Part One
Screening, Bioinformatics, Chemoinformatics, and Drug Design

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Drug Discovery Approaches Toward Anti-Parasitic Agents

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Abstract

Parasitic diseases afflict hundreds of millions of people worldwide, and are a major issue in animal health. Because most drugs available today are old and have many limitations, novel drugs for the treatment of human and animal parasitic diseases are urgently needed. Modern research disciplines such as genomics, proteomics, metabolomics, chemogenomics, and other "-omics" technologies improve the quality of the drug discovery process and influence the design of novel anti-parasitic agents. These include the application of high-throughput technologies such as DNA/RNA sequencing, microarrays, mass spectrometry, high-throughput screening, and bio/chemoinformatics. Here, an overview is provided of the drug discovery workflow, and the steps employed to generate novel drug candidates with anti-parasitic activity are briefly described.

Drug Discovery Initiatives to Accelerate the Development of Novel Anti-Parasitic Drugs for Humans and Animals

Infectious diseases, including those caused or transmitted by parasites, are responsible for substantial morbidity and mortality worldwide and affect several billion people globally, particularly in developing countries [1]. Until recently, infectious diseases were viewed as a problem of the past; however, the emergence of drug-resistant organisms makes the need for new drugs or vaccines more important than ever before. Moreover, as these diseases predominantly afflict inhabitants of poor countries, drug discovery efforts are minimal due to the lack of returns on investment. Accordingly, diseases such as malaria, leishmaniasis, Chagas disease, elephantiasis, or schistosomiasis are often called "neglected diseases" [2].

More recently, the growing realization of the humanitarian and economic consequences of neglected diseases in poor countries has spurred the establishment of new organizations specifically focused on novel anti-parasitic drug development

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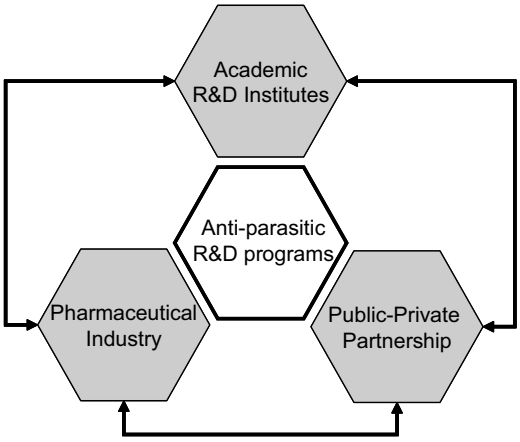


Figure 1.1 Research and development activities to fight neglected diseases. Anti-parasitic research and development programs were initiated by academic R&D centers, public–private partnerships, and the pharmaceutical industry. Intensive collaboration is key to optimizing R&D output.

[3–5]. Collaborations between the pharmaceutical industry, specialized academic drug discovery centers, and public–private partnerships (PPPs) have been initiated to support anti-parasitic drug discovery and development programs (Figure 1.1).

Public–private partnerships focus to combine the skills and resources of academia, the pharmaceutical industry, and contract research teams, with the goal of generating independent research and development (R&D) consortia. Well-known initiatives are the World Health Organization's Special Program for Research and Training in Tropical Diseases (WHO/TDR; http://www.who.int/tdr), the Drugs for Neglected Diseases *initiative* (DND*i*; http://www.dndi.org), the Institute for One World Health (iOWH; http://www.oneworldhealth.org), and the Bill & Melinda Gates Foundation (B&MGF; http://www.gatesfoundation.org). For instance, the DND*i* has built a virtual, not-for profit R&D organization for developing new drugs against kinetoplastid diseases, which include human African trypanosomiasis, visceral leishmaniasis, and Chagas disease. The partners of DND*i* are Doctors Without Borders, the Oswaldo Cruz Foundation of Brazil, the Indian Council for Medical Research, the Kenya Medical Research Institute, the Ministry of Health in Malaysia, the Pasteur Institute in France, and the WHO/TDR. DND*i* has already registered two products; in 2007 and 2008, respectively, the antimalarial drugs fixed-dose artesunate-amodiaquine (AS/AQ) and fixed-dose artesunate-mefloquine (AS/MQ) were launched [2].

Development projects organized by PPPs are often supported by the pharmaceutical industry itself. One particular project in the public eye is the Accelerating Access Initiative (AAI; http://www.ifpma.org/health/hiv/health_aai_hiv.aspx), a global initiative to broaden access to and ensure affordable and safe use of drugs for HIV/AIDS-related illnesses. Related programs, such as the Global Alliance to Eliminate Lymphatic Filariasis (GAELF; http://www.filariasis.org) and the Medicines for Malaria Venture (MMV; http://www.mmv.org), exist for many parasitic diseases. In

addition, the pharmaceutical industry also invests directly in anti-parasitic research activities, with many companies having established state-of-the-art research facilities that concentrate exclusively on the development of drugs and vaccines for neglected diseases. Prominent research centers include the Novartis Institute for Tropical Diseases (NITD; http://www.novartis.com/research/nitd), the GlaxoSmithKline Drug Discovery Center for Diseases of the Developing World (DDW; http://www.gsk.com), or the MSD Wellcome Trust Hilleman Laboratories (http://www.hillemanlaboratories.in).

Needless to say, another major source of novel drugs in anti-parasitics stems from the extensive research activities in academic facilities. A relatively recent trend has been the foundation of academic drug discovery centers that focus exclusively on R&D in the field of neglected diseases. These aim to translate basic biomedical research into candidate medicines for neglected diseases. Examples are the Drug Discovery Unit of the University of Dundee (DDU; http://www.drugdiscovery.dundee.ac.uk), the Sandler Center for Drug Discovery (formerly Sandler Center for Basic Research in Parasitic Diseases) at the University of California San Francisco (http://www.sandler.ucsf.edu), and the Seattle Biomedical Research Institute (SBRI; http://www.sbri.org).

It is worth noting that anti-parasitic drug R&D programs are not confined to human medicine. Indeed, the animal health industry also performs intensive research on novel anti-parasitics [6–8]. This is important, as the situation of people in developing countries suffering from neglected diseases is aggravated by drastic economic losses in agriculture due to parasitic infections in farm animals. In this context, some animal health companies support developing countries in the framework of corporate social responsibility activities. For example, Intervet/Schering-Plough Animal Health (ISPAH; http://www.intervet.com) has an ongoing cooperation with the Indian non-governmental organization *Bharatiya Agro Industries Foundation* (BAIF; http://www.baif.org.in), whereby poor farmers in India have access to ISPAH's range of livestock products, including vaccines and anti-parasitic agents. It is expected that more than two million rural families could benefit from this project.

Innovation from Anti-Parasitic Drug Discovery Approaches

A *parasite* is defined as an animal that lives completely at the expense of plants, other animals, or humans [9]. In general, parasites are much smaller than their hosts, show a high degree of specialization for their mode of life, and reproduce more quickly and in greater numbers than their hosts. Parasites belong to a wide range of biologically diverse organisms and, based on their interactions with their hosts, are often classified into three categories: parasitic protozoa; endo-parasites; and ecto-parasites [7, 9].

1) Parasitic protozoa are unicellular microorganisms that infect humans or animals and either live extra- or intracellularly. Representatives include *Plasmodium falciparum*, *Trypanosoma cruzi*, and *Leishmania donovani*; causing malaria, Chagas disease, and leishmaniasis, respectively [9].

2) Endo-parasites are mainly multicellular helminthes that have adapted to live in the host's gastrointestinal tract, or systemically. Well-known endo-parasites are the nematode *Brugia malayi*, the causative agent of lymphatic filariasis, and the blood-fluke *Schistosoma mansoni*, which causes schistosomiasis [9, 10].
3) Ecto-parasites are parasitic organisms that live on the surface of their hosts. In most cases, ecto-parasites do not cause fatal maladies by themselves but affect the health of their hosts by transmitting pathogenic viruses, bacteria, or protozoa [9, 11]. Taxonomically, the majority of ecto-parasites belong to the phylum Arthropoda, and include important organisms such as fleas, flies, and ticks.

Historically, all marketed anti-parasitic products have been discovered by screening synthetic and natural compounds against intact parasites, either in culture or in animal models [12]. Such physiology-based assays, bioassays, or phenotypic assays involve parasites cultured *in vitro*, and exist for many different protozoa, endo-parasites, and ecto-parasites [13–16]. The main benefit of testing candidate compounds directly on whole organisms is that compounds with anti-parasitic activity are immediately apparent, suggesting that they possess the physico-chemical properties that allow them to penetrate the membrane barriers of the parasites in order to reach their molecular targets [17]. Since the simultaneous optimization of lead compounds for optimal anti-parasitic activity and bioavailability is one of the major hurdles in the lead optimization process, the advantage of bioassays should not be underestimated. Bioassays continue to play an important role in today's drug discovery process, particularly during the identification and optimization of novel anti-parasitic compounds [7].

On the other hand, the use of bioassays as the sole screening platform has a disadvantage. Those potential drugs with a high activity against attractive anti-parasitic target molecules, but no activity in bioassays (e.g., due to disadvantageous physico-chemical properties), are discarded. For this reason, alternative target- or mechanism-based drug screening strategies have been developed [8].

The Process of Target-Based Drug Discovery

In contrast to physiology-based drug discovery screens, the target-based approach starts with the identification of a protein that is used to search for new active compounds in *in vitro* screens [18, 19]. The goal of the target-based approach, as with every drug discovery workflow, is to provide drug candidates for the downstream development process, finally ending with a newly registered drug (Figure 1.2). In principle, the target-based approach consists of four major steps: (i) target identification; (ii) target validation; (iii) lead discovery; and (iv) lead optimization, including the *in vitro* profiling of the optimized lead structures (Figure 1.2). Target-based drug discovery is a highly technology-driven process, which particularly benefits from advances in modern "omics" research areas. Omics is a neologism which refers to a broad area of study in biology of fields ending in the suffix "-omics," such as genomics, transcriptomics, proteomics, or metabolomics (http://omics.org). Omics

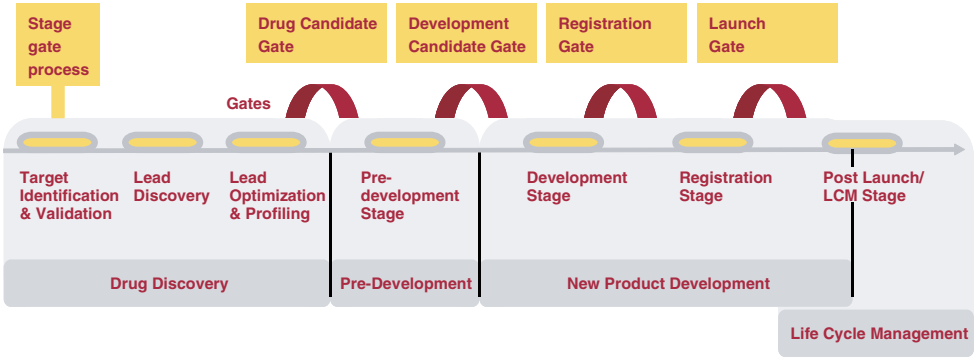


Figure 1.2 Drug discovery and development workflow. The workflow is organized as a stage-gate model; that is, a product development process is divided into stages separated by gates. At each gate, the continuation of the development process is decided by the organization. The drug discovery stage typically consists of target identification and validation, lead discovery and optimization, and profiling.

sciences apply large-scale experiments in order to analyze complete biological entities such as genomes, proteomes, metabolomes, and so on. They are enabled by major advances in modern high-throughput technologies such as DNA/RNA sequencing, microarrays, mass spectrometry, high-throughput screening, or combinatorial and medicinal chemistry – technologies that are increasingly common and affordable [20]. These technologies have already started to improve the quality and quantity of the drug discovery process [21].

Target Identification

Target identification starts with the discovery of a relevant drug target believed to be essential for the survival of a parasitic organism [22]. In order to avoid or minimize potential toxicity effects prior to the development of a new anti-parasitic drug, an optimal drug target would be absent from the host [23]. However, experience has shown that many of the existing anti-parasitic drugs act on target molecules which also exist in the host organisms [10, 24, 25].

Common methods for the selection of potential drug targets are classical biochemistry and molecular biology techniques. For example, the reverse transcriptase-polymerase chain reaction (RT-PCR) can be used to verify the expression of a potential target protein in the critical life stages of a parasite [19, 26]. Alternatively, information that can be "mined" in genome and drug target databases such as EuPathDB (http://w1.eupathdb.org/eupathdb) [27], GeneDB (http://www.genedb.org) [28], and the TDR target database (http://tdrtargets.org) [29], enables the identification of new drug targets. Such databases contain a wealth of data relating to parasite genes, proteins, homologs, transcript expression, single nucleotide polymorphisms (SNPs), cellular localization, and putative functions. It is expected that the data content in these databases will continue to increase due to advances in high-throughput technologies, and their ever-greater data output. For example, the area

Table 1.1 Published genomes of apicomplexan organisms.

Parasite	Taxonomy	Link
Babesia bovis T2Bo	Aconoidasida	Genbank accession AAXT00000000
Theileria annulata str. Ankara	Aconoidasida	http://www.sanger.ac.uk/Projects/
Theileria parva str. Muguga	Aconoidasida	Genbank accession AAGK00000000
Cryptosporidium hominis TU502	Coccidia	http://cryptodb.org/cryptodb/
Cryptosporidium parvum Iowa	Coccidia	http://cryptodb.org/cryptodb/
Plasmodium yoelii str. 17XNL	Aconoidasida	http://plasmodb.org/plasmo/
Plasmodium falciparum 3D7	Aconoidasida	http://plasmodb.org/plasmo/
Toxoplasma gondii ME49	Coccidia	http://toxodb.org/toxo/
Toxoplasma gondii TgCkUg2	Coccidia	http://toxodb.org/toxo/

of functional genomics already enables the determination of complete genomic protein functions by utilizing high-throughput experiments such as microarrays, serial analysis of gene expression (SAGE), ChIP-on-chip experiments, or proteomics [30]. Moreover, the emergence of competitive second- or next-generation DNA-sequencing techniques [31] and further advances in single-molecule DNA-sequencing technologies [32] will lead to the sequencing of additional genomes, including those of parasites [33]. Currently, over 1000 bacterial and 120 eukaryotic genomes have been reported as completely sequenced, and many more are ongoing (http://www.genomesonline.org) [34]. In examining the phylum *Apicomplexa*, approximately 50 genome sequencing projects have now been initiated, of which nine have already been published (Table 1.1). The availability of genome datasets for parasites, their vectors, and hosts provides the basis for another highly effective target identification method, the bioinformatic comparison of genomes [10, 35–38]. Such comparative genomics strategies aim to compare simultaneously two or more genomes in order to identify similarities and differences, and hence identify potential drug targets [18, 19].

Target Validation

When a particular protein has been identified as a potential drug target, the validation of its function is mandatory (Box 1.1) [39]. This involves the demonstration that

Box 1.1: Features of Optimal Anti-Parasitic Targets

A validated target:

- has a clear biological function
- has an essential role for the growth or survival of the parasite
- is expressed during the relevant life stages
- is druggable
- can be screened in a biochemical or cellular assay.

affecting the target will be sufficient for obtaining a significant anti-parasitic effect, and this can be accomplished by genetic studies that include the generation of loss-of-function (Knock-out) and gain-of-function (Knock-in) mutants in animal models [40]. Further common target validation methods are RNA interference, antisense RNA, and antibody-mediated inhibition experiments. Alternatively, the validation of a drug target is performed using chemical compounds [26, 41]. In such cases, experimental compounds with well-understood modes of action are tested directly on parasites and screened for anti-parasitic phenotypes. With positive results, it is inferred that the phenotypic effect is due to the interaction of the chemical compound with its known target. *Chemical validation* is a reliable form of target validation, although it cannot be excluded that the phenotypes resulting from the chemical validation experiment are in fact due to an interaction of the compounds with secondary, unknown, or multiple targets. One benefit in employing chemical validation is that, simultaneously, the druggability of a molecular target –that is, the ability of a target to interact with a small compound that modulates its function – is analyzed [42]. Experience has shown that this is a key prerequisite to successful drug discovery [42]. Since both genetic and chemical validation approaches have their benefits and drawbacks, a drug target is best validated using a combination of the two.

A potential drug target should fulfill additional criteria, including the ease of recombinant expression and purification, and "assayability" in automated biochemical or cellular assays (including their miniaturization) (Box 1.1) [26]. Drug target validation is a complex process that often produces ambiguous results. Accordingly, target validation is a risk-adjusted decision on the overall value of the target protein [43]. However, since the downstream steps in target-based drug discovery include very expensive and time-consuming processes, it is important that potential target molecules are validated in as large a quantity as possible.

Lead Discovery

The next step after the validation of a drug target is to identify compounds that interfere with its function [44]. A general lead discovery workflow is shown in Figure 1.3; this consists of a series of steps of hit identification, hit exploration, hit-to-lead, and lead selection [45]. The compounds should be amenable to chemical optimization, finally leading to a drug candidate. Such compounds are called "leads," and the process is called "lead discovery" [17].

Hit Identification

In order to identify small chemical compounds that interact with the target molecule, a screening campaign (often high-throughput screening, HTS) is performed [46]. Before an HTS can be started, biochemical or cellular assays must first be developed and miniaturized for optimal robotics and throughput [18]. The HTS assays are usually validated for their suitability and robustness by screening a small subset of compounds before the actual high-throughput screen is started. Depending on the type of assay, the HTS can typically involve the examination of more than one million

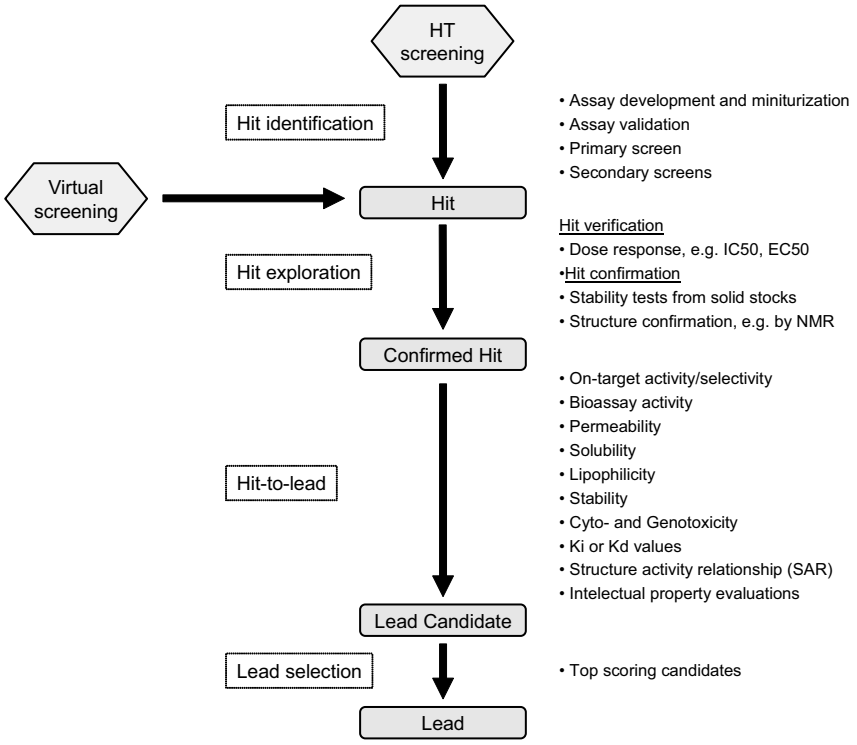


Figure 1.3 A typical lead discovery workflow.

compounds within a few weeks [47, 48]. The primary screen during the hit identification process is carried out on the initial validated target molecule, while secondary screens may be performed on further targets. For example, orthologous targets from additional parasites may be screened if the goal of the drug discovery project is ultimately to produce a compound that exhibits broad anti-parasitic activity, as is often the case in the animal health industry. Another possibility is to consider orthologs from host organisms, and to include only those compounds that show a high activity on the target molecules of the parasite. Thus, potentially toxic compounds may be filtered out in a very early stage [7]. A complementary approach is to involve chemoinformatics to identify hit compounds (Figure 1.3) [49]. Such *in silico* approaches – which are also known as "virtual screening" – deal with the automatic evaluation of large virtual compound libraries in order to prioritize compound subsets [50]. Compared to HTS, virtual screening has two major advantages: (i) the speed and throughput of *in silico* screens are much greater than in experimental setups; and (ii) more importantly, virtual screening is not limited to existing in-house compound collections, and provides a fast and cheap way to explore unknown parts of the chemical space [49]. Accordingly, virtual screening can generate target-focused and activity-enriched datasets, which can eventually be tested in experimental HTS assays [49, 51].

Hit Exploration

Hit exploration can be divided into "hit verification" and "hit confirmation," and mainly involves filtering processes to separate appropriate from inappropriate molecules (Figure 1.3) [17]. Hit verification concentrates on the experimental validation of the effectiveness of a compound by measuring the half-maximal inhibitory concentration (IC_{50}) in the case of antagonists, and the half-maximal effective concentration (EC_{50}) for agonists [46]. During hit confirmation, the stability of compounds is proofed. For example, compound solutions are freshly prepared from solid stocks and measured again in order to exclude artifacts from compound degradation in liquid stocks. Hit confirmation also includes the verification of the compound structures using techniques such as mass spectrometry (MS) and nucleic magnetic resonance (NMR) [19, 46]. The result of the hit exploration process is termed a "confirmed hit" (Figure 1.3).

Hit-To-Lead

The next phase in a lead discovery project is the "hit-to-lead" (H2L) process [52]. This is characterized by less filtering and a broader knowledge of the hits for a subsequent prioritization [17]. During the H2L process, data regarding toxicity, bioactivity, and intellectual property are assembled (Figure 1.3). One of the first actions in the H2L process is usually to purchase or synthesize structurally related compounds which are then tested together with the confirmed hits for their target activity and, by screening in bioassays against intact parasites in culture, for their anti-parasitic activity. In this way, data related to the on-target activity and initial structure–activity relationships (SARs) of the compound classes are generated. The experiments also provide the first hints regarding the bioactivity of the compound classes. If the compounds are inactive in the bioassays, then data arising from solubility, lipophilicity, or permeability experiments may explain the lack of bioactivity. Other typical H2L activities are the evaluation of toxicity data from cytotoxicity and genotoxicity tests [53], and an understanding of the patent literature for the corresponding compound classes (Figure 1.3) [54]. The H2L process concludes with a list of lead candidates that fulfill clearly defined criteria (Box 1.2), and from which a lead is selected for chemical optimization.

Box 1.2: Definition of a Lead

A lead:

- possesses specific activity in functional target assays
- exhibits a particular SAR
- shows no indication for genotoxicity
- has adjustable physico-chemical properties
- already features some bioactivity in parasitic bioassays, or at least offers favorable physico-chemical properties needed for bioactivity.

Lead Optimization and Profiling

Leads display certain effects and properties of active drugs, but not with all of the necessary attributes. The missing properties are subsequently introduced in the lead optimization phase, a process that aims to transform an active lead compound into a drug candidate [55]. Lead optimization is a multi-parametric process aimed at simultaneously optimizing several features, such as on-target activity, bioactivity, and stability (Figure 1.4) [7, 56]. Therefore, lead optimization is highly complex, and generally takes the most time in drug discovery projects, largely because the necessary medicinal chemistry is a major bottleneck in the process [57].

The lead optimization workflow can be divided into different phases connected with decision points, any of which might bring an end to the project (Figure 1.4). During the first phase, mainly *in vitro* target assays are used to control the optimization progress, and the emphasis is on improving on-target activity; this is fundamental for achieving biological activity [7]. During this phase, several thousand derivatives might be synthesized, a situation made possible by the major advances in medicinal and combinatorial chemistry that have helped to increase both diversity and yields [58]. With such large-scale synthesis, a clear SAR for a compound class can be determined. In SAR studies, the lead compounds are typically divided into specific regions, after which each in turn is chemically modified. Figure 1.5 shows the results of a SAR experiment with anthelmintic

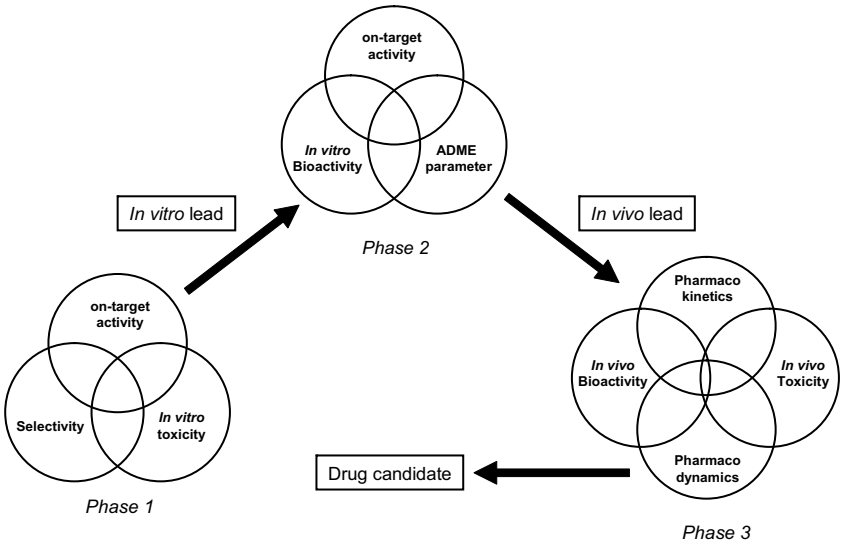


Figure 1.4 Multiparametric lead optimization. Starting with an *in vitro* lead, a chemical compound is optimized until it meets specific criteria defined for drug candidates. The process is multiparametric, and several sometimes conflicting requirements (e.g., high on-target activity, anti-parasite efficacy, low toxicity) must be accomplished simultaneously during the chemical optimization.

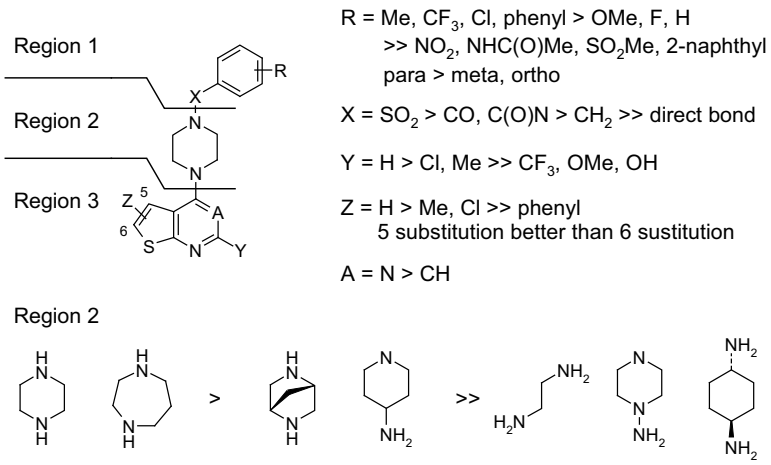


Figure 1.5 Structure–activity relationship (SAR) of thienopyrimidine analogs. The lead structure was divided into three regions, with all regions being chemically modified sequentially, varying only one motif at a time: first the aromatic head (region 1), followed by the central diamine linker (region 2), and finally the thienopyrimidine core (region 3). The anthelmintic potencies were determined in bioassays. This figure summarizes the nematocidal SARs of compound analogs. For example in region 1, substitution of the para position on the aromatic motif led to analogs with superior activity. Of these substituents, methyl, trifluoromethyl, chloride, and phenyl were observed to be the best.Reproduced with permission from Ref. [59]; © 2009, Wiley-VCH, Weinheim.

thienopyrimidine analogs, and demonstrates how different substitutions can affect nematocidal activity [59]. It is important to note that the lead optimization steps can be efficiently supported by chemoinformatic tools [60]; this is especially true if the protein structure of a target complex is available. Then, modern structure-based drug design methods can be applied to support rational lead optimization [61, 62]. Finally, it is essential to check the toxicity potential of interesting compounds in this first phase of lead optimization, usually by performing *in vitro* toxicity tests [63].

Yet, because on-target activity alone is not sufficient to achieve anti-parasitic activity, it is also essential to consider the biological activity and to ensure that the physico-chemical properties are within the desired range. This is achieved during the second phase of lead optimization, which focuses on monitoring biological activity using *in vitro* parasite models [13–15]. Critical to the biological activity, and thus to the success of any potential drugs, are the ADME parameters (this is an acronym for absorption, distribution, metabolism, and excretion) [64]. ADME deals with the disposition of a drug within organisms, with each of the four criteria having an important influence on the efficiency and pharmacological activity of a compound as a drug [65].

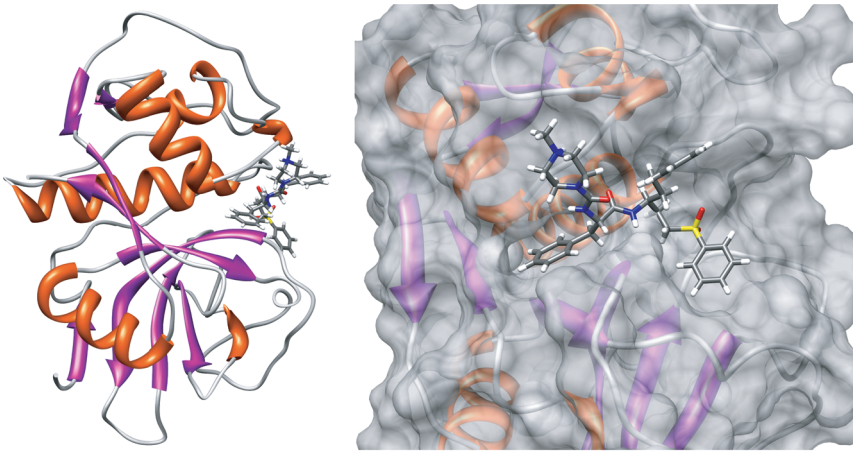
The third phase of lead optimization goes a step further, and includes animal models to assist in making the transition from *in vitro* assays to *in vivo* conditions [7]. For this, the compounds are profiled in model organisms such as mice or rats [66]. In

any animal model it is necessary to generate pharmacokinetic, pharmacodynamic, and toxicity profiles. Pharmacokinetics describes the time course of the drug in the body (namely, its ADME behavior [67]) while, in contrast, pharmacodynamics specifies the effect versus concentration relationship. In simple words, pharmacodynamics explores what a drug does to the body over time, whereas pharmacokinetics is the study of what the body does to the drug [68]. Often, the intricacies and expense of animal models limits the numbers of compounds that can be tested annually [7]. Following the successful completion of a lead optimization project, a compound is then considered to be a suitable drug candidate and transferred into a drug development program. In human health, a drug candidate has already been profiled in model organisms, whereas animal health research can go a step further and prepare a clinical profile in target animals.

Examples of Successful Target-Based, Anti-Parasitic Drug Discovery Programs

Proteases are validated as targets for therapy of a number of parasitic diseases, including malaria, leishmaniasis, African trypanosomiasis, and schistosomiasis [5, 25, 69, 70]. Several chemical structures have already been identified as protease inhibitor leads [1]; for example, promising inhibitor leads targeting the falcipain protease for the treatment of *P. falciparum* infection have been discovered by a collaboration between the pharmaceutical company GlaxoSmithKline plc and the University of California San Francisco, supported by the Medicines for Malaria Venture. Although these compounds are far along in the drug development process, their structures remain proprietary [69]. A related example of a target-based drug discovery workflow is the identification of the cysteine protease inhibitor, *N*-methyl-piperazine-phenylalanyl-homophenylalanyl-vinylsulfone-phenyl (K11777 or K777) as a small-molecule therapy of Chagas disease by targeting the parasite's cathepsin L-like cysteine protease, cruzain [24, 71]. The vinyl sulfone class of molecules was originally identified in the mid-1990s in a curtailed industrial drug discovery program (at Khepri Pharmaceuticals) to target bone loss, but the parent molecule of K777 (K11002 or K02) was subsequently transferred (including the intellectual property rights) to an anti-parasitic drug discovery program conducted at the University of California, San Francisco. Following the modification of K02 to improve its bioavailability, K777 was put through a standard development workflow which included on-target mechanism of action studies incorporating crystallography, both *in vitro* and *in vivo* anti-parasitic activity profiling (the latter in mice and dogs), and a suite of ADME and (acute and chronic) toxicity (studies in rodents and dogs). As of early 2010, a dossier is being prepared for filing K777 as an Investigational New Drug (IND) at the US Food and Drug Administration in advance of clinical trials in humans. A structure-guided medicinal chemistry program is also ongoing to identify "back-up" compounds (Figure 1.6) [72].

Another recent success story in the development of novel anti-parasitic drugs is the identification of a new class of anthelmintics, the amino-acetonitrile derivatives (AADs) [73]. In veterinary medicine, there is an urgent need for novel drugs against



(a) (b)

Figure 1.6 Ribbon (a) and surface (b) representations of the cysteine protease cruzain from *T. cruzei*, complexed with the inhibitor K11777 [72].

parasitic worms, as some nematodes have developed drug resistance against all available anthelmintic drugs; even worse, some multidrug-resistant worms have appeared [74, 75]. Thus, the development of the AADs, which were discovered in a physiological-based screen, proved to be most welcome [76]. Consequently, an extensive lead optimization program is ongoing which, to date, has resulted in over 600 compounds with different anthelmintic activities, both *in vitro* and *in vivo*, in different hosts [77]. Moreover, the compounds are effective against a wide range of livestock helminths, including several drug-resistant parasites [78]. This indicates a new mode of action for the AADs and, indeed, genetic experiments have shown that they act on unique, nematode-specific subunits of the acetylcholine receptors [79]. If the excellent pharmacokinetic properties and tolerability of the AADs in ruminants can be extended to humans, the class may offer an alternative anthelmintic for human medical practice [73].

Conclusion

The discovery of novel drugs for parasitic diseases is a high-risk, expensive, and lengthy process [22]. The past few years have seen increased financial and infrastructural support for drug discovery and development for parasitic diseases by academic institutes, PPPs, and the pharmaceutical industry [3, 5]. Already this has led to imaginative, comprehensive and dynamic drug discovery and development pipelines (even in the face of sometimes modest financial backing) when compared to those diseases directly impacting developed countries [1]. A closer look, however, at the portfolios of some of the PPPs reveals a plethora of early discovery projects for parasitic diseases that have yet to translate into late devel-

opment leads. The enormous expense and considerable expertise required to develop late leads and drug candidates (involving medicinal and combinatorial chemistry, and in-life animal studies such as A
DME and toxicology) remain major bottlenecks. This is especially true for academic institutions which, with their relatively finite resources, concentrate either on the biology or chemistry of the drug discovery workflow. Here, a closer collaboration between biology and chemistry groups may lead to an increased efficiency, and indeed a number of specialized academic centers have already arisen specifically focused on R&D for neglected diseases. Most importantly, the pharmaceutical industry, through a variety of internal and external R&D programs, has re-entered the business of drug development for infectious diseases. This is vital, given its decades-long know-how on furthering compounds through to the market. In summary, the continued collaboration between academic groups, PPPs, and the pharmaceutical industry is the key to optimizing R&D output and, eventually, the registration of novel and badly needed anti-parasitic drugs.

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