilariae) *B. pahangi*/jird (adult worms)	*B. pahangi*/dog (adult worms)	105–108
Onchocerca volvulus	*O. gutturosa* (adult worms) *O. volvulus* (adult worms)	*O. lienalis*/mouse (microfilariae)		109,110

drug discovery and has recommended a streamlined process for evaluating new compounds[41]. TABLE 7 lists some of the commonly used *in vitro* and *in vivo* models in antiparasitic disease discovery.

Although the parasitic strains used in laboratory tests are often the same or very similar to those infecting the human patient there are certain cases where important differences exist. The standard animal models for malaria infection use *P. berghei, P. chabaudi, P. yoelii* or (less often) *P. vinckei* rather than the *Plasmodium* species that infect humans. The *T. brucei brucei* parasites used in the initial African trypanosomiasis tests differ from the *T. b. rhodesiense* and *gambiense* subspecies that cause human disease. The *Onchocerca gutturosa* worms used as *in vitro* models for onchocercal infection are parasites of cattle rather than humans. These differences can be especially crucial when a molecular target-based drug discovery strategy is followed; for example, the cysteine proteases of *P. vinckei* (vinckepains) differ from those of *P. falciparum* (falcipains), so that both types of protease had to be expressed and studied in a falcipain-based programme[42]. Genetically modified parasites in which the pathogen target replaces the model target gene would be one solution to this problem.

An important consideration in choosing the appropriate animal model is the desired product profile (TABLE 3). Most of the diseases require testing in several types of animal model. The primary models generally reflect the acute form of a disease, whereas the secondary, more complex assays represent the chronic or drug-resistant disease for which treatment is being sought. For example, compounds would not be considered useful leads for late-stage African trypanosomiasis or chronic Chagas' disease unless they showed activity in the corresponding secondary infection model (TABLE 7). The primary models also serve to filter out compounds before entering the time-consuming and expensive chronic tests, in which infected animals are typically followed for 6 weeks or longer.

The helminth models offer particular challenges. *In vitro* assays of antischistosome activity are not standardized, and it is not clear how well these will predict activity in animal models. For most *in vitro* helminth screens, readout is based on modulation of parasite motility, although dye reduction can be used as a secondary criterion to assess worm viability. There is reasonable, but not complete, correlation between the motility and dye reduction readouts. Genetically modified *C. elegans* might increase throughput in early discovery. In addition, for *Onchocerca*, the primary animal model is based on microfilariae and not on the adult worms, yet the main need is a drug that would act against the adult worms (macrofilariae). Time-consuming and relatively expensive secondary assays are required to select good lead compounds for onchocerciasis or lymphatic filariasis.

For all the studies in animals, the impact of formulation on the activity of compounds needs to be considered. In order to give compounds the maximum chance of success (seeing at least some activity), it is usual to initially test compounds in water-based formulations containing 10% dimethylsulphoxide (DMSO). This can have a significant impact on oral bioavailability, particularly with water-insoluble compounds. DMSO-containing formulations are

Box 2 | **Points to consider in selecting parasite molecular targets**

Selectivity
- Is the target absent from mammals; or:
- Has the target any molecular or pharmacological properties which distinguish it from related mammalian proteins?
- Do any related mammalian proteins occur both in humans and in the animal species to be used for *in vivo* efficacy, toxicity and pharmacokinetic studies?

Validation
- Is there evidence (from RNA interference, knockouts, inhibitors and so on) to suggest that the target is essential for growth, survival, or fertility?
- Are proteins with similar properties also present in any model parasite species used?
- Is the target is expressed in a parasite life-cycle stage suitable for drug intervention?

Potential for development of resistance
- Absence of isoforms of the target within a species
- Absence of biochemical 'bypass' reactions or transport mechanisms to circumvent inhibition of the target

Biochemical Properties
- If the target protein is an enzyme involved in a multi-step pathway, is it rate-limiting to the extent that inhibition can reduce flow through the pathway to non-viable levels?
- Will the rate of target turnover allow inhibition over reasonable time periods?

Structure and 'druggability'
- Amino-acid sequence of the target known
- Crystal or NMR structure of related proteins known or obtainable, preferably with bound cofactors, inhibitors or agonists/antagonists
- Target has a small molecule ligand-binding pocket
- Target type has precedents, that is, existing drugs or ligands

'Assayability'
Important features:
- Expression precedent available
- Existing biochemistry/enzymology
- Single subunit where possible
- Specific and inexpensive readout that can be predicted, especially optical, that is compatible with high-throughput screening
- Active-site chemistry available

Other desirable features include:
- Focused chemical library already available for the class of molecule
- Cell-based assays
- Assays with functional endpoints
- Assays with few steps (for example, washes)

not normally acceptable for the assessment of toxicity and it is important to retest active compounds in non-DMSO-containing vehicles, such as 'standard suspending vehicle' (SSV)[43].

Importance of lead optimization. As outlined in FIG. 1, lead optimization is an iterative process in which medicinal chemistry is used to design and synthesize new compounds, and these are evaluated for improved properties. Increasingly, as the cycle depicted in FIG. 1 is traversed, costs escalate and time frames expand, with the lead-optimization stage being the point most crucial in constraining the drug discovery process. This stage is essentially an exercise in problem-solving in which bioavailability, metabolic and toxicity considerations come into play in the selection of a robust drug candidate. It is at this stage that involvement of the pharmaceutical industry becomes highly desirable. Lead optimization has been the most disregarded and under-funded section of the antiparasite drug discovery process during the past two decades. The advent of PPPs has dramatically improved the situation in recent years by re-engaging the pharmaceutical industry and by providing funding.

An example highlighting the role of lead optimization in producing a drug candidate is that of the antimalarial synthetic peroxide OZ277 (RBx-11160), which has now entered clinical trials[44]. Under the TDR 'malperox' programme[45], meetings were organized for TDR-funded biologists and chemists to discuss the key issues in the optimization of artemisinin-like compounds. More than 1,000 semi-synthetic and synthetic artemisinins from at least seven different laboratories were evaluated. Biological data were reported back to the chemists to assist them in the optimization process. One of the most promising projects concerned a series of synthetic peroxides. Since 2000 this project has been funded by a newly formed PPP, MMV. Under MMV, a strong 'virtual' discovery team was established that links chemists in the United States with parasitologists in Switzerland and pharmacokineticists in Australia. An optimized drug candidate was selected in 2003 and taken to an Indian pharmaceutical company (Ranbaxy) for scaled-up production to provide the material now being assessed in clinical trials[44].

This scenario illustrates how public funding for a diverse set of early-stage discovery projects led to a set of good lead compounds that were selected for further development by a PPP. The PPP managed the subsequent successful development of the lead compound into a drug candidate by using its funding power and its ability to bring together the necessary expertise, particularly from the pharmaceutical sector.

Partnerships for drug discovery

The role of PPPs in drug development for neglected diseases has been extensively discussed[4,5,46,47]. They have had encouraging successes in moving drug candidates into clinical trials, and in stimulating discussion of how industry can contribute to this process[48]. However, drug discovery is more risky than development, and the PPPs in TABLE 6 have adopted portfolio approaches that often emphasize development. Basic research and early discovery research are supported primarily by public funds. Early discovery research can involve collaborations between different public institutions as well as public–private agreements. We give some examples below of collaborative early discovery research supported by public funds, and in some cases also by in-kind contributions from industry.

The sequencing of parasite genomes involved the establishment of international genome networks (TABLE 2). To help translate the resulting knowledge into new drugs, TDR, through its Pathogenesis and Applied Genomics group, is supporting the establishment of regional networks in South America, Asia and Africa for training in bioinformatics and its applications to parasite genome studies. This includes the identification of potential drug targets. Through

its Genomics and Discovery Research group TDR can follow up potential targets with validation studies and assay development. With validated targets and suitable assay formats, the principal investigators can seek partners with expertise in HTS and access to compound collections. These partners can be located in academia — for example, at ICCB-ICG Harvard mentioned above, or at the Walter & Eliza Hall Institute of Medical Research in Melbourne, Australia. Alternatively, an industrial partner can be involved, as in the case of the screening of plasmodial lactate dehydrogenase (TABLE 6), or the ongoing collaboration between TDR and Serono, which is enabling scientists from two disease-endemic countries to screen enzyme targets from *Plasmodium* and other parasites. Several other collaborative networks aim to link suppliers of natural products with academic laboratories that can evaluate their antiparasite activity (see, for example, the Multilateral Initiative on Malaria (MIM) and the University of Mississippi websites in Further information). The Kitasato Institute in Japan has been screening thousands of natural products from its own stocks[27] and some 30,000 synthetic compounds from Japanese pharmaceutical companies for antimalarial activity under a broad collaboration between WHO/TDR, the Japanese Ministry of Health & Welfare and 14 Japanese pharmaceutical companies.

These collaborations bring together, for a specific purpose, groups that have particular expertise in different areas of drug discovery. In such collaborations, a formal agreement should define the project's objectives and each party's rights and obligations; such agreements typically require preferential pricing of any resulting products for developing countries, and define how any intellectual property rights are to be protected and made available[4]. Projects from these early drug discovery collaborations, if successful, can become candidates for support by PPPs, whose experience and concept of management through focused project development teams are tailored to advancing discovery projects further.

Outlook

Although, as stated in the introduction, very few new drugs were approved for the tropical diseases between 1975 and 1999, there has been a burst of activity since 2000, with more than 20 new agents developed or in development for parasitic diseases[49] (TABLE 6). This burst of activity is largely attributed to a new spirit of partnership between the public and private sectors, with industry and public-health interests more closely aligned. Public–private agreements, and in particular the PPP model of project management, together with increased philanthropic funding, have increased the numbers of antiparasite drug candidates reaching the clinic during the past 5 years. Even if many of these candidates are combinations or drugs already used for other indications, the pipeline of discovery projects is much richer than a decade ago. The pharmaceutical industry's historically diminished involvement is being compensated, at least partially, by their involvement in PPPs, by individual agreements concerning specific projects and, more recently, by the foundation of industry-backed research institutes with specific drug discovery mandates for indications such as malaria, tuberculosis and dengue[4].

In order to continue to be successful, the PPPs will need to identify new compounds for development. There are concerns that most of the 'low-hanging fruit' have been picked, and that early-stage discovery research needs further strengthening in both the public and private sectors, with financial support from public sources. The success of the PPP model should not obscure the fact that they need a thriving background of discovery-oriented research, itself largely dependent on public funding. Examples of relevant publicly funded early-stage research include parasite genetics and biochemistry, molecular target identification and validation, HTS against these targets, screening compounds against whole parasites, and chemistry to progress hits to lead compounds. Once good lead compounds are identified, it will be easier to attract new sources of funding — for example, from PPPs. They can provide the financial resources and expertise in drug development necessary to turn leads into drug candidates (as exemplified by the OZ compound discussed earlier[44]).

The disease-endemic countries are playing an increasingly important role in the discovery of new drugs. Some countries, such as Brazil, China, India or South Korea, already have a drug-manufacturing industry and institutions involved in drug discovery research. Other countries have research institutes with expertise in, for example, natural product chemistry, but as yet lack a pharmaceutical industry capable of moving from compounds in discovery all the way through the drug development pathway as illustrated in FIG. 1. There is a growing awareness (see, for example, REF. 50) that the countries most affected by these diseases need to be actively involved in the solutions, including research to develop new and better treatments: a country's capacity to respond to the threat of disease is closely linked to its research capacity[51]. Both private and public support are increasing: for example, the Gates Foundation recently awarded a $20-million grant to science academies in Nigeria, South Africa and Uganda, and Britain's Department for International Development is planning to increase its spending on research and development in Africa. TDR has for 30 years supported research capacity strengthening in the developing countries, with this being a key component of its mission. Many of the leaders in tropical disease research now come from the disease-endemic countries and were supported by TDR in the early stages of their careers. This core of experts and expertise that exists in the disease-endemic countries needs to be encouraged and more actively engaged, not only in early drug discovery projects, but also in more advanced drug discovery and development projects like those being supported by the PPPs.

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Competing interests statement

The authors declare no competing financial interests.

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