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Insights into Integrated Lead Generation and Target Identification in Malaria and Tuberculosis Drug Discovery

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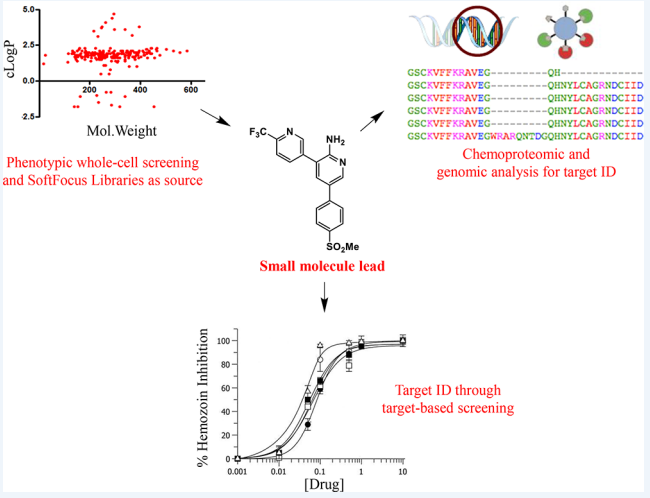
CONSPECTUS: New, safe and effective drugs are urgently needed to treat and control malaria and tuberculosis, which affect millions of people annually. However, financial return on investment in the poor settings where these diseases are mostly prevalent is very minimal to support market-driven drug discovery and development. Moreover, the imminent loss of therapeutic lifespan of existing therapies due to evolution and spread of drug resistance further compounds the urgency to identify novel effective drugs. However, the advent of new public−private partnerships focused on tropical diseases and the recent release of large data sets by pharmaceutical companies on antimalarial and antituberculosis compounds derived from phenotypic whole cell high throughput screening have spurred renewed interest and opened new frontiers in malaria and tuberculosis drug discovery.



This Account recaps the existing challenges facing antimalarial and antituberculosis drug discovery, including limitations associated with experimental animal models as well as biological complexities intrinsic to the causative pathogens. We enlist various highlights from a body of work within our research group aimed at identifying and characterizing new chemical leads, and navigating these challenges to contribute toward the global drug discovery and development pipeline in malaria and tuberculosis. We describe a catalogue of in-house efforts toward deriving safe and efficacious preclinical drug development candidates via cell-based medicinal chemistry optimization of phenotypic whole-cell medium and high throughput screening hits sourced from various small molecule chemical libraries. We also provide an appraisal of target-based screening, as invoked in our laboratory for mechanistic evaluation of the hits generated, with particular focus on the enzymes within the *de novo* pyrimidine biosynthetic and hemoglobin degradation pathways, the latter constituting a heme detoxification process and an associated cysteine protease-mediated hydrolysis of hemoglobin. We further expound on the recombinant enzyme assays, heme fractionation experiments, and genomic and chemoproteomic methods that we employed to identify *Plasmodium falciparum* falcipain 2 (*Pf*FP2), hemozoin formation, phosphatidylinositol 4-kinase (*Pf*PI4K) and *Mycobacterium tuberculosis* cytochrome *bc1* complex as the targets of the antimalarial chalcones, pyrido[1,2-*a*]benzimidazoles, aminopyridines, and antimycobacterial pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones, respectively.

In conclusion, we argue for the expansion of chemical space through exploitation of privileged natural product scaffolds and diversity-oriented synthesis, as well as the broadening of druggable spaces by exploiting available protein crystal structures, -omics data, and bioinformatics infrastructure to explore hitherto untargeted spaces like lipid metabolism and protein kinases in *P. falciparum*. Finally, we audit the merits of both target-based and whole-cell phenotypic screening in steering antimalarial and antituberculosis chemical matter toward populating drug discovery pipelines with new lead molecules.

1. INTRODUCTION

Malaria and tuberculosis (TB) remain global health problems. According to the latest World Health Organization estimates, ∼3.2 billion people are still at risk of malaria with 212 million new cases resulting in an approximate 429 000 deaths in 2015.[1] Similarly, ∼10.4 million incident cases of TB were reported in 2015, of which approximately 12% were co-infections with the human immunodeficiency virus (HIV). This led to about 1.4 million TB deaths and an additional 400 000 deaths resulting from TB disease among people living with HIV in 2015.[2]

The current first-line treatment strategies against malaria hinge on artemisinin-based combination therapies (ACTs), which comprise an artemisinin derivative, partnered with a

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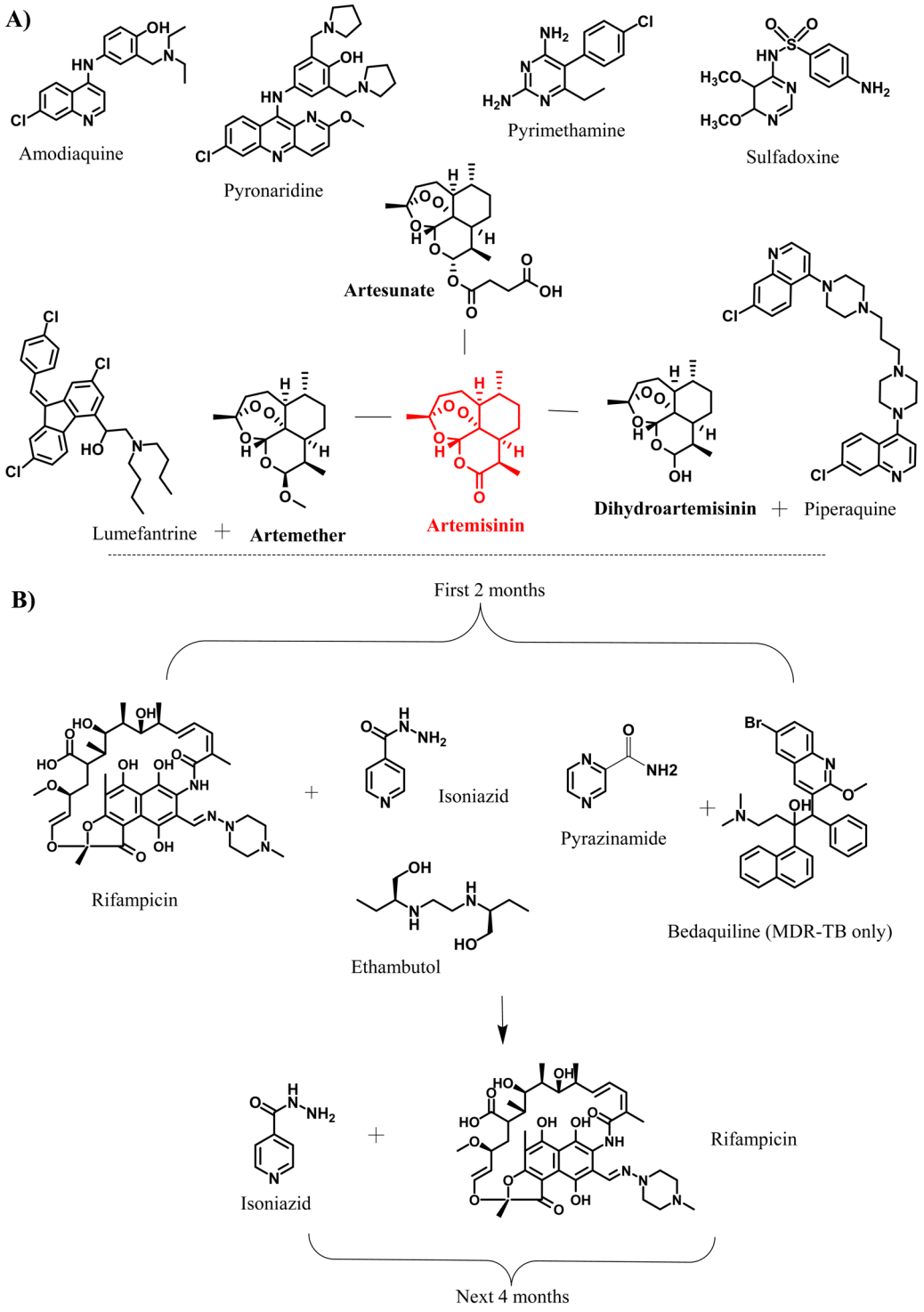

Figure 1. Recommended standard combination treatment for malaria (A) and tuberculosis (B).

longer-acting antimalarial (Figure 1A). While largely still effective, there are reports of emerging ACT-tolerance characterized by prolonged parasite clearance times,[3,4] hinting at imminent loss of therapeutic utility of ACTs. On the other hand, first-line treatment of drug-susceptible TB involves an initial phase of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) for the first 2 months, followed by a 4-month continuation phase of INH and RIF (Figure 1B). Against multidrug-resistant TB, bedaquiline (BDQ) is normally introduced to the standard treatment regimen and administered for a maximum of 6 months,[5] highlighting the cumbersome extensive treatment duration. As in malaria, the evolution and spread of drug resistance presents a major impediment to control efforts against TB, hence a pressing need for new, safe and effective drugs.

Over the last 10−15 years, a number of significant developments have stimulated ongoing efforts for new drugs against malaria and TB. These include the founding of new public−private partnerships focused on tropical diseases[6] and the release, by pharmaceutical companies, of large data sets on

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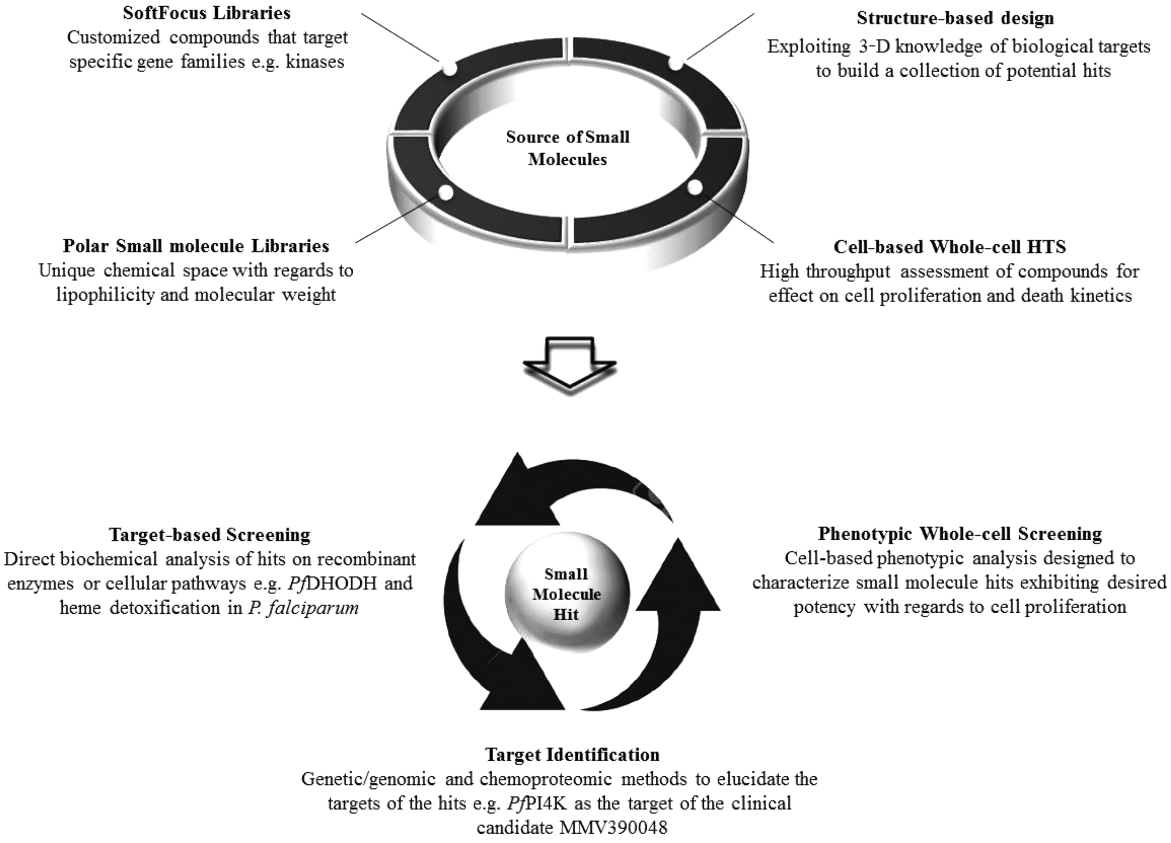

Figure 2. Breakdown of small molecule hit generation from source to target identification screening as explored within our laboratory.

antimalarial and anti-TB compounds derived from phenotypic high-throughput screening (HTS).[7−9] In addition, public−private partnership involvement in antiparasitic and antimycobacterial drug discovery by Medicines for Malaria Venture (MMV), Drugs for Neglected Diseases initiative, and the Global Alliance for TB Drug Development[6] have provided new impetus. In this Account, we appraise of the challenges facing antimalarial and anti-TB drug discovery initiatives and discuss the various strategies we have exploited toward contributing to new drugs against *Plasmodium falciparum* and *Mycobacterium tuberculosis*, the respective causative agents of malaria and TB. We conclude with a perspective on potential areas of future research in drug discovery against these pathogens.

2. CHALLENGES FACING MALARIA AND TB DRUG DISCOVERY

Antimalarial and anti-TB drug pipelines are inadequately populated due to the time-consuming nature of the process, high candidate attrition rates, insufficient financial investments, and other limitations intrinsic to the biological complexities of the pathogens. One key challenge relates to the dearth of suitable animal models to optimally assess compound activity and efficacy. In malaria, for instance, though murine parasites like *P. berghei* readily infect laboratory mice and are extensively utilized in early drug discovery projects, the species fundamentally differ from the human parasite and, as such, can present with dissimilar sensitivities to drugs tested. Moreover, biological disparities between humans and rodents make interpretation of the subsequent data speculative at best. Similarly in TB, although mice are readily infected by *M. tuberculosis*, the human disease pathology is not recapitulated in mice. Hence, the translational value of the mouse model is largely lost since the natural disease pathway between the two species is not conserved. This need for relevant in vivo models of drug efficacy, pharmacokinetics, and toxicology ushered the emergence of humanized mice, characteristically immunodeficient mice engrafted with human hematopoietic cells or tissues or transgenically expressing human genes,[10] as critical preclinical evaluation tools. Though these have contributed to a better understanding of disease pathways, they too are fraught with limitations. For instance, chimeric mice having both human and mouse liver tissues are likely to exhibit comparatively elevated extent of murine drug metabolism, which can confound analyses since mice have a higher metabolic rate. Additionally, extra-hepatic human-specific factors affecting drug metabolism or clearance cannot be identified in such chimeras. Lastly, since many aspects of mammalian biological systems, particularly immune systems, are species-specific, these models present limited value in the analysis of immune-mediated drug toxicities.

Second, the complex biology of the parasites presents a daunting challenge to the development of pan-active compounds against all parasite life cycle stages. The *P. falciparum* life cycle comprises intricate hepatic, asexual erythrocytic, sexual gametocytic, and vector host stages, while *M. tuberculosis* is characterized by two metabolically distinct growth states, an active replicative and a nonproliferative persistent one. This potentially obscures identification and characterization of druggable targets. Furthermore, the sketchy understanding of the pathogens' biology, partly attributable to incomplete annotation of their genomes, complicates drug discovery efforts since target-based screening is customarily contingent on successful ascription of biological function to targets and biochemical validation of their tractability.

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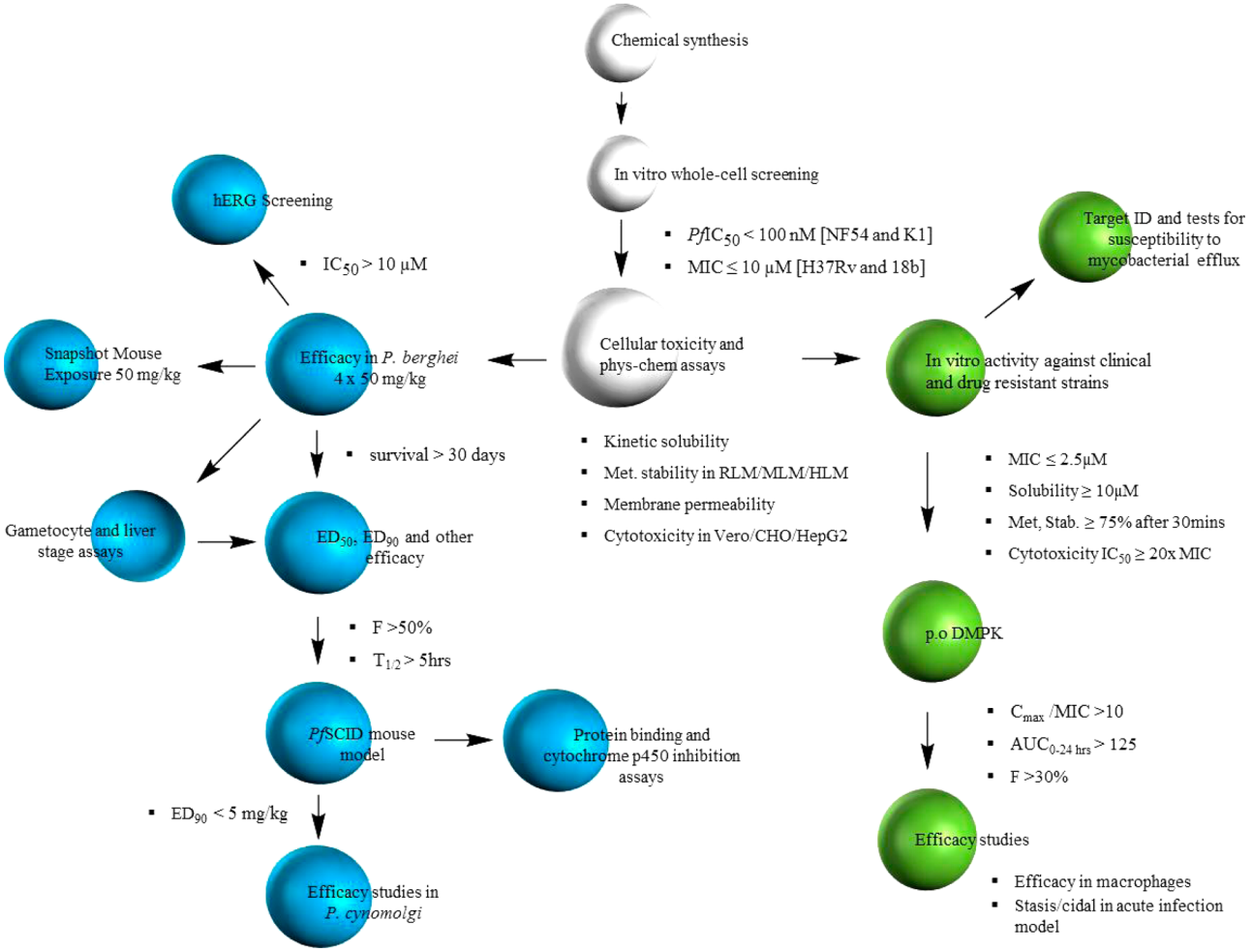

Figure 3. Hit to lead optimization screening cascade for malaria and TB chemical series highlighting *P. falciparum*- (aqua blue) and *M. tuberculosis*-specific (green) platforms. Assays or platforms common to both are indicated in gray. IC_{50} = half-maximal inhibitory concentration; MIC = minimum inhibitory concentration; NF54 and K1 = sensitive and resistant *P. falciparum* strains, respectively; H37Rv and 18b = replicating and nonreplicating *M. tuberculosis* strains; RLM/MLM/HLM = rat, mouse and human liver microsomes; CHO = Chinese hamster ovarian cells; hERG = human ether-a-go-go-related gene; ED = effective dose; *F* = bioavailability; SCID = severe combined immunodeficiency.

Another challenge involves the limited number of new chemotypes explored for clinical evaluation. Most new therapies in malaria, for example, are based on different combinations of known drugs or novel drugs based on known pharmacophores.[11] While undoubtedly effective, a higher risk of rapid loss of their useful therapeutic lifespan exists owing to the organisms' adaptation to drug pressure from prior use of their related scaffold(s). Indeed, the two pathogens are endowed with permissive genomes that can allow for polymorphisms in response to selective pressure and compensatory mechanisms that offset any subsequent loss of fitness from these mutations. All these challenges ultimately translate to poor rates of successful transitioning of drug candidates into clinical evaluation thus necessitating the need for a constant supply of novel biologically relevant chemical matter, defined as inhibitory molecules with desirable physicochemical traits and toxicity profiles that are amenable to clinical application.

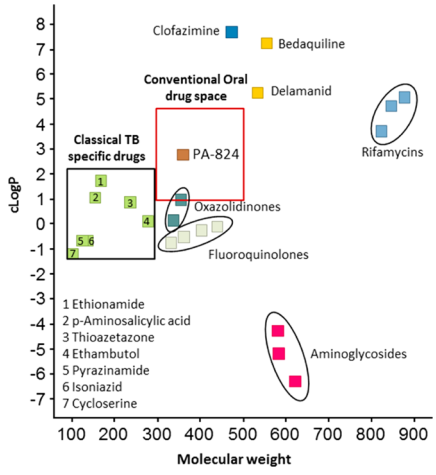

Figure 4. Plot showing the unique chemical space occupied by anti-TB compounds from low molecular weight polar libraries relative to the conventional oral drug space of classical TB drugs.

3. APPROACHES TO NOVEL ANTIMALARIAL AND ANTI-TB LEADS

Traditionally, target-directed and whole-cell phenotypic screenings represent two complementary methods of identifying viable new medicinal chemistry starting points. These approaches have recently been reviewed and contrasted within the context of antiparasitic[12−15] and antimycobacterial[16] drug discovery. This section attempts to flesh out both strategies as

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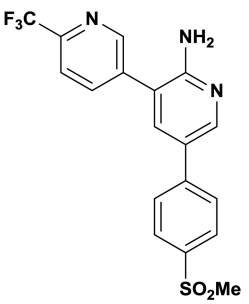
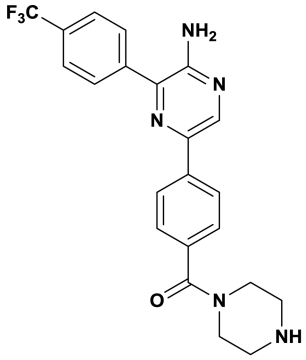


1



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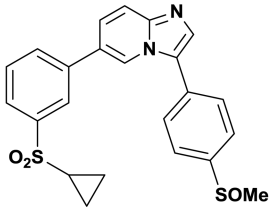
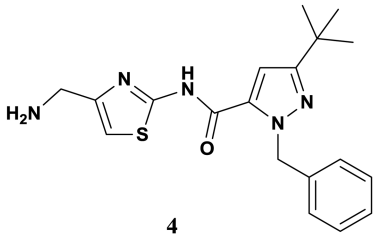


3

NF54 IC_{50} = 1.1 nM

In vivo *P. berghei* (po) at 4 x 50 mg/kg = 99.8%

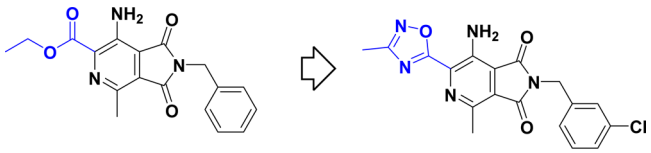
3/3 malaria-infected mice cured



4

In vivo *P. berghei* (po) at 4 x 50 mg/kg = 99.5%

Figure 5. Structures of compounds **1**, **2**, **3**, and **4**.



5 6

Figure 6. Structure of compound 5 and its derivative **6**.

pursued in our research group, specifically with regard to cell-based medicinal chemistry optimization of hits and attendant target identification efforts (Figure 2). The blueprint of our drug candidate identification approach espouses an integrated screening cascade for hit to lead optimization (Figure 3).

3.1. Cell-Based Phenotypic Whole-Cell HTS

Our cell-based phenotypic HTS screening design primarily comprises assessment of cell proliferation or death kinetics as end point, with a diverse SoftFocus library[17] against *P. falciparum*[15] and *M. tuberculosis*[18] and a low molecular weight (MW) polar library against *M. tuberculosis*[19] constituting the sources of our hits. Compared to standard empirical screening libraries, SoftFocus libraries offer the advantage of higher hit rates since compounds in these repositories are customized to be target-specific. On the other hand, libraries of small polar molecules in TB drug discovery offer the advantage of occupying a unique chemical space in terms of MW (<250 Da) and lipophilicity (clogP < 2.5), which is incidentally also occupied by classical TB drugs (Figure 4).

3.1.1. Whole-Cell Screening Hits and Identification of Putative Targets. While cell-based medicinal chemistry optimization has recently successfully delivered a number of preclinical antimalarial candidates, it has not to the same degree in TB where cell-based structure−activity relationship (SAR) exploration is more challenging. Thus, in TB drug discovery, it is important to frontload mechanism of action (MoA) studies through target identification in order to drive SAR exploration. The identification of putative targets of hit compounds discovered through phenotypic screening ideally needs to be conducted in parallel with hit optimization studies. In this regard, genetic and genomic approaches have largely been successful in target identification, especially in malaria.[20]

From a whole-cell image-based screening of a BioFocus DPI SoftFocus library of 35 000 compounds in collaboration with MMV, we identified 222 hits with >80% inhibition at an average primary and retest concentration of 1.82 μM against the sensitive (3D7) and multidrug resistant (Dd2) *P. falciparum* strains.[17] Further, cell-based medicinal optimization has led to the delivery of a clinical candidate, MMV390048 (**1**), a 2-aminopyridine,[21] and a preclinical development candidate, UCT943 (**2**), from the aminopyrazine class[22] (Figure 5). Our integrated screening cascade depicted in Figure 3 was critical in the selection and progression of these candidates and was propelled by the need to identify new chemical entities endowed with novel MoA and pan-activity against all parasite life cycle stages. The limitations of animal models were overcome in our utilization of the humanized *P. falciparum* SCID mouse model carrying the relevant human infection in the assessment of **1** and **2**. The SoftFocus libraries have also delivered other lead series. For instance, the sulfoxide-based imidazopyridazine analog **3**, arising from a prodrug-like strategy, was completely curative in the *P. berghei* mouse

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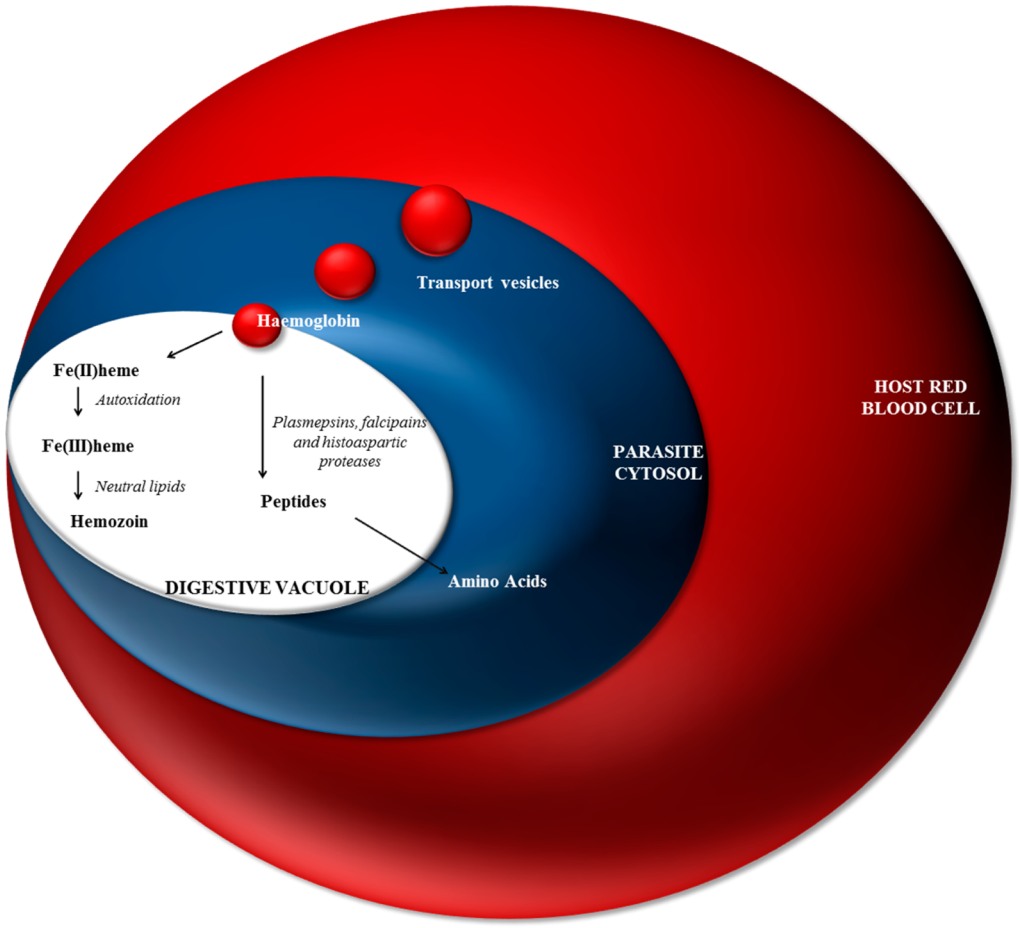

Figure 7. A schematic depiction of the hemoglobin degradation pathway, hemozoin formation and role of proteases in *P. falciparum* as described in section 3.2.1.

model at 4 × 50 mg/kg oral dose[23] as was the amino-methylthiazole pyrazole carboxamide lead, **4**[24] (Figure 5).

Employing genomic and chemoproteomic strategies, we recently identified *P. falciparum* phosphatidylinositol 4-kinase (*Pf*PI4K) as the target of **1**.[25] In this analysis, resistant mutants were generated through drug pressure using **1**, and whole-genome sequencing identified nucleotide polymorphisms in *Pf*PI4K, thus citing this protein as the potential target of and resistance determinant of **1**. Concurrent chemoproteomic mechanistic approaches using covalent immobilization on Sepharose beads and pull-down experiments to affinity-capture potential protein targets from blood stage extracts revealed **1** competitively and selectively inhibited *Pf*PI4K binding. A similar capturing experiment with kinobeads, which represent a combination of immobilized promiscuous ATP-competitive kinase inhibitors, showed *Pf*PI4K as the only *P. falciparum* protein that exhibited a dose-dependent reduction of bead binding upon addition of **1**. Furthermore, these chemoproteomic *Pf*PI4K competitive-binding data strongly correlated with antiparasitic activity. Since phosphatidylinositol 4-kinases are highly homologous across *P. falciparum* and *P. vivax* species,[26] a functional assay was conducted that also confirmed inhibition of recombinant *Pv*PI4K, thus corroborating the genomic and chemoproteomic data and affirming *Pf*PI4K as the target of **1**.

Similarly, from a whole-cell screening of a small polar library of ∼6000 compounds against *M. tuberculosis* by the Novartis Institute of Tropical Diseases, phthalimide-containing ester **5**, from the pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione series, was identified as a representative example for further hit validation (Figure 6).[19] This compound was subsequently transformed into early lead compound **6**, through optimization of potency and microsomal metabolic stability.[19]

Additionally, to identify the molecular target and potential MoA of pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones, we explored a genetic approach by attempting to raise spontaneous resistant mutants (SRMs) in the H37RvMa strain.[19] Although these mutant generation efforts were unsuccessful, the series was flagged as hyperactive against a cytochrome *bd* oxidase deletion mutant (Δ*cyd*KO) through routine hit triage, which involved screening representative compounds against known target mutants as a way to prioritize chemical series potentially acting via novel mechanisms. Supplementary analysis revealed that a Δ*cyd*KO derivative strain carrying an Ala317Thr point mutation in *qcrB* (encoding a subunit of the menaquinol cytochrome *c* oxidoreductase) was resistant to compounds in this series (MIC_{90} > 10). Considered in the light of the observed hypersensitivity of the Δ*cyd*KO deletion mutant to these compounds and the cross-resistance of the *qcrB*A317T-bearing Δ*cyd*KO variant, these observations therefore strongly hinted that these pyrrolo[3,4-*c*]pyridine-1,3(2H)-diones target the QcrB subunit of the cytochrome *bc1* complex, a validated target in *M. tuberculosis*.[19]

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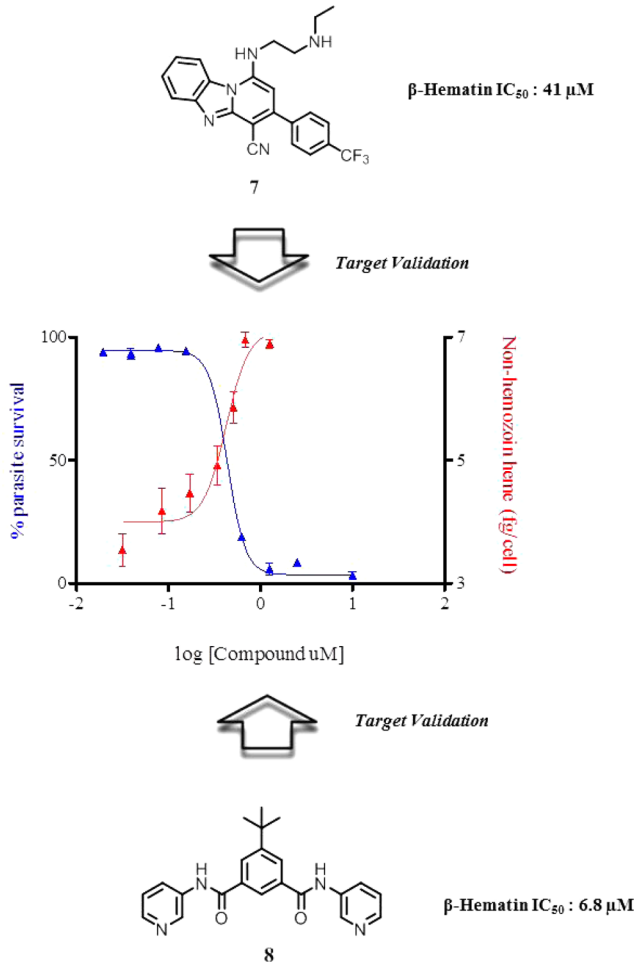

Figure 8. Parasite survival curve directly corresponding to a dose-dependent accumulation of toxic heme, validating inhibition of cellular hemozoin formation as target of pyrido[1,2-*a*]benzimidazole (**7**) and benzamide (**8**) hits against *P. falciparum*.

3.2. Target-Based Screening

Though effective in the identification of active compounds, an often-cited drawback of whole-cell phenotypic screening is the lack of clarity in the underlying MoA of the hits. Target-based screening navigates this pitfall by employing simple biochemical assays where specific binding or affinity of inhibitors to target is investigated in reactions that mimic and miniaturize real-time physiological events. In this regard, we have explored inhibition of such targets as the heme detoxification pathway, falcipain 2 (*Pf*FP2) and dihydroorotate dehydrogenase (*Pf*DHODH).

3.2.1. Hits from Target-Based Screening and Identification of Putative Targets. During the trophozoite stage of its life cycle, *P. falciparum* ingests ∼80% of host hemoglobin into its digestive vacuole (DV). As hemoglobin is degraded, its heme component is converted into hemozoin (Hz), and globin hydrolyzed to its constituent amino acids for protein synthesis. Hz is an inert crystalline form of ferriprotoporphyrin IX (Fe(III)PPIX), a cytotoxic by-product from the autoxidation of the released heme (Figure 7). Hz formation in *P. falciparum* represents an attractive antimalarial drug target since it is unique to the parasite, critical to its survival, accessible to drugs, and not genome-coded (hence immutable). Expectedly, its inhibition through canonical aminoquinolines and other heme-targeting chemotypes has therefore been of wide interest in *P. falciparum* and other hematophagous organisms.[27] The crystal structure of the Fe(III)PPIX−halofantrine complex has revealed that coordination to the iron(III) center of the heme monomer, $\pi-\pi$ stacking, and hydrogen bonding constitute the key interactions between inhibitors and Fe(III)-PPIX.[28] We have employed a pyridine-based cell-free system that mirrors the lipid-mediated process in the DV by substituting neutral lipids with the lipophilic Nonidet P-40 detergent to assess how different scaffolds inhibit β-hematin (abiotic Hz) formation[29−31] and used this as surrogate for Hz inhibition in the parasite. To further validate if the hits in the lipid-based assay are indeed bonafide inhibitors of Hz formation, a within-cell fractionation assay that examines the effect of β-hematin inhibitors on the fate of total heme in the parasite when ring stage cultures are treated with increasing doses was utilized. In true inhibitors, this is typified by a dose-dependent signature of decreasing Hz fraction matched by a corresponding increase in toxic free heme and hemoglobin.[32] We have recently validated this screen in non-quinoline based scaffolds to illustrate the Hz-inhibiting potential of pyrido[1,2-*a*]benzimidazoles (**7**)[33] and benzamides (**8**)[34] (Figure 8).

Since hemoglobin hydrolysis is the result of a concerted process involving multiple catalytic proteases, including aspartic, metallo-, and cysteine proteases[35] (Figure 7), plasmodial cysteine proteases involved in hemoglobin catabolism have also been interesting antimalarial drug targets due to the role of hemoglobin degradation as an intraerythrocytic source of nutrition and space. The parasite cysteine protease falcipain 2, *Pf*FP2, is a papain family cysteine protease and a crucial hemoglobinase whose inhibition leads to parasite death, presumably due to blockage of hemoglobin hydrolysis.[36] Studies to examine the activity of chalcones,[30] thiazolidinediones,[37] thiosemicarbazones,[38] and 4-aminoquinoline isatin derivatives[39] against *Pf*FP2 have been explored in our laboratories.

Our target-based screens have also included analysis of the parasite dihydroorotate dehydrogenase, *Pf*DHODH, which catalyzes the rate-limiting step of *de novo* pyrimidine biosynthesis (Figure 9). Pyrimidine is an essential constituent of nucleic acids and requisite in protein glycosylation, membrane lipid biosynthesis, and strand-break repair.[40] The absence of a pyrimidine-salvage pathway in *P. falciparum* suggests an absolute parasite reliance on *de novo* synthesis, consequently leading to the validity of *Pf*DHODH as target for antiplasmodial small molecules.[41] Using 3D quantitative SAR pharmacophore models, molecular docking, and enzyme inhibition experiments on *Pf*DHODH, we recently identified new inhibitors of this enzyme based on a dihydrothiophenone scaffold.[42] Since the crystal structures of *Pf*DHODH in complex with diverse inhibitors have been determined,[43,44] insights into the structural basis for inhibition by such newly identified small molecules can therefore be understood.

As a caveat, however, the application of target-based screening is limited since not all targets can be purified or prepared in a manner suitable for biochemical evaluation. Additionally, signals from biochemical assays can only capture the physiological landscape involved in intracellular drug activity and tissue-specific responses to a limited degree of fidelity. In fact, it is not uncommon for target-based screening hits to completely lack whole-cell activity or kill through an entirely unrelated target.[45] Relatably, target-based identification of new leads against *M. tuberculosis* has been largely unsuccessful as most hits are inactive in vivo due to either

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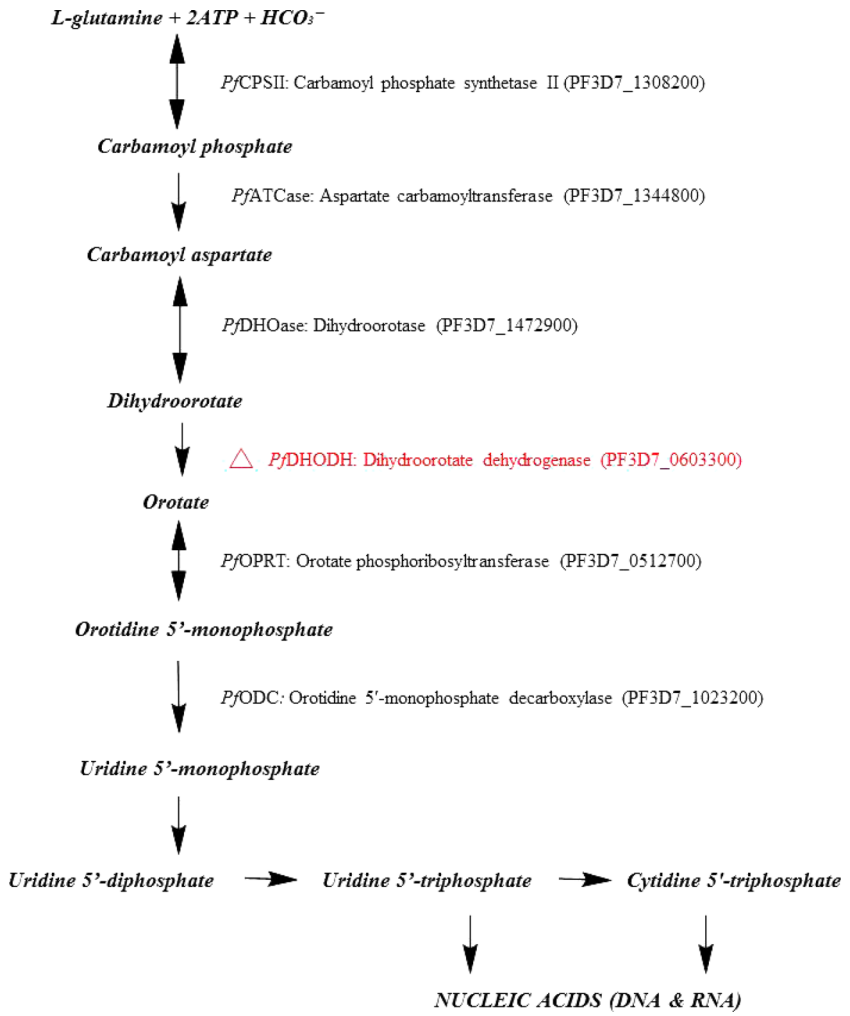

Figure 9. Schematic view of the sequential enzymatic reactions for *de novo* pyrimidine biosynthesis in *P. falciparum* with selective inhibition of *Pf*DHODH (red) able to block the necessary transfer of electrons into the second half of the pathway.

lack of cellular permeation or functional redundancy of target during infection.

4. CONCLUSION AND FUTURE PROSPECTS

Significant milestones have been achieved toward overcoming some of the aforementioned challenges associated with limitations of animal models. Of particular note is the development of diverse strains of immunodeficient mice on the mutant *Il2rg* platform, in the appreciation that no single specific model suffices in addressing the myriad of drug efficacy queries. Indeed, the successful transitioning of our candidate drug **1** into clinical testing had hinged on the successful exploitation of the humanized SCID mouse model carrying the relevant human infection.[25] Nonetheless, discourse on other considerations is noteworthy. First, most current screening libraries comprise a limited number of chemical scaffolds; an expansion of the antimalarial and anti-TB chemical spaces with novel, biologically relevant chemical matter seldom addressed in conventional screening collections is therefore imperative. One approach involves more intensive research on natural products whose inherent bioactivity confers them high affinity, specificity, relatively favorable pharmacokinetic properties, and hydrophilicity.[46] More library assemblies predicated on natural product privileged structures, like indoles, have the potential to widen the chemical space in malaria and TB drug discovery. Chemical space expansion could also invoke the concept of diversity-oriented synthesis, which employs divergent synthetic steps where the product of one complexity-generating transformation is a substrate in the next, subsequently affording structures with increased scaffold complexity better suited to probe broader biological spaces.[47]

Correspondingly, expansion of the druggable space is worth considering. In practice, a target is regarded truly validated when its inhibitor is used in the clinic for treating human disease. The product profile to build such targets would therefore comprise essentiality for organism viability in humans, complete cidality at low-concentration inhibition, and amenability to inhibition by small molecules having the appropriate physiochemical properties. Despite the completion of the *P. falciparum* and *M. tuberculosis* genomes and a growing understanding of the organisms' biological landscape, few targets have been clinically validated. For instance, only tubulin β-chain, adenosine diaminase, dihydrofolate reductase−thymidylate synthase, topoisomerase I, dihydroorotate dehydrogenase, flavoprotein subunit of succinate dehydrogenase, and inosine-5′-monophosphate dehydrogenase code for currently validated drug targets among the ∼5300 protein-encoding genes in *P. falciparum*. Similarly, only nine (cmaA1, cyp51, embA, embB, embC, folK, InhA, katG, and rpoC) of the 3999 proteins encoded by *M. tuberculosis* have been pharmaceutically

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investigated.[48] This paucity in druggable targets invites research into expanding the targetable biological space in *M. tuberculosis* and *P. falciparum*. Some of the spaces as yet untargeted by known antimalarials include lipid metabolism, which is almost nonfunctional in uninfected erythrocytes and thus unique to *P. falciparum*. Protein kinases also constitute an appealing target class due to the well-established binding of small molecules to their catalytic clefts and the indispensability of protein phosphorylation in regulating parasite physiology. The availability of genomic/proteomic data, protein crystal structures, bioinformatics infrastructure, and protein networks as well as modeling programs have tremendously facilitated target prediction in both pathogens. However, caution must be urged on predictive analyses to guard against overestimation of the possible number of drug targets the parasite genomes could encode. For instance, using a computational algorithm that identified enzymes that catalyze "chokepoint" reactions, only ∼200 *P. falciparum* genes (4%) were estimated to encode potentially suitable targets, and among these, only ∼30 shared no significant homology with any human enzyme.[49] Absence of significant homology is, however, not an absolute requisite for target identification; since a single amino acid change can theoretically confer selectivity, much as generate high level resistance as illustrated by bumped kinase inhibitors whose "bumps" offer selectivity by precluding their binding to almost all mammalian kinases, which characteristically have bulky gatekeeper residues in their ATP-binding pockets.[50]

Finally, the success of phenotypic whole-cell screening in steering antimalarial and anti-TB drug discovery in recent years is noteworthy. This approach has recently yielded thousands of antiplasmodial and anti-TB hits that have proven to be useful starting points for medicinal chemistry optimization.[7−9] Moreover, whole-cell screening on ∼20 000 diverse small molecules from the Broad Institute collection against *M. tuberculosis* recently revealed only slight overlap in molecules effective against *M. tuberculosis* and related species,[51] thus highlighting the utility of direct screening against the organism. However, the approach is not without shortcomings. First, the parasite species, strain, and life cycle stage must be clinically relevant, and in vitro maintenance of the biological cultures must be able to produce sufficient quantities for the phenotype needed for HTS. Second, considerations must be made on the choice of the best-suited assay platforms, their optimization, and the interpretation of signals obtained. Lastly, lead optimization efforts are unlikely to ride on a single-target-inhibition hypothesis as there may be multiple targets, and thus the need for more than one assay to guard against potential off-target-related compound activities. Nonetheless, these override the hallmark demerit of target-based approaches, which is the discordance between inhibition of recombinant systems and translation into whole-cell activity or clinical efficacy. In conclusion, advances in plasmodial and mycobacterial genetics, chemical biology, -omics, and other techniques now provide a platform that permits the merits of both approaches to be effectively harnessed to populate the antimalarial and anti-TB drug pipelines with high-quality leads.

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Notes

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