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Australian chemistry and drug discovery towards the development of antimalarials†

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ABSTRACT

Malaria, a disease caused by the *Plasmodium* parasite, accounts for more than 450 000 deaths annually. The devastating impact of this disease is compounded by the emergence or risk of widespread resistance to current antimalarial drugs, underscoring the need to develop new therapies. Australian scientists are at the forefront of fundamental, clinical and surveillance research, and have made significant contributions to advancing the field of malaria research. A significant component of this research has been directed toward the development of new antimalarial therapies. This perspective summarises the recent endeavours by Australian researchers in chemistry and drug discovery sciences in the identification and development of new antimalarial therapies in the global challenge to treat and eliminate malaria.

Keywords: antimalarial, Australian chemistry, Australian drug discovery, drug development, high throughput screening, malaria, parasitology, pharmacology, *Plasmodium*.

Introduction

Half of the world's population is at risk of malaria infection. In humans, malaria is a disease caused by five species of *Plasmodium* parasite. *P. falciparum* is the deadliest and most prevalent in sub-Saharan Africa, while *P. vivax* and *P. knowlesi* are endemic to Southeast Asia and the Americas. *P. malariae* and *P. ovale* are geographically widespread but have significantly lower prevalence and result in mild clinical manifestations. Approximately 241 million people are infected by *Plasmodium* with over 627,000 deaths worldwide in 2020.[1] Malaria continues to represent a major global health challenge.

The *Plasmodium* parasite is transmitted to the human host by way of a bite from an infected *Anopheles* mosquito. Sporozoites are injected into the circulatory system until they traverse and invade a liver hepatocyte. In the liver stage or pre-erythrocytic stage, the parasite develops into a schizont releasing many merozoites into systemic circulation where they invade red blood cells initiating the asexual stage. In the asexual blood stage, multiple rounds of self-replication occur leading to erythrocyte and the symptomatic signs of disease, such as lethargy and ague. On occasions, a sexual form of the parasite known as a gametocyte develops in a host erythrocyte over 14 days. Mature gametocytes are then ingested by a mosquito upon a blood meal and immediately mature into male and female gametes inside the mosquito midgut. The fertilised gametes then develop into ookinetes that invade the midgut wall and form an oocyst on the exterior of the midgut. The mature oocyst then produces ookinetes that traverse to the salivary glands of the mosquito for transmission to another human host. The multiplex lifecycle of the malaria parasite makes studying and developing new antimalarial therapies a challenging undertaking. It also opens opportunities to target different or multiple stages of the lifecycle to develop preventative, curative and transmission blocking therapies.

Current efforts to combat malaria have concentrated on mosquito control measures, the deployment of vaccines and combination drug therapies. Preventative measures are

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aimed at controlling transmission of the disease by the mosquito. The implementation of insecticide-treated bed nets has limited the prevalence of infection; however, this has not completely curbed the spread of Malaria. Recently the first malaria vaccine RTS, S (Mosquirix) was approved. While this is a major achievement, this vaccine only offers modest protection.[2] Chemo-prophylactic treatments such as doxycycline and atovaquone/proguanil (Malarone) are important in controlling the disease in malaria-endemic regions and preventing the disease in travelers visiting these countries.

Current therapies for treating malaria consist of combinations of quinolines or endoperoxides. The World Health Organisation no-longer recommends the use of the non-artemisinin combination therapies, consisting of sulfadoxine-pyrimethamine, chloroquine, mefloquine and amodiaquine due to widespread resistance to these therapies. Artemisinin combination therapies (ACTs) are now the frontline therapy to treat malaria. Concerningly, there are recent reports of emerging resistance to ACTs in South-East Asia[3] and more recently in sub-Saharan Africa.[4] Reports of malaria parasites developing resistance to combination drug treatments stresses the need to identify novel therapies to combat malaria infection. Therefore, there is an urgent need to develop drug candidates that have novel chemotypes and mechanisms of action and that target multiple stages of the parasite lifecycle aligned with treatment, prophylaxis or elimination target candidate profiles.[5]

Only recently has significant benevolent funding been available to develop new treatments for malaria. These initiatives have allowed industry and academia to contribute significant in-kind infrastructure and research support that has enabled the identification, mainly through phenotypic high throughput screening, and the development of novel antimalarial chemotypes to populate pre-clinical and clinical pipelines. The most notable of the new chemical chemotypes now in clinical trials are OZ439 **1**, an endoperoxide,[6] DSM-265 **2** a dihydroorotate dehydrogenase (DHODH) inhibitor, KAF156 **3** likely a SEC61 inhibitor, MMV048 **4** a phosphatidylinositol 4-kinase (PI4K) inhibitor and KAE609 **5** a PfATP4 inhibitor (Fig. 1).[7] Concerningly, resistance has been observed with several candidates in human clinical trials, and with the natural attrition rate of candidates when progressing through the clinic, there is a continued need to populate the drug discovery pipeline with novel chemotypes for the treatment and elimination of malaria.

Australian biomedical research has been central to the fundamental understanding of biological mechanisms and pathways important for parasite development across the lifecycle. A recent review by Doolan gave a historical perspective and highlighted the groundbreaking scientific breakthroughs by Australian scientists in malaria research.[8] The advances in parasitology, pathology and epidemiology of the malaria parasite have influenced the decisions by international governing organisations on the implementation and changes in policies, and agendas in malaria control, treatment and eradication. Furthermore, the research by Australian scientists has led to advances in new technologies that have enabled the study and biological evaluation of antimalarials across the entire parasite lifecycle and has catalysed small molecule and biologic drug discovery initiatives in Australia and across the globe. This appraisal seeks to highlight chemistry and drug discovery endeavours by Australian scientists over the last 15 years that have contributed to the identification and development of new antimalarials.

Biological evaluation

Screening for antimalarials

A call to arms at the start of the twentieth centenary brought together industry and academia to enable phenotypic screening of large compound libraries against the malaria parasite. This undertaking has led to the mass screening of natural product, vendor and industry small molecule libraries sampling a large diversity of chemical space.

The Avery group at Griffith University are world leaders in antimalarial high throughput screening techniques and has established multiple platforms to enable the screening of large compound libraries against two different stages of the malaria parasite lifecycle. One assay allows the evaluation of compounds against the asexual stage parasite and drug resistant forms of the asexual stage parasite.[9] This assay platform has been used to screen multiple compound libraries and has uncovered several new chemotypes for further optimisation.[10–13] Another assay technology using an imaging-based platform was developed to evaluate compounds against both early and late sexual gametocytes.[14,15] This technology also culminated in a new method to produce sufficient numbers of gametocytes to screen large libraries of compounds[16] and is now universally applied in laboratories worldwide. Both assays have been used to support the screening of boutique compound libraries, such as Medicines for Malaria Venture (MMV) 'Box' sets, which include the 'Malaria Box' and 'Pathogen Box'[17–20] and to support medicinal chemistry efforts in numerous antimalarial development campaigns (many cited throughout this review). More recently, the Screening Facility at the Walter and Eliza Hall Institute (WEHI) led by Dr Lowes has adapted an existing *P. falciparum* lactate dehydrogenase assay format to screen compound libraries and to support medicinal chemistry efforts in industry and academic partnerships (also cited throughout this review).

Understanding the mechanism by which antimalarials kill *Plasmodium* parasites is important for their development as it allows an improved understanding of potential resistance mechanisms, of the pharmacodynamic and pharmacokinetic relationship, and in turn safety, and, in some cases, enables structure-based optimisation. Target identification is usually undertaken by forward genetic studies, but several mechanistic-based screening assays have also been

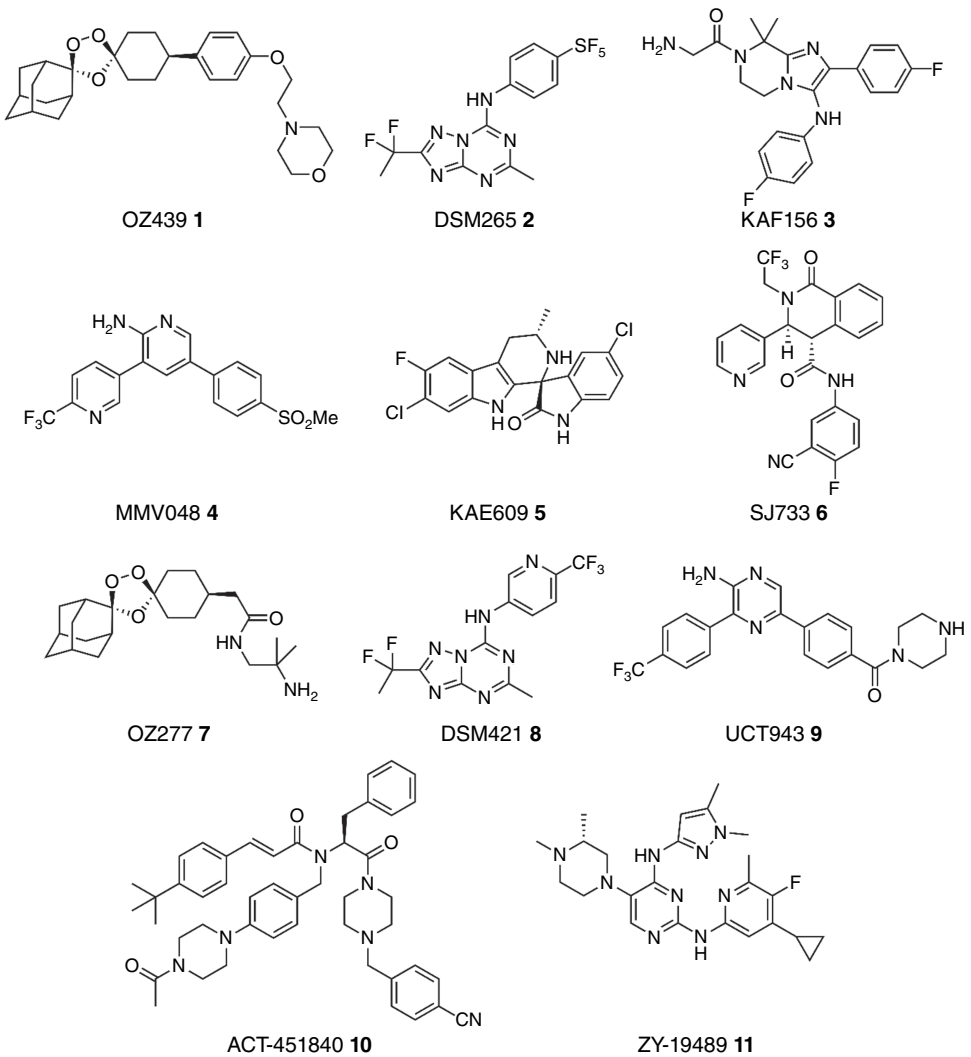


Fig. 1. Structures of recently developed antimalarials in clinic trials.

developed to identify pathway specific compounds. Typically, these are secondary or low throughput assays used to evaluate and characterise small boutique compound sets with known antimalarial or antiparasitic activities but have an unknown mechanism of action.

The Gilson/Crabb group at the Burnet Institute developed a luciferase-reporter assay to assess the ability of compounds to block the export of parasite proteins to the host red blood cell.[21] Assays were also established to identify compounds that inhibit asexual parasite invasion and egress from the host red blood cell,[22] and to uncover compounds that inhibit the new permeability pathway[23] – indispensable for nutrient exchange in the host red blood cell. Several compounds from MMV 'Box' sets were independently identified that block these pathways and will be important tools for unravelling new molecular targets central to parasite survival and that can become starting points for antimalarial optimisation.

The Kirk/Lehane groups at the Australian National University developed an assay to screen for compounds that lead to an increase in Na^+ levels and pH in the parasite cytosol as a characteristic marker for PfATP4 inhibition.[24] PfATP4 is a plasma membrane P-type ATPase essential for controlling parasite cytosolic levels of Na^+ and in turn pH. The assay was important for characterising the PfATP4 activity in the development of clinical candidates KAE609 **5** and SJ733 **6** (Fig. 1).[24–26] The assay was also used to screen the MMV Malaria Box and uncovered 28 compounds with diverse scaffolds that inhibited PfATP4,[27,28] including derivatives of the clinical candidates KAE609 **5** and SJ733 **6**. This assay was also used to uncover several new chemotypes in the MMV Pathogen Box that inhibit PfATP4.[29] The Kirk/Lehane groups also developed an assay and screened the MMV Malaria Box to find compounds that affect transporters responsible for a decrease in cytosolic pH.[30] The screen found 15 compounds that affected parasite cytosolic

pH, and subsequently determined by forward genetic studies that two compounds exert their effects through inhibition of *P. falciparum* formate nitrite transporter (PfFNT). This research led to the characterisation of the PfFNT transporter as a druggable antimalarial target.[31]

The Andrews/Poulson groups at Griffith University developed an assay where parasites were cultured under hypoxic or reduced bicarbonate conditions as an indicator of *P. falciparum* carbonic anhydrase activity. This assay was applied in a screen of the MMV Malaria Box and identified a set of quinoline-like compounds.[19] It was hypothesised that although the activity of these quinoline compounds was affected by both hypoxic and reduced bicarbonate conditions, the mechanism of action was likely independent of carbonic anhydrase. The McFadden group have a longstanding interest in dissecting pathways of apicoplast targeted antimalarials. They (and others[32]) have been able to show that isopentenyl pyrophosphate (IPP) biosynthesis is the sole function of the apicoplast. As part of this research, an IPP supplementation assay was employed to characterise the on-target apicoplast activity of many slow-acting antimalarials.[33]

Target-based approaches have also been applied to interrogate the on-target activities of antimalarials and library compounds against proteins important for parasite survival. The McGowan group and colleagues at Monash University deployed biochemical assays to measure compound inhibition of *P. falciparum* metallo-aminopeptidases M1, M17 and M18. Two compounds in the Malaria Box were found to moderately inhibit both PfA-M1 and PfA-M17,[34] although it was suspected that these peptidases are not the primary targets responsible for parasite activity of these compounds. The Wilks/Lucet group recombinantly expressed thirteen *P. falciparum* kinases, PfCDPK1, PfPK6, PfCK2, PfGSK3, PfCLK1, Pfmap2, PfPK7, PfNek2, PfNek4, FIKK4.2, FIKK10.2, PfCK1 and PfPK5, considered important for parasite development.[35] A thermal shift assay and ADP-Glo luciferase assay was used to assess compounds from the Malaria Box against each of these kinases. The screen found three compounds that showed modest inhibition of PfGSK3, PfPK6 and PfPK5,[19] that did not correlate with parasite activity implying these kinases were not the primary targets of these compounds. The Cowman/Sleebs groups at WEHI established a high throughput screening assay to identify binders of the ligand reticulocyte binding protein homolog 5 (Rh5) that is indispensable for parasite invasion of the host red blood cell.[36] The assay was used to screen a known drug library and the Malaria Box and found that the leukotriene inhibitor pranlukast inhibited the interaction with Rh5 with its host cell ligand basigin. Further characterisation of pranlukast and pranlukast analogues revealed Rh5 inhibitory activity did not robustly correlate with blocking parasite invasion or killing the parasite.

The Quinn group at the Eskitis Institute implemented a target-based approach using native mass spectrometry to screen a library of natural product-based fragments to find binders of high priority antimalarial drug targets. Remarkedly, 69 parasite proteins were recombinantly expressed in soluble forms amenable to native mass spectrometry detection.[37] Overall, 96 fragments were found to differentially bind to 32 of the parasite proteins, of which 79 fragments displayed modest asexual parasite activity. Native mass spectrometry was also applied to *P. falciparum* dUTPase to screen the same fragment library.[38] The screen found several securinine natural product derivatives that inhibited the catalytic activity of PfdUTPase and showed modest activity against stage V gametocytes.

Collectively, the screen of boutique compound sets using target-based and pathway-based assays have uncovered several starting points for further investigation. Even though the target is implied in a target-based strategy, further characterisation and target deconvolution of compounds is generally required to ensure the parasite activity observed is on target. Overall, the target-based screening assays developed provide a launch pad to screen larger compound libraries to uncover new chemotypes to feed the antimalarial drug discovery pipeline.

Antimalarial target identification

Forward genetic and chemo-proteomic methods are typically employed in the target deconvolution of antimalarials (examples provided in the chemistry section below). Metabolomic methods have also been useful in revealing details of pathway aberrations by antimalarials. The McConville, Creek and Ralph groups independently and collaboratively have established metabolomic methods[39] and determined metabolomic signatures of frontline antimalarials.[40–44] The contributions by these groups are numerous, many of which are integrated into studies mentioned in this review. One notable collaborative example was the metabolomic analysis of 96 compounds from the Malaria Box.[45] This study unveiled that a significant proportion of the 96 compounds had metabolome fingerprints consistent with that of artemisinin, quinolines, PfATP4, DHODH or isoprenoid biosynthesis inhibition, providing key information on the likely mechanism of action of these antimalarial starting points.

ADME and pharmacokinetic evaluation of antimalarials

A key component of antimalarial development is the evaluation of ADME and pharmacokinetic properties. The Charman group at Monash University has been instrumental in providing expertise and key data for many antimalarial programs across the globe, and in the development of antimalarial property guidelines.[46] The exceptional contribution to antimalarial research by the Charman group is too expansive to completely cover in this review (many examples are referenced in the chemistry section). One notable example was a program focused on the development of a synthetic ozonide

scaffold to overcome the short half-life and supply demands of the artemisinin derivatives. Several iterations around the ozonide scaffold led to the lead compound OZ277 **7** (Fig. 1) which was characterised by an improved pharmacokinetic profile compared to artesunate.[47] Further critical feedback on ADME and PK culminated in the development of OZ439 **1** with a significantly prolonged half-life in pre-clinical models[6,48] enabling human dose modelling for development in clinical trials.[49] Other notable contributions were to the pre-clinical development of the DHODH inhibitors DSM265 **2**[50] and DSM421 **8**,[51] the PI4K inhibitors MMV048 **4**[52] and UCT943 **9**[53] and the PfATP4 inhibitor SJ733 **6** (Fig. 1).[25]

Clinical evaluation of antimalarials

Clinical development of antimalarials in Australia has largely been driven by Edstein and colleagues at the Australian Defence Force Malaria and Infectious Disease Institute since the mid-1980s. Additionally, they have provided key *in vitro* antimalarial data for many early-stage programs many of which are mentioned in the chemistry section. The extensive works of Edstein and colleagues are too numerous to comprehensively cover in this appraisal but have contributed to pharmacokinetic/pharmacodynamic analyses in the pre-clinical and clinical development of many antimalarial therapies that are used in the field today. McCarthy and colleagues at QIMR Berghofer Medical Research Institute (now at the Doherty Institute and WEHI) have also been instrumental in developing and conducting controlled blood-stage and gametocyte challenge models in human clinical trials. Most notably these have been used to evaluate antimalarials recently developed, including artefenomel (OZ439) **1**,[54,55] cipargamin (KAE609) **5**,[56] DSM265 **2**,[57,58] MMV048 **4**,[59,60] SJ733 **6**,[61] Actelion-451840 **10**[62] and ZY-19489 **11**[63] (Fig. 1).

Chemistry

Natural product antimalarials

Historically, natural products have been an important component of antimalarial research. Key examples are the discovery of quinine, the impetus for quinoline drugs, and artemisinin. Several natural product scaffolds originating from Australian research have provided novel starting points for antimalarial development.

The Quinn and Davis groups at Griffith University have been at the forefront of curating and screening a library of natural isolates and products against the malaria parasite. This has led to the discovery of natural products with interesting antimalarial properties. A bispyrroloiminoquinone alkaloid, tsitsikammamine C **12** (Fig. 2), isolated from an Australian marine sponge showed potent activity against the *P. falciparum* parasite (EC_{50} 13 nM) and efficacy in a *P. berghei* mouse model of malaria.[64] In another study, a screen of the Eskitis Institute Nature Bank library against the *P. falciparum* parasite uncovered the natural products alstonine **13** and himbeline **14** (Fig. 2) with EC_{50}s of 0.17 and 0.58 μM, respectively.[65] Another example was a serrulatane diterpenoid **15** (Fig. 2) isolated from the Australian desert plant *Eremophila microtheca*. Synthetic amide derivatives of this diterpenoid scaffold were shown to have modest antimalarial activity (EC_{50}s 1.25–5.65 μM).[66] Further screening of the natural product isolate library identified

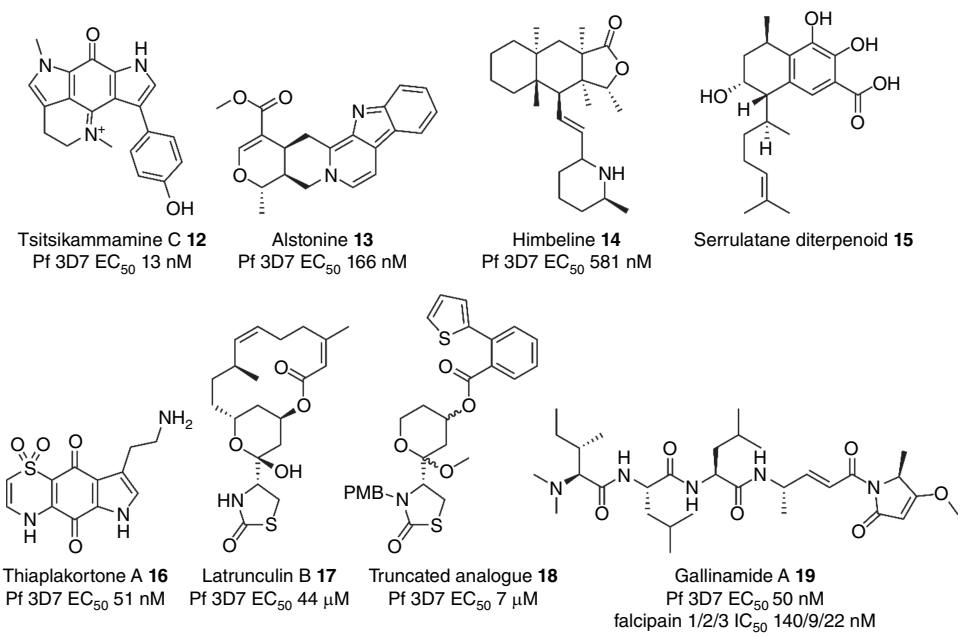


Fig. 2. Structures of natural products and their antimalarial activity.

the thiazine-derived alkaloids, thiaplakortones A–D, from the Australian marine sponge *Plakortislita*.[67] Thiaplakortone A **16** (Fig. 2) was the most potent (EC_{50} 51 nM) against *P. falciparum*. Subsequent synthesis of thiaplakortone A and its derivatisation led to analogues with improved metabolic stability and efficacy in a *P. berghei* mouse model.[68,69]

The Baell group at Monash University synthesised truncated derivatives (**18**) of the natural alkaloid latrunculin B **17** (Fig. 2) isolated from Red Sea marine sponge and showed they have modest activity against the malaria parasite (EC_{50} 7 μM).[70] The latrunculin scaffold was shown to reduce parasite motility through disruption of actin dynamics. The Payne group at the University of Sydney completed the total synthesis and stereochemical assignment of gallinamide A **19** (Fig. 2),[71,72] a depsipeptide isolated from the cyanobacterium *Schizothrix*. Gallinamide A **19** and synthetic analogues were found to potently inhibit parasite cysteine proteases, falcipain 2 and 3, which are critical for the processing of haemoglobin in the parasite digestive vacuole providing sustenance for the developing asexual parasite.[73] Accordingly, gallinamide A **19** was shown to cause swelling of the food vacuole in the parasite, a characteristic of falcipain inhibition, and potently killed the *P. falciparum* parasite (EC_{50} 50 nM). Overall, these natural products provide unique scaffolds as tools to investigate new malaria biology or starting points for antimalarial development.

Antimalarials discovered via phenotypic screening

Commonly, starting points for antimalarials are discovered by phenotypic screens of small molecule libraries on *P. falciparum*. The Baell group identified MIPS-0004373 **20** (Fig. 3) with a bis-3-alkylthio-1,2,4-triazine scaffold originating from a screen of an internal library, that showed fast-acting and potent activity against *P. falciparum* asexual stage parasites (EC_{50} 8 nM).[74] Subsequently, MIPS-0004373 **20** was shown to have potent activity against sexual stage gametocytes and gametes and to potently inhibited liver stage development.[75] MIPS-0004373 **20** was characterised by low metabolic stability and a short half-life in mice, but remarkably showed high efficacy (ED_{50} 1.5 mg kg^{-1}) by oral administration in a *P. berghei* mouse model.[76] The metabolic stability of the bis-triazine series was improved by replacing the liable thioether groups. The lead compound **21** was shown to maintain parasite potency *in vitro* (EC_{50} 31 nM) and efficacy in a *P. falciparum* humanised SCID mouse model.[77] MIPS-0004373 **20** has a high barrier to resistance and further investigation is ongoing to pinpoint the mechanism of action.

The Todd group at the University of Sydney (now located at University College London) initiated an Open Source Drug Discovery program whereby any laboratory from across the globe could collaboratively contribute chemistry

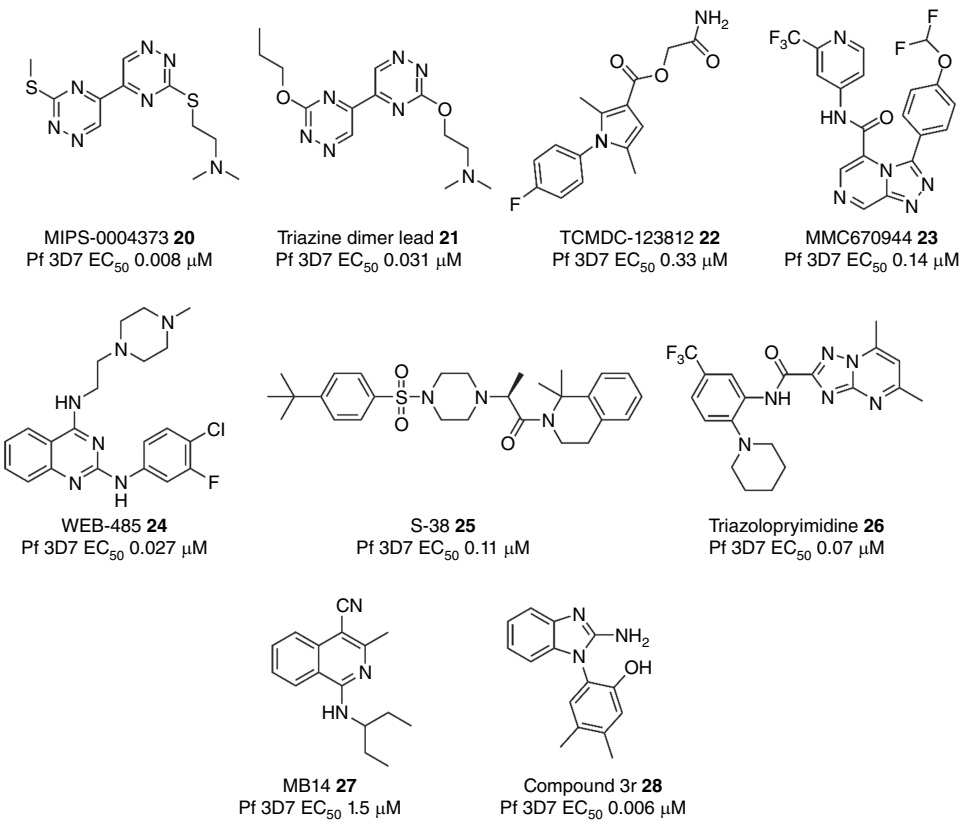


Fig. 3. Structures of compounds discovered from phenotypic screens or via a repurposing exercise, and their antimalarial activity.

and biological resources and expertise towards the optimisation of selected antimalarial scaffolds. One starting point with a pyrrole scaffold (**22**) (Fig. 3) that originated from a GSK Tres Cantos antimalarial screen showed potent asexual activity and gametocyte activity.[78] A concerted and creative effort by the consortium replaced certain structural motifs on the scaffold that were viewed as chemical liabilities. It was found that these changes were largely detrimental to parasite activity and eventually the series was 'parked'. Other compound scaffolds were also investigated as part of the Open Source consortium, including the triazolopyridine series that targets PfATP4.[79] Optimised compounds from this series such as **23** (Fig. 3) have potent asexual parasite activity (EC_{50} 0.14 nM) and oral efficacy in a *P. falciparum* humanised SCID mouse model. Laboratories from across the globe are continuing to work collaboratively on the optimisation of several other scaffolds.

The Sleebs/Gilson groups also initiated a program that started with a hit scaffold from the GSK Tres Cantos screen. Optimisation of the potency and ADME properties of the 2,4-amino quinazoline scaffold to produce the frontrunner compound WEB-485 **24** (Fig. 3) that has potent asexual antimalarial activity (EC_{50} 0.027 μM) and modest efficacy in a *P. berghei* and *P. falciparum* mouse models.[80,81] The future challenge of this series is to overcome the dose-limiting *in vivo* toxicity.[82] Target deconvolution studies on this series are ongoing to assist in mitigating toxicity. The Sleebs/Gilson groups continue to work on scaffolds identified from parasite red blood cell invasion and export screens on boutique small molecule libraries (mentioned in the Screening section).[21,22] One example was the phenylsulfonyl piperazine scaffold that was optimised to give frontrunner compound *S*-38 **25** (Fig. 3) with an IC_{50} of 0.11 μM. However, the restrictive SAR negated improvement in the *in vitro* metabolism.[83] Therefore, in the future, this series will be used as a tool to investigate the mechanism responsible for the invasion phenotype. The Sleebs/Cowman groups at WEHI screened the Janssen Jumpstarter small molecule library and identified several hits with novel chemotypes, including the triazolopyrimidine hit scaffold.[84] The optimisation of this scaffold led to the triazolopyrimidine **26** (Fig. 3) with potent slow-acting antimalarial activity (EC_{50} 0.07 μM). Future work will focus on correcting the metabolic stability of this scaffold which remains a challenge.

The Abbott group at La Trobe University was able to repurpose a human protein kinase A scaffold as a starting point to target kinases in *P. falciparum*.[85] Optimisation of the 4-cyano-3-methylisoquinoline class produced the frontrunner compound MB14 **27** with modest parasite activity (EC_{50} 1.5 μM) (Fig. 3).[86] It was subsequently deduced from forward genetic and chemo-proteomic studies that the optimised scaffold killed the malaria parasite by targeting PfATP4.[87] The Scammells/Norton group at Monash University also used a similar strategy whereby they started with a benzimidazole scaffold identified from a fragment screen against the important parasite invasion ligand AMA1.[88] Optimisation of the benzimidazole scaffold against the *P. falciparum* parasite gave rise to the lead compound 3r **28** (Fig. 3) with potent activity (EC_{50} 0.006 μM) and good physicochemical properties. It was found that this compound series acts through a mechanism independent from AMA1 and is the focus of future research.[89]

Target based antimalarials

Phenotypic approaches are the most common avenue to develop antimalarials, but target-based screening strategies have also produced starting points for antimalarial development, including the clinical candidate DSM265 **2**.[50] The target-based approach is heavily reliant on using reverse genetics to determine the essentiality of the target for parasite survival and in turn its validity for antimalarial drug discovery. The Guddat group at the University of Queensland has a longstanding program targeting the hypoxanthine-guanine-[xanthine]-phosphoribosyltransferase (HG[X]PRT), protein essential for the function of the parasite nucleoside salvage pathway. This group has used substrate mimetics at the core of their program which has been facilitated by multiple X-ray structures of HGXPRT enabling structure-based optimisation.[90,91,92–94] This program has generated several mimetic scaffolds such as **29** (Fig. 4) with potent biochemical inhibition of both PfHGXPRT and PvHGPRT (K_is 0.15–72 μM).[95] The challenge with these mimetics is the requirement for a polar phosphate (or acidic) group limiting membrane permeability. To overcome this challenge, the team has employed several prodrugs (**30**) to enhance membrane permeability and achieve modest parasite activity (EC_{50}s 2.5–12.1 μM).

A target-based approach was implemented to discover inhibitors of the metallo-aminopeptidases, PfA-M1 and PfA-M17.[96] McGowan and colleagues at Monash University initiated this program by obtaining biological data to show the importance of both proteases for parasite development.[97–99] X-Ray structural data of both proteases in complex with known peptidomimetic inhibitors of aminopeptidases initiated a structure-guided design program.[100,101] The Scammells/McGowan groups started with a phosphonic arginine mimetic **31** (Fig. 4) that has potent and selective inhibition of PfA-M17 (K_i 0.011 μM).[102] The phosphonic acid was then replaced with a hydroxamic acid group and optimisation of the P'_1 and P_1 positions led to a potent dual inhibitor **32** (Fig. 4) of PfA-M1 and PfA-M17 (K_i 0.27 and 0.10 μM) and potent *P. falciparum* activity (EC_{50} 0.015 μM).[103,104] Furthermore, the lead compound **32** has selectivity against human metalloproteases and robust metabolic stability, stimulating the next phase of development.

The Andrews/Ryan groups, while investigating a strategy to block the metabolism of proguanil **33** to cycloguanil **34**, a DHFR inhibitor, designed the derivative tBuPG **35** (Fig. 4). Unlike proguanil and cycloguanil, the potent slow-acting

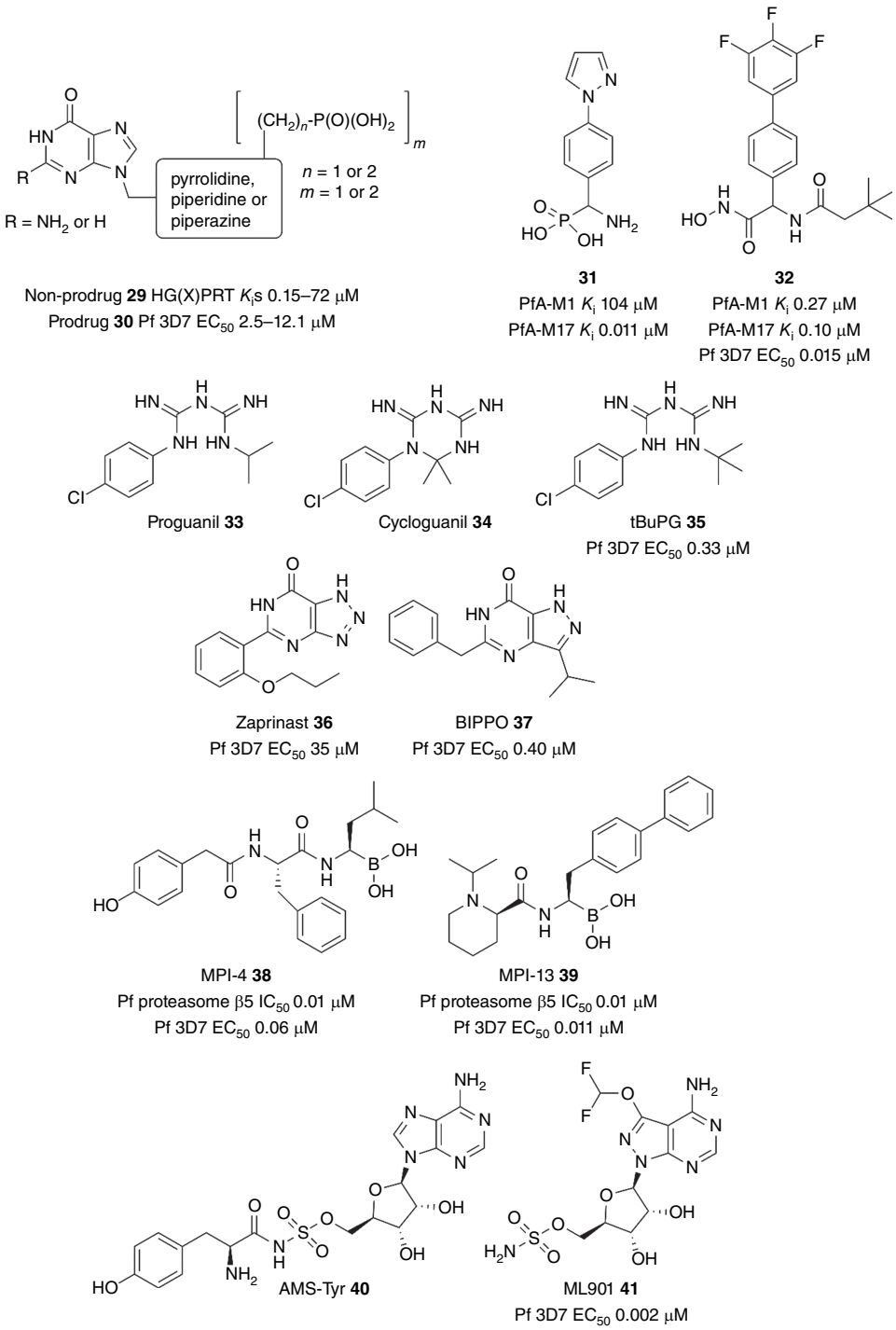


Fig. 4. Structures of compounds developed employing target-based strategies, and their antimalarial activity.

parasite activity of tBuPG **35** (72 h IC_{50} 0.33 μM; 96 h IC_{50} 0.05 μM) was independent of folate and isoprenoid biosynthesis.[105] It was found that the parasite activity of tBuPG **35** in combination with cytochrome bc1 inhibitors atovaquone and ELQ300 was synergistic and as a result tBuPG **35** is under investigation as a potential replacement of proguanil as the partner agent with atovaquone in the antimalarial therapy Malarone.

The Gilson/Tonkin groups uncovered the role of cyclic-AMP and cyclic-GMP in activating protein kinase A (PKA) and protein kinase G (PKG) in parasite invasion and egress of the host RBC. 3′-5′-Cyclic nucleotide phosphodiesterases (PDEs) are key regulators of this process by hydrolysing cyclic-AMP and cyclic-GMP and stalling invasion and egress. The Thompson group used structural modelling of human and *P. falciparum* PDE to repurpose and fine-tune the

parasite potency of the known human PDE inhibitor, zaprinast **36** (Fig. 4).[106] This strategy produced the compound, BIPPO **37** (Fig. 4), which was 90-fold more potent than zaprinast against the *P. falciparum* parasite (EC_{50} 0.40 μM). In a mechanism-dependent manner, BIPPO was shown to affect the premature egress of merozoites from the asexual schizont and to prevent the capacity of the immature merozoites to invade the host erythrocyte. BIPPO serves as an important tool in the investigation of processes that underpin parasite invasion and egress.

The Tilley group's interest in targeting the parasite proteasome was sparked by their discovery that artemisinin resistance is overcome by inhibiting the proteolytic function of the proteasome.[107] To unearth novel inhibitors of the proteasome, the Takeda library of peptide boronates was screened against *P. falciparum*. The hit compounds were further triaged by screening against the Pf20S and human 20S proteasome subunits. Among the hit compounds, MPI-4 **38** (Fig. 4) was shown to have potent inhibition of the β1, β2 and β5 subunits of the proteasome (IC_{50} 0.6, 0.06 and 0.01 μM, respectively) but equally potent inhibition of the human constitutive proteasome and immunoproteasome.[108] MPI-4 **38** potently killed the malaria parasite (EC_{50} 0.06 μM), but also reduced mammalian cell viability with similar potency, underlining the challenge of selectively targeting the parasite proteasome. CryoEM structures were then obtained of the *P. falciparum* proteasome β5 subunit in complex with peptide boronates that provided insight into optimising selectivity. Optimisation produced the frontrunner compound MPI-13 **39** (Fig. 4) with 19-fold selectivity against the *P. falciparum* versus human proteasome β5 subunit (IC_{50} 0.012 versus 0.23 μM), and 84-fold selectivity against the parasite versus mammalian cells (IC_{50} 0.011 versus 0.93 μM).[109] MPI-13 **39** showed robust efficacy in a *P. falciparum* humanised SCID mouse model and activity against male gamete and liver schizont development demonstrating that the proteasome is a multi-stage antimalarial drug target.

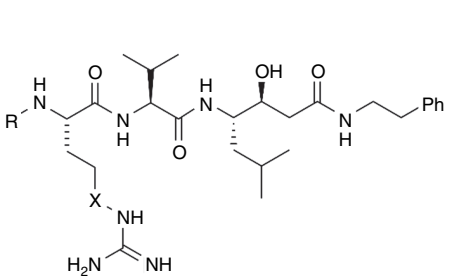
More recently Tilley and colleagues initially explored the application of adenosine 5′-sulfamate (AMS) as a substrate mimetic of adenosine 5′-monophosphate and as a potential modality to block protein synthesis by way of targeting parasite aminoacyl tRNA synthetases (aaRS).[110] Proteomic studies of AMS treated parasites primarily detected the AMS-Tyr adduct **40** (Fig. 4) signifying that AMS commandeered the mechanistic function and subsequently selective inhibition of TyrRS. To discover compounds with improved parasite specificity a screen of the Takeda library of nucleoside sulfamates identified ML901 **41** (Fig. 4) with potent antimalarial activity (EC_{50} 0.002 μM) and 5000-fold selectivity against mammalian cells. Forward genetic, chemo-proteomic and structural biology methods were then employed to show that ML901 **41** was a selective inhibitor of TyrRS. ML901 **41** was shown to potently inhibit both gamete and liver stage development and has efficacy in a *P. falciparum* humanised SCID mouse model, demonstrating that TyrRS is an attractive multi-stage antimalarial drug target.

The Cowman/Boddey groups researching protein trafficking by the malaria parasite within the host erythrocyte discovered that the aspartyl protease plasmepsin V is necessary for processing of the *Plasmodium* export element (PEXEL) on the N-terminus of parasite proteins exported to host RBC.[111] This fundamental finding led Cowman/Boddey/Sleebs to develop peptidomimetic inhibitors that mimic the PEXEL motif and inhibit plasmepsin V. The peptidomimetics initially designed including WEHI-916 **42** (Fig. 5) helped define the substrate specificity of plasmepsin V, show the requirement of plasmepsin V in protein export and pharmacologically validate plasmepsin V as an antimalarial drug target.[112,113] X-Ray structures of these peptidomimetics in complex with plasmepsin V engendered an in-depth understanding of substrate recognition and inhibition of the protease, enabling the design of the peptidomimetics WEHI-842 **43** and WEHI-601 **44** (Fig. 5) with improved biochemical inhibition of plasmepsin V (IC_{50} 0.019 and 0.005 μM, respectively) and parasite activity (EC_{50} 0.43 and 0.09 μM, respectively).[114–117] These peptidomimetics were then employed to demonstrate the importance of plasmepsin V and protein export in gametocyte maturation establishing plasmepsin V as a multi-stage antimalarial target.[118]

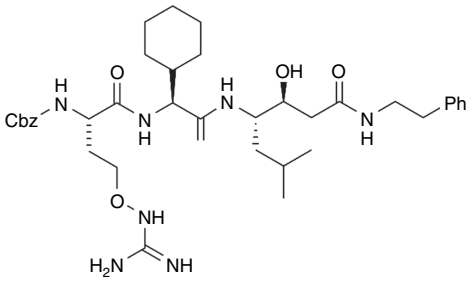
In search for novel drug-like plasmepsin inhibitors, Cowman screened the MSD (Merck and Co.) library of aspartyl protease inhibitors and identified the imino pyrimidinone hit WM5 **45** (Fig. 5) with potent antimalarial activity (EC_{50} 0.01 μM).[119] Forward genetic and chemoproteomic studies identified plasmepsin X as the molecular target. Optimisation of the imino pyrimidinone scaffold by large teams at both MSD, Wuxi and WEHI with the support of Wellcome led to the compound WM382 **46** (Fig. 5) that had potent asexual stage activity (EC_{50} 0.5 nM). Further phenotypic and chemo-proteomic experiments showed that WM382 **46** potently inhibited both plasmepsin IX and X (IC_{50} 0.51 and 0.035 nM).[119,120] WM382 **46** was shown to block transmission of the parasite to the mosquito and was orally efficacious in asexual and liver stage mouse models, demonstrating that dual inhibition of plasmepsin IX and X is an attractive strategy to develop antimalarials.

Conclusion

Malaria was declared eradicated from Australia in 1981, however, there are still approximately 700–800 cases of infection each year, mostly arising from overseas-acquired malaria. Despite the low incidence of malaria, Australia has an important responsibility to support malaria elimination programs in endemic countries in the neighbouring region such as Papua New Guinea, the Solomon Islands and Vanuatu, and more broadly South-East Asia. In addition to nearby regions, the fundamental malaria research and

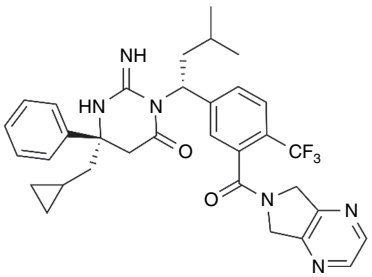


R = SO_2CH_2Ph, X = CH_2 WEHI–916 **42**
Plasmepsin V IC_{50} 0.12 μM
Pf 3D7 EC_{50} 4.3 μM

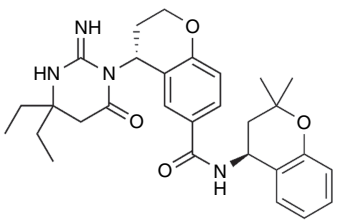


WEHI-601 **44**
Plasmepsin V IC_{50} 0.005 μM
Pf 3D7 EC_{50} 0.09 μM

R = Cbz, X = O WEHI-842 **43**
Plasmepsin V IC_{50} 0.019 μM
Pf 3D7 EC_{50} 0.43 μM



WM5 **45**
Plasmepsin X IC_{50} 1.9 nM
Pf 3D7 EC_{50} 10 nM



WM382 **46**
Plasmepsin X IC_{50} 0.51 nM
Plasmepsin X IC_{50} 0.035 nM
Pf 3D7 EC_{50} 0.51 nM

Fig. 5. Structures of compounds targeting plasmepsins, and their antimalarial activity.

antimalarial development undertaken in Australia and by Australian researchers overseas has contributed considerably to the worldwide effort to treat and eliminate the disease in malaria-endemic regions across the globe. The research outlined in this review further highlights that Australia's chemistry and drug discovery sector is world-class and that continued collaborative efforts and new partnerships with Australian organisations will add significant value to antimalarial development and support the global effort to treat and eliminate malaria.

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Biography

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