

REVIEW

Aminopeptidase Inhibition in Drug-Resistant *Plasmodium falciparum*: Structural, Functional, and Pharmacological Rationale for Targeting PfA-M1 and PfA-M17

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Correspondence: Sharoen Yu Ming Lim (lymsharoen@unimas.my)**Received:** 2 March 2026 | **Revised:** 2 July 2026 | **Accepted:** 4 July 2026**Keywords:** aminopeptidases | antimalarial drug targets | dual-target inhibition | malaria | PfA-M1 | PfA-M17

ABSTRACT

Malaria remains a significant global health burden, increasingly threatened by the emergence of artemisinin-resistant *Plasmodium falciparum* strains that compromise the efficacy of frontline combination therapies. Hemoglobin digestion constitutes a central metabolic pathway sustaining intraerythrocytic parasite growth, in which the terminal processing of hemoglobin-derived peptides and liberation of free amino acids are mediated by two zinc-dependent metalloaminopeptidases, PfA-M1 (M1 alanyl aminopeptidase) and PfA-M17 (M17 leucyl aminopeptidase). This review synthesizes current evidence supporting PfA-M17 and PfA-M1 as validated antimalarial targets, with particular emphasis on the structural biology, catalytic regulation, and cellular essentiality of PfA-M17. We discuss how the oligomerization-dependent activation of PfA-M17, governed by metal-ion-mediated quaternary assembly and a dynamic regulatory loop (L13), imposes structural constraints that may limit resistance evolution. We further evaluate selective and dual-target inhibitors, including MMV1557817 and compound 26, that demonstrate cross-species antiparasmodial efficacy with substantial fitness costs on resistant parasites. The mechanistic interplay between aminopeptidase inhibition and established antimalarial drug classes, including quinolines and artemisinins, is also examined. This review supports dual PfA-M1/PfA-M17 inhibition as a promising approach for next-generation antimalarial drug development.

1 | Introduction

Malaria continues to pose a significant global health threat in 2024, with an estimated 282 million cases and 610,000 deaths reported in 80 countries, predominantly in the African region that was home to 95% (265 million) cases [1]. The efficacy of existing antimalarial therapies, including artemisinin-based combination therapies (ACTs), is increasingly compromised by the emergence and spread of drug-resistant *Plasmodium falciparum* (*P. falciparum*) strains, highlighting an urgent need for novel therapeutic strategies that circumvent existing resistance mechanisms [2]. A promising approach to antimalarial drug development involves targeting parasite-specific enzymes essential for nutrient acquisition and survival within erythrocytes. Hemoglobin digestion represents a central metabolic pathway in *P. falciparum*,

providing amino acids required for protein synthesis, parasite growth, and the maintenance of intracellular osmotic balance [3–5]. Within this pathway, metalloaminopeptidases, particularly *P. falciparum* M1 alanyl aminopeptidase (PfA-M1) and M17 leucyl aminopeptidase (PfA-M17), play pivotal roles in the terminal stages of peptide processing, sequentially cleaving hemoglobin-derived peptides to release free amino acids [6, 7]. Hemoglobin degradation in *P. falciparum* is a highly coordinated proteolytic cascade involving multiple enzyme classes, including vacuolar endopeptidases such as plasmepsins and falcipains, followed by downstream exopeptidases responsible for peptide trimming and amino acid liberation. This pathway has been extensively characterized through biochemical and genetic studies over several decades (e.g., Goldberg laboratory; Rosenthal and colleagues),

establishing it as a central metabolic vulnerability of the parasite and a validated target for antimalarial intervention.

PfA-M1 and PfA-M17 function as cytosolic exopeptidases involved in terminal peptide processing downstream of vacuolar hemoglobin degradation. Although they share overlapping substrate specificity, they differ in regulatory control, oligomeric state, and relative contribution to amino acid homeostasis [8, 9]. Both enzymes exhibit substrate specificity and structural divergence from their human homologs, providing opportunities for selective inhibitor design with minimal host toxicity [10, 11]. During the intraerythrocytic stage, *P. falciparum* relies heavily on host hemoglobin degradation to obtain amino acids required for parasite growth and replication. Hemoglobin catabolism occurs within the acidic food vacuole through a coordinated proteolytic cascade involving several classes of proteases. Initial cleavage of hemoglobin is mediated primarily by aspartic proteases (plasmepsins I–IV) and cysteine proteases (falcipains), generating large peptide fragments. These intermediates are further processed by additional proteases, including falcilysin, into shorter oligopeptides that are subsequently hydrolyzed by the aminopeptidases PfA-M1 and PfA-M17 to release free amino acids for parasite metabolism (Figure 1).

Over the past decade, multiple lines of evidence have validated PfA-M1 and PfA-M17 as essential and druggable targets. Genetic approaches, including conditional knockdowns and overexpression studies, have revealed stage-specific growth defects and impaired parasite development upon enzyme perturbation

[8, 12]. Complementary chemical biology studies employing selective and dual inhibitors have further demonstrated that disruption of aminopeptidase activity results in potent antiplasmodial effects, accumulation of hemoglobin-derived peptides, and sensitization to other antimalarials [13, 14]. Structural studies, including high-resolution X-ray crystallography and cryo-EM, have elucidated key active site features, metal coordination requirements, and regulatory loops that govern oligomerization and enzyme activity, informing rational inhibitor design [9, 15]. Importantly, PfA-M1 and PfA-M17 differ in their redundancy and intracellular roles. Whereas selective PfA-M1 inhibition is often sufficient to impair parasite growth, PfA-M17 provides a structurally constrained metabolic node whose inhibition confers significant vulnerability but may require dual-target strategies for maximal efficacy [8, 14]. These insights have spurred the development of both selective inhibitors, such as T5, and dual-target compounds, including MMV1557817 and compound 26, highlighting the potential for next-generation antimalarial agents that exploit parasite-specific aminopeptidase biology [13, 16].

Hemoglobin digestion has long been recognized as a major therapeutic vulnerability in *P. falciparum* and has been the subject of extensive antimalarial drug-discovery efforts spanning several decades. Early studies focused primarily on digestive vacuole endoproteases, particularly the aspartic proteases plasmepsins I–IV and the cysteine proteases falcipains, which initiate hemoglobin cleavage within the acidic food vacuole [17–20]. Numerous potent inhibitors of these enzymes demonstrated compelling

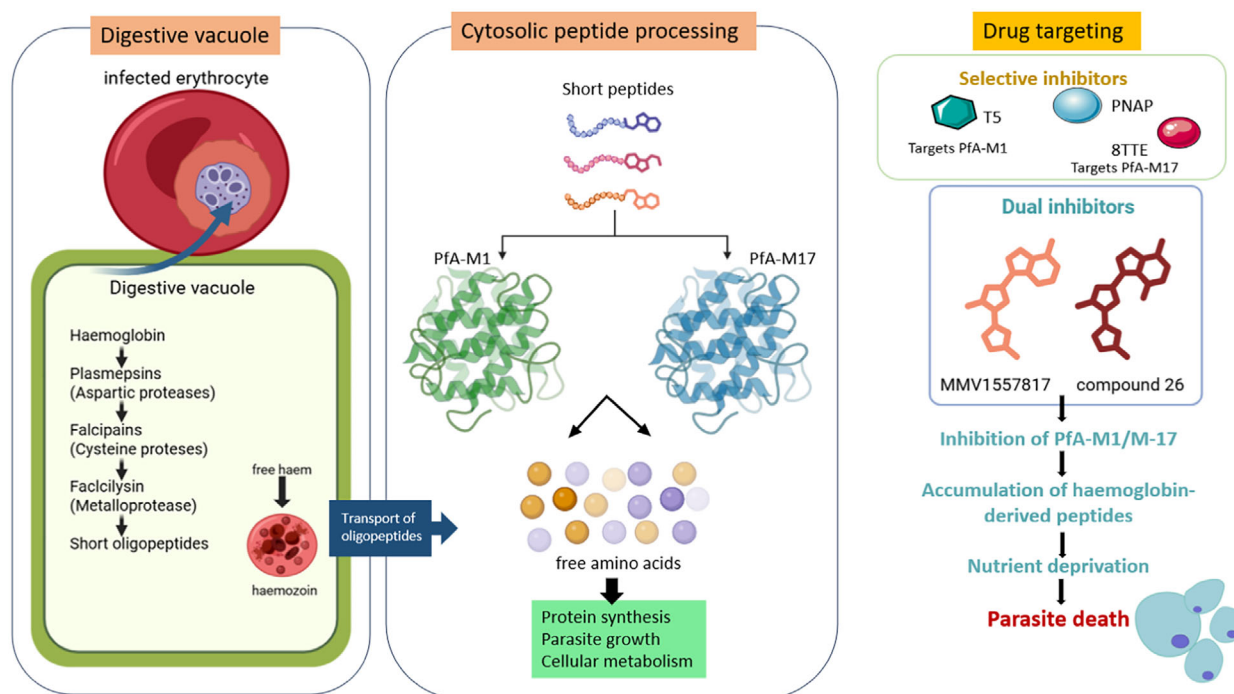


FIGURE 1 | Position of PfA-M1 and PfA-M17 within the hemoglobin digestion pathway of *Plasmodium falciparum*. Hemoglobin degradation begins within the digestive vacuole through the sequential action of plasmepsins, falcipains, and falcilysin, generating short peptide intermediates. These peptides are subsequently processed in the parasite cytosol by the aminopeptidases PfA-M1 and PfA-M17 to release free amino acids required for protein synthesis and parasite growth. Aminopeptidase inhibition disrupts terminal peptide processing, resulting in peptide accumulation, impaired nutrient acquisition, and parasite death. Quinoline antimalarials act upstream by interfering with heme detoxification. Created with BioRender.com and Servier Medical Art is licensed under CC BY 4.0.

biochemical and antiparasitic activity in vitro, yet their clinical translation was ultimately limited by two principal factors: functional redundancy among protease families, whereby genetic disruption or chemical inhibition of individual plasmepsins or falcipains could be compensated by remaining family members, and difficulties in achieving sufficient selectivity and in vivo efficacy to support advancement [20]. The metalloprotease falcipain, which contributes to downstream oligopeptide processing intermediate between vacuolar endoproteases and cytosolic aminopeptidases, was similarly investigated but faced analogous challenges [21, 22]. These cumulative experiences established two important lessons for the field: first, that redundancy within the hemoglobin digestion cascade must be carefully considered when selecting single enzymatic targets; and second, that terminal aminopeptidases such as PfA-M1 and PfA-M17, which occupy nonredundant positions at the final step of peptide processing, may offer distinct advantages precisely because they cannot be bypassed by alternative proteolytic routes.

Although numerous inhibitors targeting these enzymes demonstrated promising biochemical and antiparasitic activity, functional redundancy among protease families and challenges in translating enzyme inhibition into clinical efficacy limited their development. More recently, attention has shifted toward terminal aminopeptidases such as PfA-M1 and PfA-M17, which occupy downstream positions in the pathway and are directly responsible for the liberation of amino acids required for parasite growth [6, 8]. Importantly, hemoglobin digestion is also closely linked to the activity of established antimalarial drugs. Quinoline compounds, including chloroquine, interfere with heme detoxification following hemoglobin degradation [23, 24], while artemisinin activation depends on iron- and heme-derived species generated during this process [25, 26]. Consequently, disruption of hemoglobin digestion may influence parasite susceptibility to other antimalarial classes and has important implications for combination therapies, drug interactions, and resistance mechanisms.

In this review, we synthesize current evidence on PfA-M17 and PfA-M1 as validated antimalarial drug targets, with particular focus on the structural organization, catalytic regulation, and cellular essentiality of PfA-M17. We examine the chemical landscape of selective and dual-target inhibitors developed against these enzymes, and discuss how their unique structural and functional properties have informed rational inhibitor design. The broader context of aminopeptidase inhibition within the hemoglobin

digestion pathway is also considered, including its mechanistic relationship with established antimalarial drug classes and implications for combination therapy and resistance management. Taken together, the evidence positions dual PfA-M1/PfA-M17 inhibition as a scientifically well-grounded and therapeutically compelling strategy for next-generation antimalarial drug discovery.

2 | Biological Role of PfA-M17 in Parasite Metabolism

PfA-M17 (leucyl aminopeptidase) is a zinc-dependent metallo-peptidase expressed throughout the intraerythrocytic life cycle of *P. falciparum*, with peak expression during the trophozoite stage when hemoglobin digestion and protein synthesis are maximal [27]. The enzyme sequentially removes N-terminal amino acids from short peptides derived from hemoglobin catabolism, thereby contributing substantially to the parasite's intracellular amino acid pool. Genetic and chemical perturbation studies, including conditional knockdown approaches and small-molecule inhibition, have demonstrated that PfA-M17 is required for normal intraerythrocytic development, particularly during trophozoite maturation [8]. Chemical inhibition phenocopied this genetic knockdown, confirming PfA-M17 as the primary intracellular target of selective inhibitors. Metabolomic profiling revealed accumulation of hemoglobin-derived peptides and sensitization under amino acid-restricted conditions, providing functional evidence that PfA-M17 is essential for nutrient acquisition rather than bulk hemoglobin degradation [8]. Notably, PfA-M17 inhibition also resulted in the formation of multiple membrane-bound digestive vacuoles containing haemozoin, uncovering a previously unrecognized role for this cytosolic enzyme in maintaining digestive vacuole organization [8]. However, the extent to which PfA-M17 functions redundantly with PfA-M1 under physiological conditions remains incompletely resolved, with some studies suggesting partial compensatory activity depending on metabolic context.

3 | Structural Organization and Catalytic Regulation of PfA-M17

PfA-M17 activity is tightly linked to its quaternary structure, metal coordination, and regulatory loop dynamics (Table 1),

TABLE 1 | Structural features of PfA-M17 relevant for drug design.

Structural feature	Functional significance	Drug-design implication	References
Binuclear Zn ²⁺ metal center	Catalysis; coordinates inhibitors	Zinc-binding groups (hydroxamates, phosphonates) essential for potency	[28, 29]
Hexameric assembly	Required for catalytic activity	Resistance mutations destabilizing hexamer reduce fitness	[5, 10]
L13 regulatory loop	Links active sites; controls active/inactive states	Potential allosteric inhibition site	[9]
S1 pocket with Met392 gate	Substrate access and hydrophobic ligand accommodation	Bulky hydrophobic groups can enhance selectivity	[28, 30]
Flexible S1' pocket	Allows diverse ligand binding	Scaffold modifications to increase dual inhibition	[30]

providing a framework for rational inhibitor design. PfA-M17 functions as a homohexamer, with catalytic activity strictly restricted to the hexameric state [10, 31]. Each subunit contributes to a conserved binuclear metal center, typically coordinated by Zn^{2+} , Mn^{2+} , or Co^{2+} ions, which is essential for catalysis and structural stability. Recombinant PfA-M17 predominantly exists as inactive monomers or small oligomers in solution, highlighting the importance of metal-dependent oligomerization in enzyme activation [31].

Recent crystallographic and molecular dynamics studies have revealed an additional regulatory layer involving a flexible loop (L13) that links neighboring active sites within the hexamer [9]. In inactive monomeric forms, this loop is highly mobile, destabilizing catalytic water and metal coordination. A newly identified inactive PfA-M17 conformation showed L13 occluding the active site and reorganizing the metal center, suggesting a dynamic equilibrium between inactive and active states governed by oligomerization and loop positioning [9].

Mutagenesis studies further demonstrated that residues such as Lys386 and Asp394 play dual roles in catalysis and hexamer stability, reinforcing the concept that PfA-M17 activity is tightly coupled to its quaternary structure [9]. Importantly, this oligomerization-dependent regulation imposes structural constraints that limit resistance development, as resistance-associated mutations often compromise enzyme stability and parasite fitness [5].

4 | PfA-M17 as a Drug Target: Inhibitor Design and Validation

4.1 | Structure-Based and QSAR-Guided Inhibitor Development

PfA-M17 has been firmly established as a promising antimalarial target, and extensive structure-based and computational strategies have significantly advanced inhibitor design. X-ray crystallography, QSAR modeling, and pharmacophore-guided approaches have consistently highlighted the importance of zinc-binding groups, particularly phosphonate and hydroxamate moieties, as critical determinants of inhibitory potency [28, 29]. Inhibitor binding is primarily driven by enthalpic contributions arising from coordination with the catalytic metal ions and π -stacking interactions within the S1 pocket, often reinforced by hydrophobic contacts with residues such as Met392 and Lys386 [28]. These interactions underscore the necessity of precise active-site complementarity for achieving high-affinity and selective inhibition.

Complementing these approaches, large-scale virtual screening and induced-fit docking studies have identified multiple small molecules with measurable inhibitory activity against PfA-M17, demonstrating selectivity over the human homolog hLAP3 and reinforcing the feasibility of parasite-specific targeting [11]. Structural differences between the parasite and human M17 enzymes, particularly in solvent-exposed pockets and flexible regulatory loops, further enable the rational design of inhibitors that exploit parasite-specific features, providing avenues for maximizing potency while minimizing host off-target effects [10].

4.2 | Chemical and Cellular Validation

Beyond biochemical potency, effective validation of PfA-M17 as a therapeutic target requires demonstration that enzyme inhibition translates into meaningful antiparasitic activity within the parasite. Multiple chemically distinct PfA-M17 inhibitors have shown robust *in vitro* activity against *P. falciparum*, confirming that disruption of M17-mediated amino acid metabolism is lethal to the parasite [4]. Early-stage developmental arrest observed upon treatment with selective inhibitors, such as PNAP, provided compelling functional evidence of PfA-M17 essentiality and underscored its role in sustaining parasite growth during the intraerythrocytic cycle [27].

More recently, the natural-product-derived compound 8TTE has expanded the chemical space of PfA-M17 inhibitors. This compound selectively inhibits PfA-M17 with a micromolar inhibition constant ($K_i = 1.1 \mu\text{M}$) and induces stage-dependent developmental abnormalities, including disrupted trophozoite maturation and delayed parasite growth [32]. Metabolomic profiling of 8TTE-treated parasites revealed accumulation of hemoglobin-derived peptides and perturbations in amino acid metabolism, providing biochemical signatures consistent with impaired terminal peptide processing and reinforcing PfA-M17 as the primary intracellular target.

Crucially, the application of unbiased cellular target-engagement approaches, particularly thermal proteome profiling combined with metabolomics, has transformed PfA-M17 validation from correlative to causal. These systems-level analyses have confirmed PfA-M17 as the sole cellular target of selective inhibitors and demonstrated that chemical inhibition phenocopies genetic depletion, producing convergent metabolic and developmental outcomes [8]. Such integrative validation frameworks ensure that observed antiparasitic effects arise from on-target activity and not off-target toxicity, thereby strengthening confidence in PfA-M17 as a bona fide druggable vulnerability.

5 | PfA-M1 and PfA-M17

5.1 | Comparative Overview of PfA-M1 and PfA-M17

PfA-M1 and PfA-M17 are zinc-dependent aminopeptidases that participate in the terminal stages of hemoglobin-derived peptide degradation in *P. falciparum* [14]. Although both enzymes contribute to amino acid acquisition and parasite metabolism, they belong to distinct metalloprotease families and exhibit important differences in structure, cellular organization, and catalytic regulation that influence their suitability as antimalarial drug targets. PfA-M1 belongs to the M1 family of metalloproteases and functions as a monomeric enzyme containing a characteristic HEXXH zinc-binding motif [12, 33]. It exhibits broad substrate specificity and has been implicated in the hydrolysis of oligopeptides generated during hemoglobin digestion. In contrast, PfA-M17 belongs to the M17 leucyl aminopeptidase family and functions as a homohexamer, with catalytic activity dependent on oligomerization and dynamic metal-ion-mediated

conformational regulation [8, 31]. Recent structural studies have revealed complex allosteric mechanisms governing PfA-M17 activity, highlighting features that may be exploited for selective inhibitor design.

Both enzymes have been genetically and chemically validated as potential antimalarial targets. Gene disruption and conditional knockdown studies have demonstrated that aminopeptidase activity is required for efficient parasite growth, while numerous small-molecule inhibitors have shown potent antiparasitic activity [16, 34]. However, recent chemoproteomic investigations suggest that the intracellular target engagement profiles of aminopeptidase inhibitors may differ substantially from predictions based solely on biochemical assays [14]. These findings have renewed interest in understanding the distinct biological contributions of PfA-M1 and PfA-M17 and in determining whether selective or dual-targeting strategies offer the greatest therapeutic advantage. The complementary but nonidentical functions of PfA-M1 and PfA-M17 have stimulated the development of both selective and dual inhibitors. Selective PfA-M1 inhibitors have demonstrated potent parasite killing, whereas emerging evidence indicates that PfA-M17 inhibition may impose substantial fitness costs on resistant parasites. Consequently, current antimalarial drug-discovery efforts increasingly focus on understanding the interplay between these aminopeptidases and exploiting their unique structural and functional properties for therapeutic development.

5.2 | Selective versus Dual Targeting Strategies

PfA-M1 and PfA-M17 share overlapping substrate preferences and structural homology, which historically complicated selective inhibitor development. Early hydroxamate compounds were broadly assumed to act as dual PfA-M1/PfA-M17 inhibitors based on biochemical enzyme assays alone. However, chemoproteomic target engagement profiling of the hydroxamate inhibitor MIPS2673 revealed predominant intracellular engagement of PfA-M1 over PfA-M17 in intact parasites [14]. It is important to note that this finding is specific to MIPS2673 and reflects its particular physicochemical and binding properties; it does not establish a class-wide selectivity profile for hydroxamate inhibitors, whose intracellular target engagement may vary substantially depending on compound permeability, potency differential, and active-site complementarity. These observations nonetheless underscore a critical methodological point: biochemical IC_{50} values against purified enzymes are insufficient to predict intracellular target hierarchy, and chemoproteomic validation should be considered an essential component of future aminopeptidase inhibitor characterization pipelines. This concept was further supported by the discovery of the amino-benzosuberone derivative T5, which selectively inhibits PfA-M1 ($K_i = 50$ nM) with no detectable activity against PfA-M17, yet produces significant antimalarial effects in vitro and in vivo [16]. Structural studies revealed that selectivity is driven by interactions within the S1' and S1 pockets, which differ substantially between the two enzymes [33].

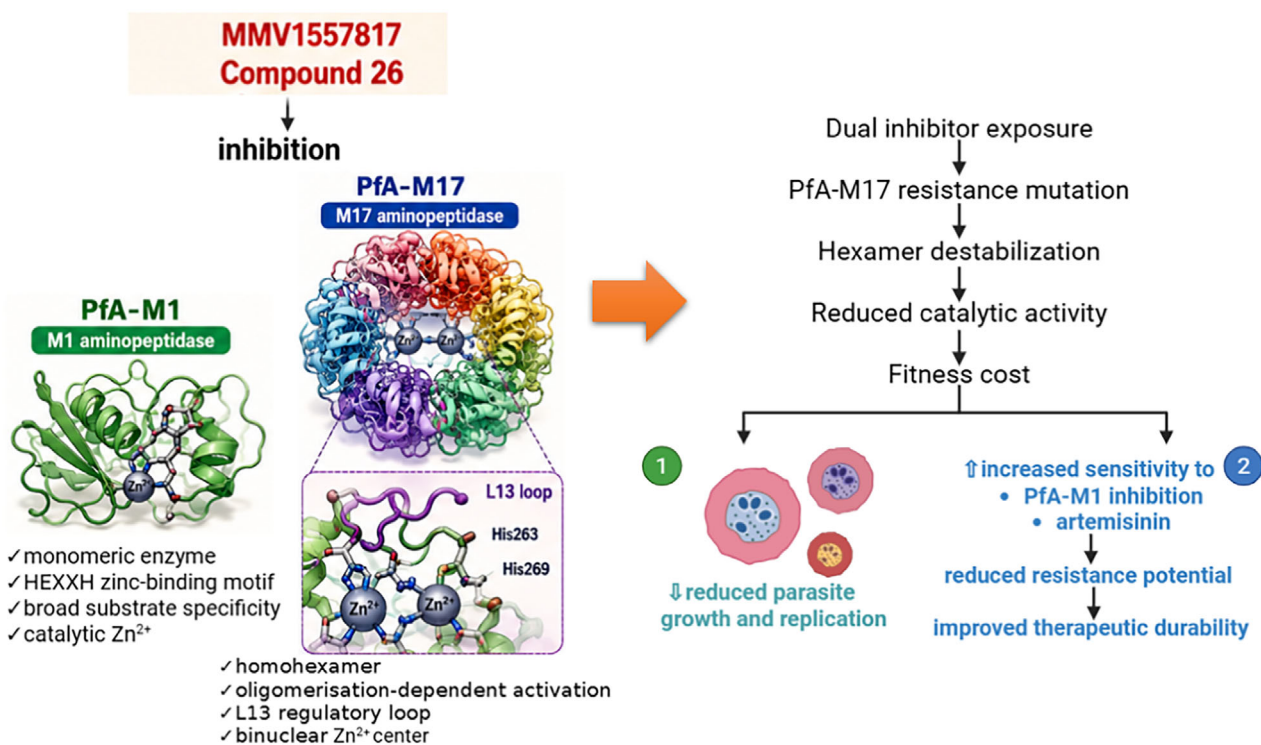


FIGURE 2 | Structural and therapeutic rationale for dual PfA-M1/PfA-M17 inhibition. PfA-M1 is a monomeric M1-family aminopeptidase, whereas PfA-M17 functions as a catalytically active homohexamer regulated by metal-dependent oligomerization and the L13 regulatory loop. Selective inhibition of either enzyme impairs parasite growth, while dual inhibitors simultaneously target both aminopeptidases to maximize disruption of amino acid acquisition. Resistance-associated mutations in PfA-M17 destabilize hexamer assembly, reduce catalytic efficiency, and impose substantial fitness costs that may increase parasite susceptibility to PfA-M1 inhibition and artemisinin, thereby limiting resistance evolution and supporting the therapeutic value of dual-target strategies. Created with BioRender.com.

Conversely, multiple studies support the therapeutic potential of dual PfA-M1/PfA-M17 inhibition. Structure-guided optimization of hydroxamate-based inhibitors demonstrated that dual nanomolar potency can be achieved through distinct binding chemistries within the same ligand [30]. Compounds such as MMV1557817 and compound 26 exhibit potent dual inhibition, broad cross-species activity, favorable selectivity over mammalian aminopeptidases, and robust antiparasitic efficacy against drug-resistant strains [5, 13]. Resistance selection experiments revealed that PfA-M17 resistance mutations incur substantial fitness costs by destabilizing hexamer assembly and reducing catalytic efficiency, thereby sensitizing parasites to PfA-M1 inhibition and even artemisinin [5]. Dual aminopeptidase inhibition may offer enhanced efficacy while constraining resistance evolution (Figure 2).

The therapeutic implications of aminopeptidase inhibition should also be considered within the broader context of hemoglobin digestion-targeted antimalarial therapy. Hemoglobin degradation is closely linked to the activity of several established antimalarial drug classes. Quinoline compounds, including chloroquine, interfere with heme detoxification following hemoglobin digestion, whereas artemisinin activation is dependent on iron- and heme-derived species generated during this process. Consequently, perturbation of hemoglobin digestion through aminopeptidase inhibition may influence parasite susceptibility to other antimalarial agents and could affect the outcomes of combination therapies. Understanding these interactions will be important for evaluating potential synergistic effects, minimizing antagonistic drug combinations, and assessing the risk of cross-resistance associated with targeting interconnected metabolic pathways.

5.3 | Aminopeptidases in Hemoglobin Digestion

PfA-M1 and PfA-M17 should also be considered within the broader context of hemoglobin digestion-targeted drug discovery. Earlier efforts focused on plasmepsins, falcipains, and falcilysin, whereas aminopeptidases occupy the terminal stages of peptide processing and may present distinct opportunities for combination therapy and resistance management.

Among these, aminopeptidase P (PfAPP) plays a unique and indispensable role by cleaving X-Pro peptide bonds, which are resistant to processing by conventional M1 or M17 aminopeptidases [34]. The inability of PfA-M1, PfA-M17, or other metalloaminopeptidases to substitute for PfAPP underscores the specialized and nonredundant nature of this enzyme in completing hemoglobin catabolism.

High-resolution structural analyses have revealed that PfAPP possesses a parasite-specific N-terminal domain and a conserved dimanganese catalytic core, features absent in human homologs. This structural divergence provides opportunities for selective inhibitor design, enabling targeting of PfAPP without off-target effects on host enzymes [34]. Functional studies using the prototypical PfAPP inhibitor, apstatin, demonstrate a conserved metal-coordinated binding mode at the active site, while distal substrate-binding regions differ substantially from human

APP1, further enhancing the potential for selective pharmacological intervention.

Collectively, these findings highlight the integrated proteolytic network underlying hemoglobin digestion in *P. falciparum*, in which upstream endoproteases and downstream aminopeptidases such as PfA-M1 and PfA-M17 cooperate in peptide degradation and amino acid release. Understanding the complementary roles of M1, M17, and APP enzymes provides a broader framework for rational antimalarial drug development, suggesting that combination targeting of multiple non-redundant aminopeptidases may achieve maximal disruption of parasite metabolism while limiting compensatory resistance mechanisms.

5.4 | Comparative and Critical Perspective on Aminopeptidase Targeting in Protozoan Parasites

Although PfA-M1 and PfA-M17 have emerged as leading aminopeptidase targets in *P. falciparum* drug discovery, it is important to place these enzymes within the broader context of peptide-processing pathways across protozoan parasites. Homologous M1- and M17-family aminopeptidases are conserved in other clinically relevant pathogens, including *Leishmania* spp., *Trypanosoma* spp., and *Toxoplasma gondii*, where they contribute to protein turnover and amino acid homeostasis [27, 35, 36]. However, their essentiality, subcellular localization, and degree of functional redundancy vary substantially between species, reflecting divergent evolutionary adaptation to distinct host environments.

In *Plasmodium*, genetic and chemical validation studies support a critical role for PfA-M1 and PfA-M17 in hemoglobin-derived peptide processing, yet the relative contribution of each enzyme to parasite survival remains incompletely resolved, particularly under different metabolic or pharmacological stress conditions. Furthermore, while dual-target inhibition has been proposed as a strategy to enhance efficacy and delay resistance [5, 13, 30, 33], it is not yet fully established whether simultaneous inhibition of both enzymes provides a consistent therapeutic advantage over selective targeting of PfA-M1, which in several studies has demonstrated strong standalone antiparasitic activity.

More broadly, most aminopeptidase inhibitor development has been concentrated within a limited number of research groups, and independent validation across diverse laboratories and parasite systems remains comparatively limited. This highlights an important gap in the field: the need for systematic cross-parasite comparisons, standardized validation pipelines, and head-to-head evaluation of selective versus dual-target strategies. Addressing these limitations will be essential for determining whether aminopeptidase inhibition represents a universally exploitable antiparasitic strategy or a *Plasmodium*-specific vulnerability.

5.5 | Relationship Between Aminopeptidase Inhibition and Established Antimalarial Mechanisms

The therapeutic potential of PfA-M1 and PfA-M17 inhibitors should be considered within the broader context of antimalarial drug mechanisms that converge on hemoglobin utilization and digestive vacuole biology. Hemoglobin digestion is not only essential for amino acid acquisition but also generates free heme and iron-containing intermediates that influence the activity of several established antimalarial drug classes [37–39]. Consequently, disruption of this pathway may have important implications for drug interactions, combination therapies, and resistance evolution.

Among the most extensively studied examples are the quinoline antimalarials, including chloroquine, amodiaquine, and piper-aquine, which accumulate within the digestive vacuole and inhibit heme detoxification [40, 41]. During hemoglobin degradation, toxic ferriprotoporphyrin IX (free heme) is released and subsequently converted into inert haemozoin crystals [42, 43]. Quinoline compounds interfere with this detoxification process, leading to the accumulation of cytotoxic heme species and parasite death [44]. Because aminopeptidase inhibitors act downstream of hemoglobin proteolysis [5, 33], perturbations in peptide turnover and digestive vacuole homeostasis may indirectly influence heme generation and vacuolar physiology. This is supported by the observation that selective PfA-M17 inhibition results in the formation of multiple aberrant membrane-bound digestive vacuoles containing haemozoin, suggesting that cytosolic aminopeptidase activity is functionally coupled to vacuolar organization in ways that extend beyond simple peptide catabolism [8]. The precise downstream consequences of this vacuolar disruption for heme accumulation, quinoline drug distribution, and ultimately quinoline susceptibility represent an important and unresolved question warranting direct experimental investigation.

Hemoglobin digestion is also closely linked to the activity of artemisinin and its derivatives. Activation of artemisinin requires cleavage of its endoperoxide bridge by ferrous iron or heme-derived species generated during hemoglobin catabolism [45]. Experimental evidence has shown that perturbations affecting hemoglobin uptake, digestion, or heme availability can influence artemisinin susceptibility [46]. Notably, several resistance-associated adaptations in *P. falciparum*, including mutations in Kelch13 and related pathways, have been associated with altered hemoglobin endocytosis and reduced heme-mediated drug activation [47, 48]. Critically, and most directly relevant to the present review, resistance selection experiments targeting PfA-M17 demonstrated that mutations conferring reduced aminopeptidase activity paradoxically sensitized parasites to artemisinin [5]. This finding provides direct in-parasite evidence that disruption of terminal peptide processing has upstream consequences for heme-dependent drug activation, and strongly suggests that dual aminopeptidase inhibition may be leveraged synergistically with artemisinin-based regimens, particularly in the context of emerging artemisinin resistance.

From a therapeutic perspective, aminopeptidase inhibitors offer a mechanistically distinct mode of action relative to current

frontline therapies, targeting terminal peptide processing rather than heme detoxification, folate metabolism, mitochondrial electron transport, or direct artemisinin activation. This mechanistic orthogonality reduces the likelihood of direct cross-resistance with existing drug classes and supports their rational incorporation into combination regimens. However, the shared dependence of multiple drug classes on hemoglobin-derived metabolic intermediates means that aminopeptidase inhibition is unlikely to be pharmacologically neutral with respect to companion drugs—interactions may be synergistic, antagonistic, or context-dependent, varying with parasite genetic background, drug exposure sequence, and metabolic state. Future studies integrating chemoproteomics, quantitative metabolomics, and resistance-selection approaches will be essential for systematically mapping these interactions and determining how aminopeptidase inhibition can be optimally combined with existing antimalarial therapies while constraining resistance development.

6 | Conclusions and Future Perspectives

PfA-M17 and PfA-M1 have emerged from the past decade of research as structurally distinct, genetically validated, and chemically tractable antimalarial targets occupying critical terminal positions in the *P. falciparum* hemoglobin digestion pathway. The convergence of genetic, chemical, structural, and systems-level evidence has firmly established that disruption of terminal aminopeptidase activity is lethal to the intraerythrocytic parasite, and that dual inhibition of both metalloaminopeptidases offers compelling therapeutic advantages over selective targeting alone. PfA-M17's unique hexameric architecture, metal-dependent conformational regulation, and the substantial fitness costs imposed by resistance-associated mutations collectively position this enzyme as an exceptionally promising node for pharmacological intervention. Similarly, PfA-M1's monomeric architecture and broad substrate specificity, together with its demonstrated essentiality across multiple parasite strains, reinforce the therapeutic rationale for selective and combination aminopeptidase targeting strategies. Crucially, resistance selection experiments have demonstrated that mutations conferring reduced PfA-M17 activity destabilize hexameric assembly, compromise parasite fitness, and paradoxically sensitize parasites to artemisinin, a finding with significant implications for combination therapy design and resistance management.

Despite these advances, several important gaps remain that must be addressed to translate aminopeptidase inhibitor programmes into clinical candidates. First, the precise functional redundancy between PfA-M1 and PfA-M17 under varying metabolic and pharmacological conditions requires further quantitative resolution. Conditional genetic systems combined with quantitative metabolomics will be essential for dissecting the relative contributions of each enzyme to amino acid homeostasis across the intraerythrocytic life cycle. Second, while advanced dual inhibitors such as MMV1557817 and compound 26 demonstrate promising in vitro and cross-species antiparasitic efficacy, comprehensive in vivo pharmacokinetic and pharmacodynamic profiling in humanized murine models and nonhuman primate systems will be critical for prioritizing candidates for preclinical advancement. Third, the structural

basis of inhibitor selectivity over human leucyl aminopeptidase (hLAP3) and alanyl aminopeptidase (hAPN) must be more systematically exploited through structure-guided optimization of parasite-specific binding pockets, particularly the S1' region and allosteric interfaces of PfA-M17. Emerging cryo-EM approaches and fragment-based screening platforms offer powerful complementary strategies to expand the chemotype diversity of aminopeptidase inhibitor scaffolds.

Looking ahead, several directions are particularly promising. The identification of allosteric inhibition sites, notably the L13 regulatory loop and hexamer interface of PfA-M17, represents an underexplored but mechanistically rational strategy for achieving selective inhibition with reduced susceptibility to active-site resistance mutations. The integration of chemoproteomics and thermal proteome profiling with in situ target engagement validation should become standard in future inhibitor characterization pipelines, ensuring that observed antiparasmodial phenotypes reflect bona fide on-target activity. Additionally, the nonredundant and structurally divergent aminopeptidase P (PfAPP) warrants co-investigation as a combination target, given its unique role in cleaving proline-containing peptides that are refractory to PfA-M1 and PfA-M17 processing. Systematic evaluation of triple aminopeptidase inhibition may offer a maximal disruption strategy with high barriers to resistance evolution.

From a broader therapeutic perspective, the mechanistic intersection of aminopeptidase inhibition with heme metabolism and artemisinin activation pathways underscores the importance of evaluating combinatorial drug interactions within this interconnected network. Understanding whether aminopeptidase inhibitors synergize with or antagonize artemisinin-based regimens will be essential for rational combination therapy design. Furthermore, cross-parasite validation of M1 and M17 aminopeptidases in *Leishmania*, *Trypanosoma*, and *Toxoplasma* spp. may reveal shared pharmacological vulnerabilities amenable to broad-spectrum antiparasitic strategies. In conclusion, PfA-M17 and PfA-M1 represent mature, mechanistically well-understood, and increasingly well-validated antimalarial targets. With continued investment in structure-guided inhibitor optimization, integrative target validation, and rational combination therapy design, dual aminopeptidase inhibition holds substantial promise as a cornerstone of next-generation antimalarial drug discovery.

Author Contributions

Sharoen Yu Ming Lim: conceptualization, writing – original draft, methodology, visualization, writing – review and editing.

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Conflicts of Interest

The author declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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