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REVIEW

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Epigenetic regulation as a therapeutic target in the malaria parasite *Plasmodium falciparum*

Thibaud Reyser^{1,2,3}, Lucie Paloque^{1,2,3}, Jean-Michel Augereau^{1,2,3}, Luisa Di Stefano⁴ and Françoise Benoit-Vical^{1,2,3*}

Abstract

Over the past thirty years, epigenetic regulation of gene expression has gained increasing interest as it was shown to be implicated in illnesses ranging from cancers to parasitic diseases. In the malaria parasite, epigenetics was shown to be involved in several key steps of the complex life cycle of *Plasmodium*, among which asexual development and sexual commitment, but also in major biological processes like immune evasion, response to environmental changes or DNA repair. Because epigenetics plays such paramount roles in the *Plasmodium* parasite, enzymes involved in these regulating pathways represent a reservoir of potential therapeutic targets. This review focuses on epigenetic regulatory processes and their effectors in the malaria parasite, as well as the inhibitors of epigenetic pathways and their potential as new anti-malarial drugs. Such types of drugs could be formidable tools that may contribute to malaria eradication in a context of widespread resistance to conventional anti-malarials.

Keywords *Plasmodium*, Epigenetics, Histone modifications, Epidrugs, Antiplasmodial drugs

Background

According to the latest World Health Organization (WHO) malaria report, an estimated 608,000 people died from malaria in 2022 [1]. Even though malaria cases are significantly lower than in 2000, and after years of increases until 2019, the number of deaths is falling again compared with 631,000 in 2021 [1]. Artemisinin-based combination therapy (ACT) has significantly helped to reduce malaria death toll since its introduction in 2001. However, emergence of artemisinin resistance in Southeast Asia threatens the use of artemisinin-based

combinations. The worst fears of the scientific community [2, 3] are beginning to manifest as evidenced by the recent emergence of artemisinin resistance in Africa [4–6], where the parasite already kills 95% of its victims [7]. Therefore, widening the drug portfolio by identifying new anti-malarial drug targets is paramount. Among them, epigenetic mechanisms stand out because they are involved in the regulation of gene expression, which is closely linked to many key biological processes of the *Plasmodium* parasite [8]. Therefore, drugs targeting epigenetic pathways, or epidrugs, in the malaria parasite could be a winning strategy towards malaria eradication.

Epigenetics: some generalities

Epigenetics which literally means “outside the genome”, has become a very trendy field of research in the past twenty years. However, its origin dates back to the 1940s, when Conrad Waddington coined the term of epigenotype to describe the link “between genotype and phenotype (that) lies a whole complex of development processes” [9]. Epigenetics was more precisely defined as

*Correspondence:

Françoise Benoit-Vical

Francoise.Vical@inserm.fr

¹ LCC-CNRS, Laboratoire de Chimie de Coordination, CNRS, Université de Toulouse, Toulouse, France

² MAAP, Inserm ERL 1289, Team “New Antiplasmodial Molecules and Pharmacological Approaches”, Toulouse, France

³ Institut de Pharmacologie et de Biologie Structurale, IPBS, CNRS, UPS, Université de Toulouse, Toulouse, France

⁴ MCD, Centre de Biologie Intégrative (CBI), CNRS, UPS, Université de Toulouse, Toulouse, France



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the study of the mechanisms regulating gene expression without causing any change in the DNA sequence while being transmissible to the following generations [10, 11]. The most studied epigenetic mechanisms, mainly histone post-translational modifications (HPTMs) and DNA modifications, are thus involved in controlling the accessibility of the coding genetic sequence to transcriptional effectors, and depend on three different sets of proteins: writer proteins that deposit modifications on histone or DNA, reader proteins that recognize and bind to the modifications and eraser proteins able to catalyze the removal of the modifications [10]. It is important to keep in mind that these levels of regulation of gene expression overlap with several other processes such as RNA modifications, RNA degradation or non-coding RNA [10] challenging the strict association between specific epigenetic marks and gene expression/silencing.

Histone post-translational modifications

DNA is wrapped up around histone proteins (Fig. 1), forming the basic structural unit of genome packaging, the nucleosome. Gene expression relies in part on the degree of compaction of chromatin, which depends on the state of the nucleosome. The histone protein is made up of two protein domains: a core structure whose main role is to mediate histone/histone interactions, and a N-terminal tail mediating histone/DNA electro-philic interaction [12]. N-terminal tail of histones can also serve as a binding site for various proteins involved in chromatin remodeling, transcriptional regulation,

and other cellular processes. These interactions can be modulated by the presence of specific post-translational modifications on the tail, allowing for dynamic and context-dependent regulation of gene expression. These post-translational modifications of histone tails are of several types and include acetylation, methylation, phosphorylation, ubiquitylation, citrullination, sumoylation, ADP-ribosylation, propionylation, butyrylation, formylation, proline isomerization and crotonylation of various amino acid [13, 14]. When nucleosomes are tightly packed, the chromatin is in a condensed state called heterochromatin, which was originally thought to prevent gene expression. When nucleosomes are less compacted, the chromatin is in a loose state called euchromatin, which allows regulatory proteins (including transcription factors) to easily access the DNA sequence and allow gene expression. However, it has been shown that gene expression can occur in heterochromatic domains, challenging the strict view of heterochromatin as a "silent" component of eukaryotic genomes [15].

The two most largely studied histone modifications are histone tail acetylation (on lysine residues) and methylation on both lysine or arginine residues [13] mainly located on the N-terminal part of histones H3 and H4 [16] (Fig. 2). The addition of an acetyl moiety (CH₃-CO-) on a positively charged lysine reduces the histone tail interaction with DNA leading to a more open chromatin conformation and, therefore, favouring gene activation. Conversely, the electronic charge of the amino-acid side chain is not altered by the methyl moiety (CH₃),

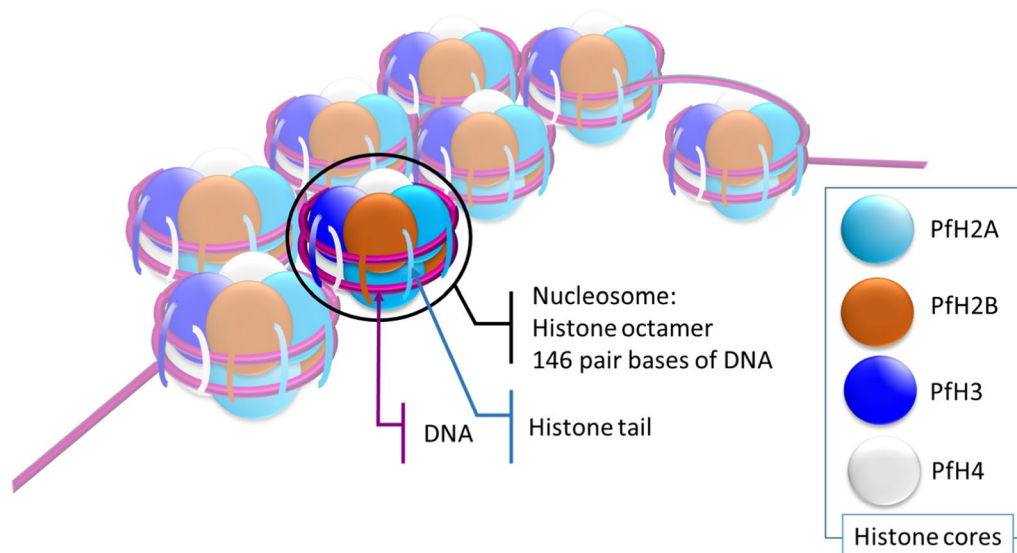


Fig. 1 Schematic view of a *Plasmodium falciparum* nucleosome organization. DNA is wrapped up around nucleosome, a histone core octamer structure, made of two H2A-H2B dimers bound to an H3-H4 tetramer [12]. Histone tails and in particular lysine and arginine residues, on which several post-translational modifications can occur, are generally positively charged allowing strong binding to DNA which possesses negatively charged phosphate groups

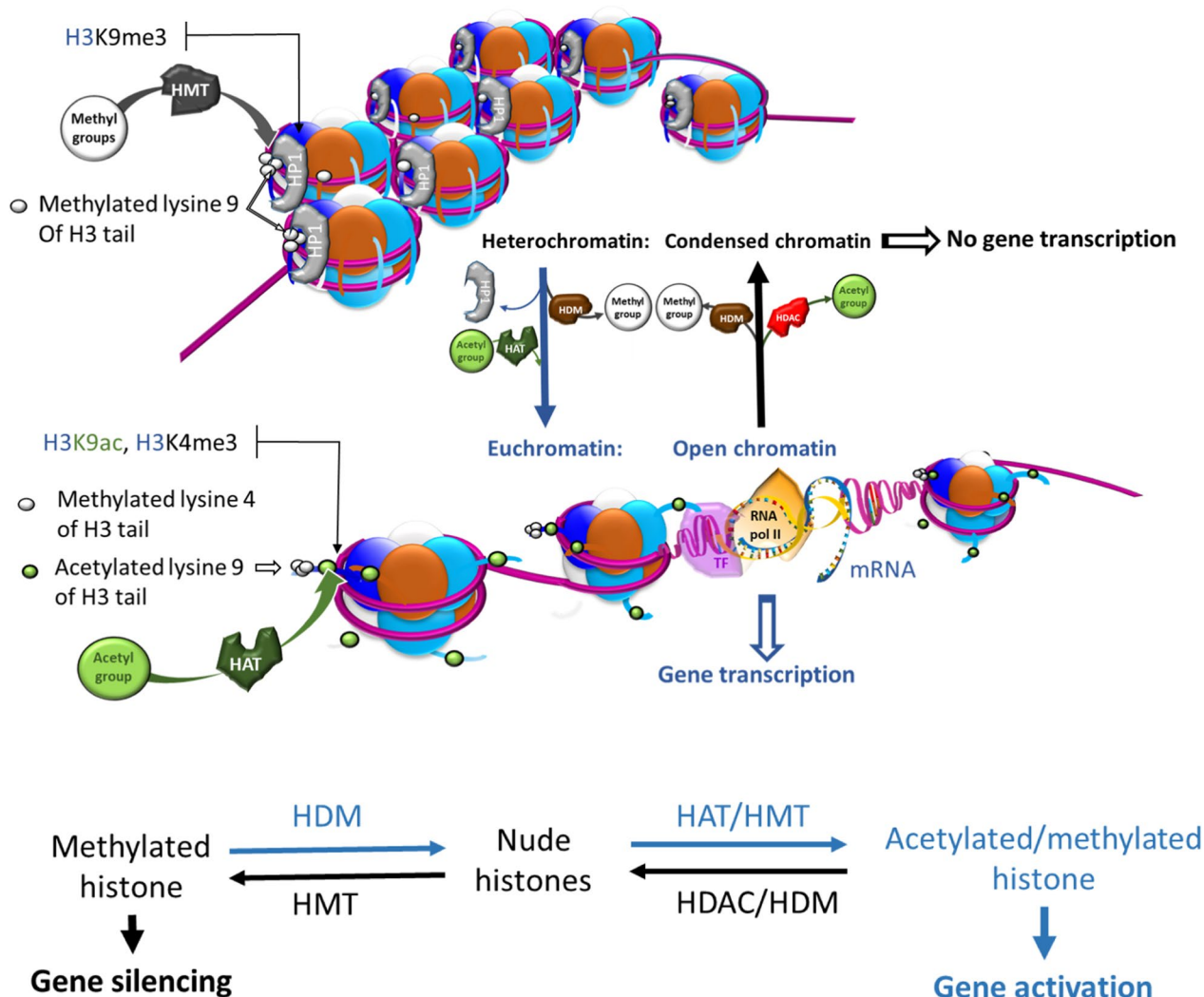


Fig. 2 Relationship between acetylation and methylation levels for the switch from heterochromatin to euchromatin. Histone methylation as the tri-methylated of the 9th lysine residue of histone 3 tail, via histone methyltransferases (HMT), is globally involved in gene silencing. These modifications tend to recruit histone binding proteins (such as heterochromatin protein 1 (HP1)) that avoid chromatin relaxation, thus preventing transcription factors from accessing DNA. This methylation state is reversible and mediated by histone demethylases (HDM) [12, 22, 23]. However, histone methylation is not always associated with gene silencing. Trimethylation on the 4th lysine of histone 3 (H3K4me3) is involved in gene expression [17–19]. When a histone tail is acetylated by histone acetyltransferases (HAT), this tends to neutralize the lysine positive charge interacting with the negative phosphate groups of DNA and pushes away histone cores therefore “opening” the chromatin. That allows transcription factors (TF) to recognize and bind to promoters and RNA polymerase II (RNA pol II) to initiate transcription. This acetylation level is reversible and mediated by histone deacetylases (HDAC)

and histone methylation has been correlated to either gene repression or activation depending on the residue affected. Thus, trimethylation on the 9th lysine of histone 3 (H3K9me3) is abundant in heterochromatic domains and correlates with gene repression while trimethylation on the 4th lysine of histone 3 (H3K4me3) is abundant in euchromatin and correlates with active transcription [17–19]. It is thought that reader proteins are able to recognize the different states of methylation and acetylation [20]. Acetylation and methylation on lysine residues are

mutually and dynamically exclusive for the same amino groups depending on the cell cycle development. Therefore, histone deacetylases/methyltransferases and histone demethylases/acetyltransferases are tightly linked in order to fine-tune gene expression [21].

DNA modifications

DNA base modifications generally affect the accessibility of genomic regions for regulatory effectors of gene expression (Fig. 3). The most common modification

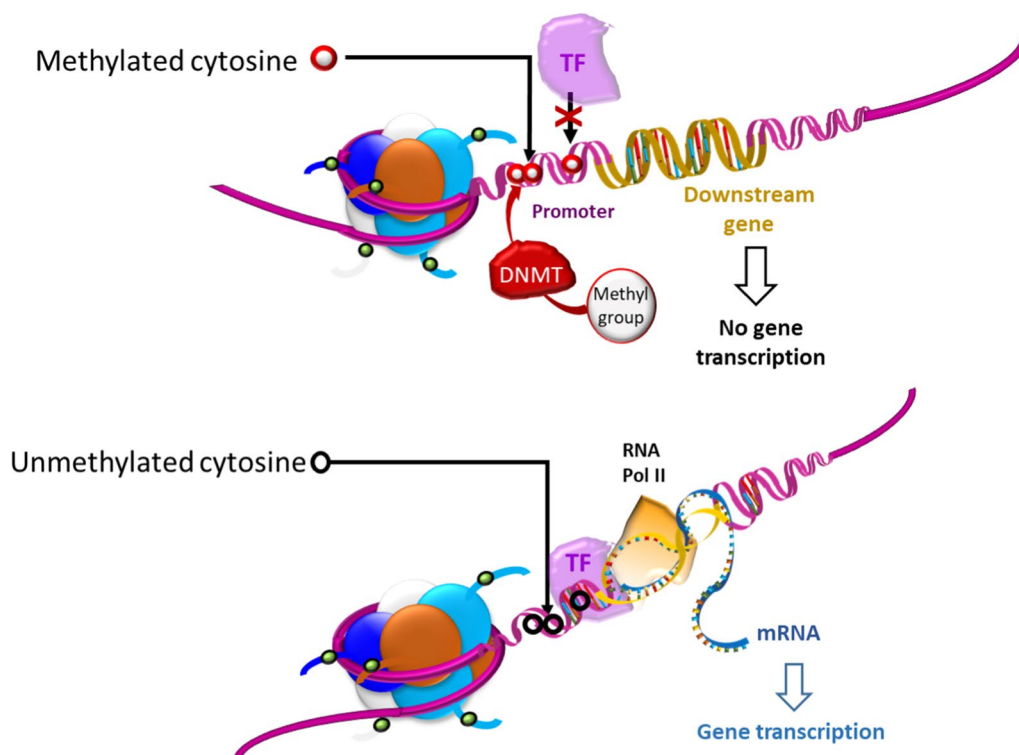


Fig. 3 DNA methylation is mediated by DNA methyltransferases (DNMTs). Methylated cytosines have an impact on gene expression when they are located in promoter regions of genes. When present, this modification prevents transcription factors (TF) from binding to promoter regions and starting transcription and thus downstream gene expression. In the absence of DNA methylation (unmethylated cytosine), transcription factor can bind to the DNA strand and transcription of the gene concerned can proceed

consists in the addition of a methyl group to the carbon 5 of a cytosine (5mC) [24] and is generally associated with loss of gene expression [25]. DNA methylation is recognized and bound by specific methylated cytosine-binding proteins, which can in turn recruit co-repressor complexes. Through steric hindrance, these protein complexes could then prevent transcription factors from binding to promoter regions, thereby silencing downstream gene expression [26].

Epigenetic regulation in the malaria parasite

In a general framework, regulation of gene expression occurs at multiple levels: basal (or constitutive) transcription is assured by general transcription factors while sequence-specific transcription factors bind to cis-regulatory regions of genes (enhancers, promoters) and allow individual genes to be turned on or off in specific cell types. Twenty-seven Apicomplexan-specific AP2 (ApiAP2) DNA-binding proteins have been identified in *Plasmodium falciparum* and they are the main factors regulating transcription. Although they are not as numerous as in other eukaryotic organisms with comparable genome sizes, such as yeast, their function is essential to the parasite’s life cycle and its ability

to adapt to changes in its environment [27–30]. PfAP2-P is involved in the regulation of gene expression during parasite development growth and pathogenesis [30] and PfAP2-G in the switch from asexual to gametocytes [31, 32]. Thus, in *Plasmodium*, epigenetic regulations could represent a main form of regulation of gene expression close to those in ancestral eukaryotic groups [33] at each step of the parasite life cycle, either at the intra-mosquito, hepatic or intraerythrocytic stage [34–37]. Epigenetics regulates key processes of *Plasmodium* biology (recently reviewed [8, 38]) such as: (i) immune evasion through the “one at a time” expression of clonally variant genes coding for surface antigen like PfEMP1 [39], (ii) the “just in time” regulation of gene expression required for the cell cycle progression during the intraerythrocytic stage [40], (iii) DNA repair mechanisms [41, 42], (iv) sexual commitment [43–46], or (v) adaptation to environmental changes [47]. While histone post-translational modifications in malaria parasites were described some 30 years ago [48, 49], DNA modifications in the parasite have been recently discovered, and although they are lowly abundant, their role in regulating the transcriptional state of the parasite genome is starting to be elucidated [50–52]. Two other epigenetic mechanisms, relying on

RNA modifications [53, 54] and on non-coding RNAs [55] also exist in the malaria parasite. In this way it has been recently shown the importance of long non-coding RNAs in pathogenicity and sexual differentiation [56, 57].

Epigenetic marks in *Plasmodium*

Histone post-translational modifications (HPTMs) in *Plasmodium*

At least 232 histone post-translational modifications have been identified in *P. falciparum* [58], including ubiquitylation and phosphorylation [59, 60], but the role of many of these marks remains unclear. *P. falciparum* has a very original epigenetic signature, with a significant number of activating histone marks yet only a handful of repressive marks. Indeed, activating histone marks are abundant and scattered throughout the genome, allowing the transcriptionally-permissive state of the genome along the intra-erythrocytic development cycle [60]. The number of HPTMs can vary, i.e., on average of 3 per histone tail but which can go up to 7 [61]. Among them, H3K9ac and H3K4me3 are the most abundant ones [62, 63] and their dynamic distribution is tightly linked with the “just in time” pattern of gene expression along the 48-h of the parasite intraerythrocytic developmental cycle, in other words only at a time it is required [40]. In this sense, a specific HPTM profile of gametocytes can also be seen, with a high abundance of acetylated histones H3 and H4 [64].

Indeed, highly transcribed genes of *P. falciparum* are associated with enriched H3K9ac marks in their promoter and 5' coding sequences of active genes. H3K4me3 is stage-specific i.e. low at early stages, peaking at late stages, does not appear to be correlated with gene expression [65] [63] as it is not dynamically enriched at active promoters, but is upregulated at intergenic regions especially at trophozoite and schizont stages [63]. Repressive histone marks, such as H3K9me3, are specifically associated with clonally variant genes, such as *var*, *rifin* and *stevor*, that are localized on subtelomeric and some chromosome internal regions [66].

Other repressive marks include H3K36me2, H4K20me3 and H3K27me3, identified in gametocytes [67]. Moreover, it has been shown that phosphorylation of the histone H2A on serine 121 occurs in case of DNA damage before the DNA repair systems are activated and removed once the repair process has started [41].

DNA modifications in *Plasmodium*

For a long time, the existence of methylated DNA within the parasite was highly debated. After its identification in 2013, its level was estimated at 0.01–0.05% to 0.58% of genomic cytosines in *P. falciparum*. 5-methyl cytosines (5mC) were later identified in *Plasmodium berghei*

[50–52]. Very recent data have shown that 5mC is in fact at a level of 0.1–0.2% during the intra-erythrocytic cycle [68] close to that of other apicomplexans, such as *Toxoplasma gondii* (0.27–0.41%) [69], but far behind that of mammals and birds (5%), fish (around 10%) or plants (as high as 30%) [70]. Hydroxy-methylated cytosines (5hmC) have also been identified and seem to be correlated with gene expression. They could represent 0.2 to 0.4% of genomic cytosines in malaria parasites, significantly more than the 0.03 to 0.06% in other organisms [50] but it is not confirmed in another study [68]. These DNA modifications could be also concomitant with histone marks [51], similar to what has already been seen in model organisms such as *Xenopus* [26].

Epigenetic effectors in *Plasmodium*

Histone and DNA modifications are under the control of specific enzymes which are responsible for ‘writing’ and ‘erasing’ a wide range of modifications on histone tails among which acetylation and methylation are the most studied ones.

Histone acetyltransferases/histone deacetylases

Histone acetylation is catalyzed by histone acetyltransferases (HATs) (Table 1). Four different HATs have been identified in the *Plasmodium* genome: *PfHAT1*, *PfELP3*, *PfGCN5* and *PfMYST* [71], but only the activity of the last two was determined. *PfGCN5*, is a nucleolar enzyme [72, 73] also active in the regulation of clonally variant gene expression [74]. It has recently been shown that *PfGCN5* can be found in different protein complexes especially in the later stages of the erythrocytic cycle of the parasite. Multiple variants of a *PfGCN5*-containing complex could be capable of performing different biological functions [74]. *PfMYST* is essential for gene expression, cell cycle progression and DNA repair [42] and its over expression entails a significant hyperacetylation at H4K5, K8, K12 and K16 which is associated with shortened intra-erythrocytic developmental cycle and reduced growth rate [75]. However, this HAT is not specific to histones since it also acetylates cytoplasmic proteins [42].

Histone deacetylation is mediated by histone deacetylases (HDACs) (Table 1) [49, 76, 77]. Five HDACs have been identified in *P. falciparum* and subdivided in 3 categories based on their phylogenetic relationship to their yeast orthologues [71]. Class I and Class II enzymes have a zinc-dependent HDAC activity and act on intrachromosomal domains whereas class III HDACs are NAD⁺ dependent and are involved in silencing genes in sub-telomeric regions [39, 78–80]. PfHDAC1 (class I) could be involved in the reversible changes of euchromatin mediating the intraerythrocytic developmental cycle of the parasite [81]. The class II includes PfHDA1

Table 1 Histone writers and erasers enzymes in *P. falciparum*

Name	PlasmoDB ID	Inferred/Known activity
Histone acetyltransferases (HATs)		
<i>PFGCN5</i> [72]	PF3D7_0823300	H3K9, H3K14 acetylation
<i>PfMYST</i> [75]	PF3D7_1118600	H4K5, K8, K12, K16 acetylation
<i>PfHAT1</i> [71]	PF3D7_0416400	Unknown
<i>PfELP3</i> [71]	PF3D7_1227800	Unknown
Histone deacetylases (HDACs)		
<i>PfHDAC1</i> (Class I) [81]	PF3D7_0925700	Parasite's progression through intraerythrocytic developmental cycle
<i>PfHDAC2</i> (Class II) [45]	PF3D7_1008000	H3K9 deacetylation involved in gametocyte commitment, virulence gene silencer
<i>PfHDA1</i> (Class II) [82]	PF3D7_1472200	Putative HDAC
<i>PfSIR2A</i> (Class III) [85, 86]	PF3D7_1328800	Telomere maintenance and <i>var</i> gene silencing
<i>PfSIR2B</i> (Class III) [88, 143]	PF3D7_1451400	Silencing of <i>var</i> genes
Histone methyltransferases (HKMTs) [90, 94, 144]		
<i>PfSET1</i>	PF3D7_0629700	H3K4 methylation
<i>PfSET2</i>	PF3D7_1322100	H3K36 methylation
<i>PfSET3</i>	PF3D7_0827800	H3K9 methylation
<i>PfSET4</i>	PF3D7_0910000	H3K36 methylation
<i>PfSET5</i>	PF3D7_1214200	Unknown
<i>PfSET6</i>	PF3D7_1355300	H3K36 methylation
<i>PfSET7</i>	PF3D7_1115200	H3K36, H3K9, H3K4 methylation
<i>PfSET8</i>	PF3D7_0403900	H4K20 methylation
<i>PfSET9</i>	PF3D7_0508100	H3K36 methylation
<i>PfSET10</i>	PF3D7_1221000	H3K4 methylation
<i>PfPRMT1</i>	PF3D7_1426200	H4R3 methylation
Histone lysine demethylases [91, 96]		
LSD1 family		
<i>PfLSD1</i>	PF3D7_1211600	H3K4me1 and me2 demethylation
<i>PfLSD2</i>	PF3D7_0801900	H3K4me1 and me2 demethylation
Jumonji-related family		
<i>PfJmjC1</i>	PF3D7_0809900	Histone Lysine Demethylase
<i>PfJmjC2</i>	PF3D7_0602800	Histone Lysine Demethylase
<i>PfJmjC3</i>	PF3D7_1122200	Histone Lysine Demethylase
DNA methyltransferase (DNMT) [51, 53, 101]		
<i>PfDNMT2</i>	PF3D7_0727300	DNA and tRNA methylation

and *PfHDA2* which are key players of gametocyte commitment and play a role in irreversible changes of chromatin structure involved in this key step of the life cycle [45, 82–84]. In class III, *PfSir2A* has the ability to deacetylate both histone 3 and histone 4. This *SIR2* deacetylase activity is necessary for virulence gene silencing [85]. Conversely acetylation of histones, in particular H4, can occur when *PfSIR2* is removed from the promoter region of the subtelomeric *var* gene [86]. *PfSIR2A* can be considered as a major *var*-associated deacetylase. Another histone deacetylase *Sir2* has been identified, *PfSIR2B* which like *PfSIR2A* regulates silencing of *var* genes in *P. falciparum* but for a different subset [84, 87, 88]. Similarly

to their orthologs in other eukaryotes, these two sirtuin enzymes might also play a role in the adaptation of the parasite to its environment [89].

Histone methyltransferases / histone demethylases

Methylation on histones can take place either on amino-groups of lysine or on guanido nitrogen atoms of arginine and is mediated by histone lysine methyltransferases (HKMT) or protein arginine methyltransferases (PRMT) (Table 1). Three putative PRMTs (*PfPRMT1*, *PfPRMT5*, *PfCARM1*), most likely involved in protein maturation than regulation of gene expression, have been identified in *Plasmodium* but only *PfPRMT1* has been

characterized [90] and recently *PfPRMT5* which plays a key role in merozoite invasion [56]. Ten *PfHKMTs* (also known as SET1 to SET10) have been identified by computational analysis [91, 92]. It should be noted that these proteins do not only methylate histones but a large range of proteins, which can be found either in the nucleus or the cytoplasm. *PfSET10* may play a role in the regulation of *var* genes expression through its ability to methylate the lysine K4 of histone H3 [92], but this remains a matter of debate [93].

Histone demethylation is mediated by histone lysine demethylases (HKDM) (Table 1). Five HKDMs have been identified and sub-categorized into two categories: LSD1 and Jumonji (JmjC) demethylases [91, 94]. While LSD1 demethylases can be involved in the removal of mono- and dimethylated groups from lysines [95], JmjC demethylases (*Pfjmc1*, *Pfjmc2* and *Pfjmc3*) are the only family capable of the demethylation of trimethylated lysine residues like H3K4me₃, H3K9me₃ and H4K20me₃ in the parasite [96]. As previously mentioned, acetylation and methylation patterns of histones are linked. For example, sexual commitment regulation relies on a switch of H3K9me₃ to H3K9ac depending on *PfSET3* and *PfGCN5* [97, 98]. This leads to the dissociation of H3K9–HP1 (heterochromatin protein 1) complex and the subsequent triggering of parasite commitment to gametocytogenesis through de-repression of *pfap2-g* [99, 100]. The return to a silencing state of this transcription factor depends on *PfHDA2* responsible for deacetylation of H3K9ac tail prior to its methylation [45].

DNA methyltransferases

Only one gene with a predicted DNA methyltransferase activity has been identified in *P. falciparum* genome (*PfDNMT2*) coding for an enzyme related to the DNA methyltransferase 2 family but with a low methylation activity on DNA cytosines in vitro [51, 53] (Table 1). Expressed all along the intraerythrocytic cycle with a peak at the trophozoite stage, *PfDNMT2* is able to methylate tRNA cytosine including C38 of tRNA^{Asp} [68, 101]. The tRNA methylation participates in maintaining stable protein synthesis by protecting tRNAs from endonucleolytic degradation during stress situations experienced by the parasite [53].

Plasmodium epigenetic effectors as a source of therapeutic targets

Case of parasite resistance to anti-malarials

The first demonstrations that epigenetics could be involved in anti-malarial drug resistance processes in *Plasmodium* was obtained in relation to the antibiotic blasticidin S [102, 103] and the bis-thiazolium salts T3 and T16 [104]. Blasticidin S, an antibiotic, with an IC₅₀

of 530 nM against the *Plasmodium* 3D7 strain, and T3 and T16 with IC₅₀ values of 26 and 10 nM, respectively, all enter into the parasite through the solute transporter plasmodial surface anion channel (PSAC) [104]. This transporter is formed by a CLAG3 protein, either CLAG3.1 or CLAG3.2 with different solute uptake efficiency [47, 105]. Within a clonal population, *clag3.1* and *clag3.2*, localized head-to-tail in the same locus, are stochastically and mutually exclusively expressed in each parasite. When one is expressed, depending of H3K9ac, the other one is repressed (marked with H3K9me₃) [106, 107]. The subset of parasites stochastically expressing no *clag3* gene or only CLAG3.1 (which has a low solute uptake efficiency) is able to withstand exposure to blasticidin S and T3 illustrating how epigenetics can mediate drug resistance in *Plasmodium* within isogenic parasite population where only few individuals can survive to the drug exposure [102, 104]. This is reminiscent of parasite resistance to artemisinins, since only a subpopulation within a clonal population, mutated for the *pfk13* gene involved in the endocytosis of haemoglobin from the host cell by the parasite [108, 109], can resist exposure to these anti-malarial drugs by entering quiescence [110, 111] (Fig. 4). It has also recently been shown that *PfGCN5*, a histone acetyl transferase, is involved in the resistance of *P. falciparum* to artemisinins by increasing the unfolded protein response pathway (UPR) and controls 300–400 genes involved in stress responses [112, 113]. On this basis, it could be hypothesized that resistance to artemisinins in *P. falciparum* may also rely on epigenetic regulation.

Given the alarming increase in resistance to known available treatments finding new anti-malaria compounds is urgent. It has already been shown for many years that apicidin is able to inhibit histone modifying enzymes such as HDACs in all the stages of the *P. falciparum* intraerythrocytic cycle leading to parasite death [35, 49]. Moreover, chaetocin, a histone methyl transferase inhibitor, is able to reverse blasticidin S resistance [114]. Targeting histone modification thus appears to be an effective way to eliminate the malaria parasite, including resistant forms associated with diverse resistance mechanisms. Therefore, epigenetic drugs are very promising candidates because they target both the mechanisms of adaptability of the parasite to variations in its environment and its cell cycle regulation system. Interestingly, this approach can be extended at any stage of the parasite development cycle since epigenetics plays a crucial role throughout the parasite life cycle.

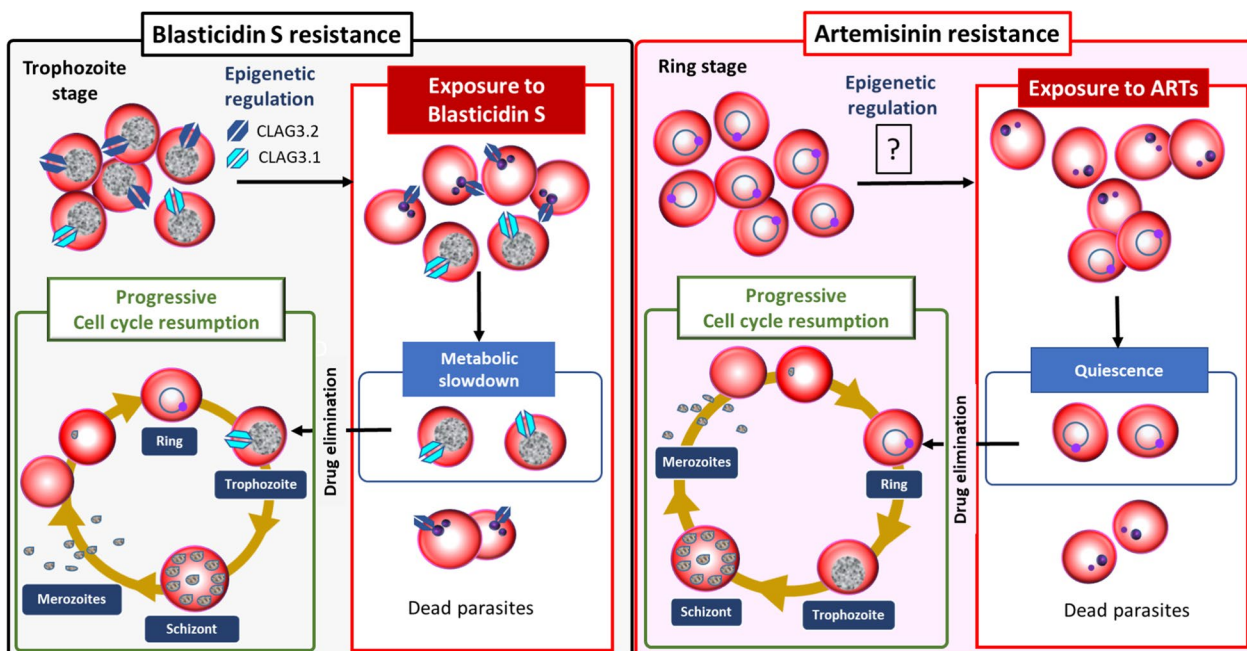


Fig. 4 Role of epigenetics in the acquisition of drug resistance, a parallel between resistance to blasticidin S and artemisinin resistance. Upon the exposure of a trophozoite population to Blasticidin S, a majority of them dies because most of them express the CLAG3.2 protein at their cell surface. Only a subset of parasites express the CLAG3.1 protein, which allows them to withstand drug exposure at the cost of a slower metabolism. This regulation of CLAG3 genes is epigenetically-mediated, allowing a stochastic number of parasites to survive drug pressure [102, 103]. Following exposure to artemisinins, a subset of ring-stage parasites can enter a quiescent state by slowing down their metabolism. After drug removal, the parasites can resume their life cycle [110]

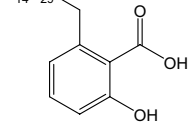
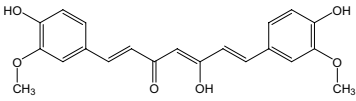
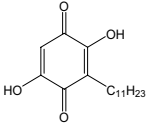
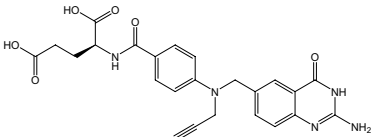
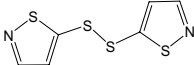
Targeting histone modification

Targeting histone acetyltransferases/histone deacetylases

Inhibition of *P. falciparum* HATs has been largely described with anacardic acid, curcumin and embelin, resulting in hypoacetylation of lysine residues (Table 2). However, these inhibitors are highly unspecific since they can have many other effects such as on lipooxygenase activities, reactive oxygen species production, disruption of chaperone expression [115, 116]. At the opposite CB3717 was identified as showing strong selective inhibition of *PfGCN5* (which differs strongly from its human orthologue [74]) leading to a decrease of H3 acetylation level at K9 position. This compound with an IC_{50} at 200 nM range in parasite growth assay is tenfold less active against human cancer cells and shows no effect against mammalian fibroblast cells up to 20 μ M [117]. *PfMYST* is potentially another interesting target as it also differs significantly from its human orthologue and NU9056, a thiazole derivative, inhibiting *PfMYST* catalytic activity is lethal for the parasite at a micromolar range [118] (Table 2). Although few *Plasmodium* HAT inhibitors have been described to date, new compounds designed to target HATs, involved in different pathologies, remain to be evaluated on the parasite and may provide interesting chemical starting points [119].

Among antiplasmodial epidrugs, HDAC inhibitors are the most numerous with a wide variety of chemical structures (cyclic tetrapeptides, 2-aminosuberic acid derivatives or L-cysteine derivatives) (Table 3). Most of them were initially designed to target human cancer cells, and were later found to have high activities against *P. falciparum* with IC_{50} values ranging from low nanomolar to sub-micromolar but low selectivity for most of them [120, 121]. SAHA and CTP-NPDG 19, a cyclic tetrapeptide like apicidin display better activities against *P. falciparum* than towards cancer cells but the selectivity index remains weak [78, 122]. Use of HDAC inhibitors, such as trichostatin A and apicidin results in a significant increase in H4K8ac and H4Ac4 levels across the *Plasmodium* genome, both in asexual and sexual stages of *Plasmodium* [35, 123]. These changes in histone marks lead in turn irreversibly to a collapse of the tightly regulated transcriptional cascade in the early hours of drug exposure and ultimately to parasite death upon longer exposures [35, 122]. FNDR-20123, a hydroxamate derivative like SAHA and trichostatin A, appears to be a very promising HDAC inhibitor in a nanomolar range, with a good PK/PD and excellent safety profile [124]. Derivatives of the clinical anticancer drug candidate quisinostat, such as JX21108, a *PfHDAC1* inhibitor, present a good

Table 2 Histone acetyltransferases (HAT) inhibitors in *P. falciparum*

Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	CC ₅₀ on mammalian cells in μM (Cell type)
Anacardic Acid		30 [115]	Targets lipoxygenase activity [116]	> 100 (HeLa) [145]
Curcumin		25 [97]	No specificity, targets all HATs and involved in production of ROS [97]	4 (HL60) [146]
Embelin		10 [73]	Specific towards GCN5	> 40 (mammalian) [73]
CB3717		0.225; 1 [117]	Specific towards PfGCN5	> 20 (NIH3T3) [117]
NU9056		0.9 [118]	Specific towards PfMyst	-

antimalarial activity and promising selectivity in vitro as in vivo in the *P. yoelli* mouse malaria model [125, 126] (Table 3).

Targeting histone methyltransferases/histone demethylases

The inhibition of HKMTs and HKDMs has been significantly less studied than the inhibition of HATs or HDACs in *P. falciparum*, probably due to their late identification. Nevertheless, some HKMT inhibitors have shown promising results with largely better selectivity index than the above-mentioned molecules and have gone so far as to be tested in vivo [127, 128] (Table 4). BIX-01294 and TM2-115 were shown to affect particularly H3K4me3 [127]. Although TM2-115 and BIX-01294 had a long-lasting effect on both *P. berghei* and *P. falciparum* parasites in mice models, they did not completely cure mice. Because of their oral bioavailability and their rapid ability to kill parasites, they nevertheless represent a good starting point for further development of the diaminoquinazoline compound series [128]. TM2-115 was also shown to activate dormancy exit of hypnozoites in *Plasmodium vivax* [129]. Collectively, these results suggest that HKMT inhibitors are very promising since they seem to target most of the life-cycle stages of the parasite. A medicinal chemistry approach could help to improve their efficacy and their pharmacokinetic profiles. There are currently few reports of drug discovery efforts specifically targeting

arginine methylation in *P. falciparum* [90]. The search for new chemical starting points by an orthologue approach is not always successful as a recent study has shown on the evaluation of human histone demethylase inhibitors on their *P. falciparum* counterparts [130] (Table 5).

Targeting DNA modifications

For a time, targeting DNA methylation was generally overlooked in *P. falciparum*, mostly because of its low abundance [50]. Recently however, several quinazoline derivatives identified as human DNMT3 inhibitors were found to be active in vitro against *P. falciparum* and in vivo against *P. berghei* infected mice [131]. In a same way, series of quinoline-quinazoline bisubstrate analogues (Table 6), with an inhibitory activity towards human DNMT3a and DNMT1, has shown promising activities in the nanomolar range on *P. falciparum* strains [132].

Targeting transcription factors

The twenty-seven main factors regulating transcription in *P. falciparum* can also be considered as very interesting targets. Their binding domains are different from human homologs which can be a guarantee of specificity for an inhibitor of these transcription factors. In silico prediction combined with biochemical and genetic studies have led to the identification of compounds with

Table 3 Histone Deacetylase (HDAC) inhibitors in *P. falciparum*

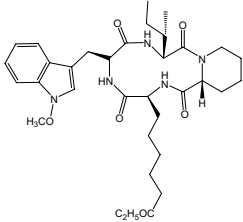
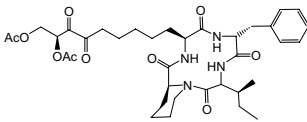
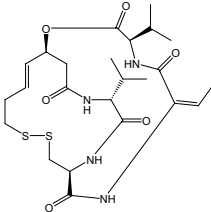
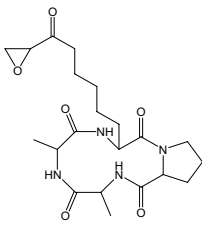
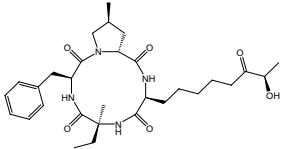
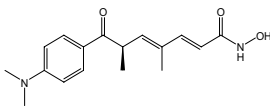
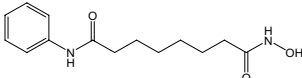
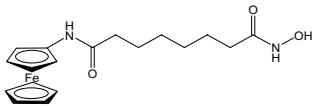
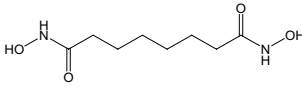
Targeting Class I and II HDACs					
Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
Cyclic tetrapeptides					
Apicidin		0.03; 0.04 [76, 147]	Targets class I and II HDACs [76]	–	10 (Jurkat) [148]
CTP-NPDG 19		0.3 [122]	Targets class I HDACs	–	> 25 (HepG2) [122]
Romidepsin		0.09 [149]	Targets class I and II HDACs	FDA-approved in cancer therapy	0.001 (NFF) [149]
HC-toxin		< 0.01 [49]	Targets class I and II HDACs	–	0.9 (TFK-1) [150]
FR235222		0.01 [151]	Targets class I HDACs	–	0.13 (HFF) [152]
Hydroxamates					
Trichostatin A		0.03; 0.08 [122, 147]	Targets class I and II HDACs	–	0.2 (HeLa) [153]
SAHA (Vorinostat)		0.2; 0.5 [76, 147]	Targets class I and II HDACs	FDA-approved in cancer therapy	5.5 (NFF) [149]
JAHA		0.5 [147]	Targets class I and II HDACs [154]	–	2.4 (MCF7) [154]
SBHA		1 [120]	Targets class I and II HDACs	–	12 (HEK) [155]

Table 3 (continued)**Targeting Class I and II HDACs**

Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
WR301801		0.001 [156]	Targets class I and II HDACs	Tested in vivo	0.6 (RAW) [156]
Belinostat		0.06 [149]	Targets class I and II HDACs	FDA-approved in cancer therapy	2.37 (NFF) [149]
Panobinostat		0.01 [149]	Targets class I and II HDACs	FDA-approved in cancer therapy	0.07 (NFF) [149]
FNDR-20123		0.04 [124]	Targets class I HDACs	–	> 100 (HepG2 & THP-1) [124]
JX21108		0.004 [125]	Targets class I HDACs	–	> 4 (HepG2) [125]
Compound 29		0.45 [139]	Targets class I HDACs	–	17 (Hela) [139]
Peptoid-based hydroxamic acids 2h		0.005 [138]	HDACs	–	4.6 (HepG2) [138]
2-aminosuberic acid derivatives		0.01; 0.3 [120]	Targets class I HDACs	–	0.2–5.8 (NFF) [120]
L-cysteine derivatives		0.05; 0.34 [120]	Targets class I HDACs	–	0.35–2.2 (NFF) [120]

sub-micromolar antiparasitic properties, demonstrating

Table 3 (continued)

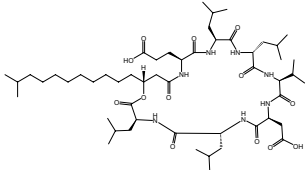
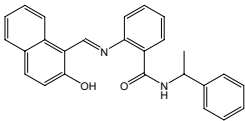
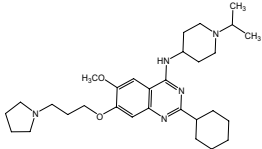
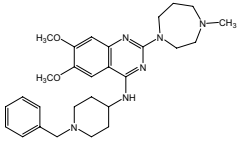
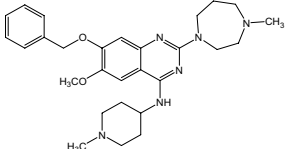
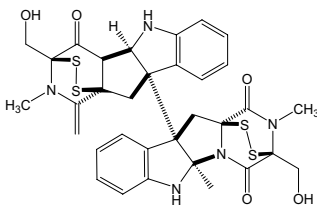
Targeting Class I and II HDACs					
Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
Targeting Sir2 HDAC (Class III)					
Surfactin		9.3 [79]	Unknown off-targets since it induces apoptosis	–	9.6 (MCF-7) [157]
Sirtinol		10 [120]	No off-targets known Inhibits both PfSir2A and B	–	> 25 (NFF) [120]

Table 4 Histone methyltransferase (HKMT) inhibitors in *P. falciparum* targeting lysine methylation

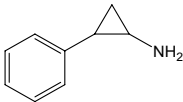
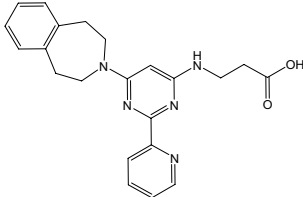
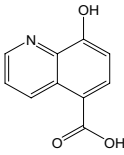
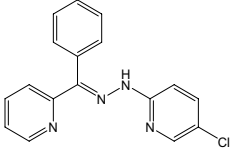
Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
UNC0638		0.028 [130]	Targets G9a methyltransferases in mammalian cells	–	23 (HL-60) [158]
BIX-01294		0.056; 0.075 [127, 147]	No target properly identified but inhibits H3K4me3	Tested on a humanized mouse model	6.1 (HFF) [127]
TM2-115		0.130; 0.137 [127, 147]	Identical to BIX-01294	Tested on a humanized mouse model	5.7 (HFF) [127]
Chaetocin		0.64; 0.95 [147, 159]; 14 [90]	No target properly identified but inhibits H3K9me2/me3	–	0.13 (HL-60) [158]

the relevance of this approach [29].

Epidrugs: a promising future?

Despite the fact that seven epidrugs such as DNMT (5-azacytidine) and HDAC inhibitors (SAHA) [133, 134] have been approved by government agencies (e.g. the FDA) in cancer therapies, lack of selectivity towards *Plasmodium* has been the major Achilles' heel in the development of antiplasmodial epidrugs. While a very good

Table 5 Histone Demethylases (HKDM) inhibitors in *P. falciparum* targeting lysine demethylation

Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
Tranylcypromine		> 10 [160]	Inhibits human LSD1 [161]	FDA-approved monoamine oxidase inhibitor	> 200 (HEK293) [162]
GSK-J1		> 10 [130]	Inhibits human Jumonji demethylases	–	9 (Human primary macrophages) [163]
IOX 1		1; 10 [160]	Inhibits human Jumonji demethylases [164]	–	86.5 (HeLa) [164]
JIB-04		0.6; 1.6; 0.47 [96, 147, 160]	Inhibits human Jumonji demethylases [96, 165]	–	> 10 (Human mesenchymal stem) [166]

activity has been observed in vitro, in the nanomolar range on the parasite, the in vivo results in the *P. berghei* infected mouse model are often more than modest and administration of these compounds cannot cure the mice completely [135]. Nevertheless, as more and more efforts are being made to understand the mechanisms of epigenetic regulation of *P. falciparum*, the repositioning of anti-cancer drugs in the context of the search for new anti-malarial drugs remains very topical [125, 136].

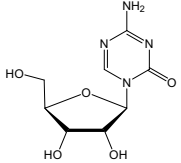
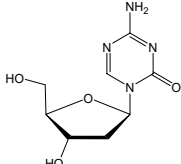
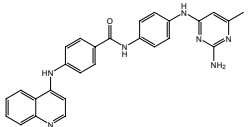
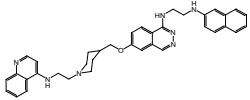
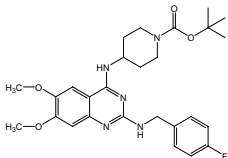
In silico approaches, through molecular docking, also led to the discovery of a new portfolio of parasite specific HAT inhibitors [77, 117] and quantitative structure–activity relationship (QSAR) models have been developed as useful tools for in silico screening of *Plasmodium* HDAC inhibitors [137]. However, recent progress has been made in the field of malaria epidrugs in order to overcome potential toxicity issues in the mammalian host. SAR studies in vitro have led to the discovery of parasite specific compounds targeting HDACs and DNMTs with inhibitory values in sub-micromolar and nanomolar ranges and good selectivity indexes (>100) [124, 132, 138, 139]. A recent review of inhibitors targeting *Plasmodium* HDACs and DNMTs lists the best compounds based on scaffold from a screening of epidrug libraries or from molecular docking studies. It highlights, in particular, the interest for in silico studies to optimize

selectivity, pharmacokinetic properties and cost of goods [140].

Combining two different inhibitors in the same molecule is a very interesting approach, especially if they have a strong synergy. The hybrid compound procainamide-SAHA, which combines a DNMT inhibitor (Procainamide) with an HDAC1 inhibitor (SAHA), has been shown to be highly active against *P. falciparum* multidrug-resistant field strains and lacks cytotoxicity against human cancer cells [141].

Therefore, epidrugs present several characteristics that are very desirable for an anti-malarial drug. Indeed, they can be fast-acting and could have high parasite-killing rates, probably due to the need for continual gene activation along the life-cycle stages. Pharmacomodulation work has allowed notable increase of selectivity against *Plasmodium*. Moreover, due to the high conservation of histone modification enzymes and their assumed conserved role in transcriptional regulation across *Plasmodium* species, epidrugs are likely to be efficient on all human malaria pathogens, among which *P. falciparum* and *P. vivax* [72, 91, 142] and one could envisage a unique compound to treat all types of malaria. Epidrugs are very promising candidates because their interest lies in their ability to target both one of the major mechanisms of adaptability of the parasite to variations

Table 6 DNA Methyltransferases (DNMT) inhibitors in *P. falciparum*

Inhibitors	Structure	<i>P. falciparum</i> IC ₅₀ in μM	Specificity	Development stage	CC ₅₀ on mammalian cells in μM (Cell type)
5-azacytidine		1.5.10 ³ [51]	Human DNMT [167]	FDA-approved in cancer therapy [167]	2.3 (KG-1a acute myeloid leukemia) [168]
Decitabine		8.10 ² [51]	Human DNMT [167]	FDA-approved in cancer therapy [167]	0.4 (KG-1a acute myeloid leukemia) [168]
SGL-1027		0.063; 0.051 [147, 160]	Human DNMT 1, 3A, 3B [169]	-	> 50 (H4IIE rat hepatoma) [169]
Quinoline-quinazoline derivative		0.06 [132]	PfDNMT	Tested in standard chemosensitivity assays on ART-resistant strains	2.5 (HepG2) [132]
Quinazoline derivative		0.019 [131]	PfDNMT3	Tested in standard chemosensitivity assays on multidrug resistant strains	> 1 (Splenic) [131]

in its environment and its cell cycle regulation system. Whether during the intraerythrocytic cycle (pathogenicity), the induction of gametocytogenesis (transmission) or even in the DNA repair mechanisms following stress due to a change in the environment, epigenetics offers new opportunities for therapeutic targets covering all the different states of the parasite.

Author contributions

TR, LP, JMA, LDS and FBV wrote the main manuscript text and JMA prepared figures. All authors reviewed the manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. WHO. World malaria report. Geneva: World Health Organization; 2023.
2. Ouji M, Augereau JM, Paloque L, Benoit-Vical F. *Plasmodium falciparum* resistance to artemisinin-based combination therapies: a sword of Damocles in the path toward malaria elimination. *Parasite*. 2018;25:24.
3. Dhorda M, Amaratunga C, Dondorp AM. Artemisinin and multidrug-resistant *Plasmodium falciparum* - a threat for malaria control and elimination. *Curr Opin Infect Dis*. 2021;34:432–9.
4. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyanza T, et al. Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *Lancet Infect Dis*. 2021;21:1120–8.

5. Uwimana A, Legrand E, Stokes BH, Ndikumana JM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med*. 2020;26:1602–8.
6. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. *N Engl J Med*. 2021;385:1163–71.
7. WHO. World malaria report. Geneva: World Health Organization; 2022.
8. Serrano-Durán R, López-Farfán D, Gómez-Díaz E. Epigenetic and epitranscriptomic gene regulation in *Plasmodium falciparum* and how we can use it against malaria. *Genes*. 2022;13:1734.
9. Waddington CH. The epigenotype. *Int J Epidemiol*. 2012;41:10–3.
10. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet*. 2016;17:487–500.
11. Almouzni G, Cedar H. Maintenance of epigenetic information. *Cold Spring Harb Perspect Biol*. 2016;8: a019372.
12. Mariño-Ramírez L, Kann MG, Shoemaker BA, Landsman D. Histone structure and nucleosome stability. *Expert Rev Proteomics*. 2005;2:719–29.
13. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011;21:381–95.
14. Tan M, Luo H, Lee S, Jin F, Yang Jeong S, Montellier E, et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell*. 2011;146:1016–28.
15. Marsano RM, Giordano E, Messina G, Dimitri P. A new portrait of constitutive heterochromatin: lessons from *Drosophila melanogaster*. *Trends Genet*. 2019;35:615–31.
16. Miao J, Fan Q, Cui L, Li J, Li J, Cui L. The malaria parasite *Plasmodium falciparum* histones: organization, expression, and acetylation. *Gene*. 2006;369:53–65.
17. Black Joshua C, Van Rechem C, Whetstone JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell*. 2012;48:491–507.
18. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001;293:1074–80.
19. Wang H, Fan Z, Shliaha PV, Miele M, Hendrickson RC, Jiang X, et al. H3K4me3 regulates RNA polymerase II promoter-proximal pause-release. *Nature*. 2023;615:339–48.
20. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. *Exp Mol Med*. 2017;49:324.
21. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007;128:693–705.
22. Peppenella S, Murphy KJ, Hayes JJ. Intra- and inter-nucleosome interactions of the core histone tail domains in higher-order chromatin structure. *Chromosoma*. 2014;123:3–13.
23. Iizuka M, Smith MM. Functional consequences of histone modifications. *Curr Opin Genet Dev*. 2003;13:154–60.
24. Breiling A, Lyko F. Epigenetic regulatory functions of DNA modifications: 5-methylcytosine and beyond. *Epigenetics Chromatin*. 2015;8:24.
25. Baubec T, Schübeler D. Genomic patterns and context specific interpretation of DNA methylation. *Curr Opin Genet Dev*. 2014;25:85–92.
26. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci*. 2006;31:89–97.
27. Balaji S. Discovery of the principal specific transcription factors of Apicomplexa and their implication for the evolution of the AP2-integrase DNA binding domains. *Nucleic Acids Res*. 2005;33:3994–4006.
28. Jenning M, Quinn J, Petter M. ApiAP2 transcription factors in Apicomplexan parasites. *Pathogens*. 2019;8:47.
29. Russell TJ, De Silva EK, Crowley VM, Shaw-Saliba K, Dube N, Josling G, et al. Inhibitors of ApiAP2 protein DNA binding exhibit multi-stage activity against *Plasmodium* parasites. *PLoS Pathog*. 2022;18: e1010887.
30. Subudhi AK, Green JL, Satyam R, Salunke RP, Lenz T, Shuaib M, et al. DNA-binding protein PfAP2-P regulates parasite pathogenesis during malaria parasite blood stages. *Nat Microbiol*. 2023;8:2154–69.
31. Quansah E, Pappoe F, Shen J, Liu M, Yang S, Yu L, et al. ApiAP2 gene-network regulates gametocytogenesis in *Plasmodium* parasites. *Cell Microbiol*. 2022;2022:5796578.
32. Bancells C, Llorca-Battle O, Poran A, Notzel C, Rovira-Graells N, Elemento O, et al. Revisiting the initial steps of sexual development in the malaria parasite *Plasmodium falciparum*. *Nat Microbiol*. 2019;4:144–54.
33. Coulson RM, Hall N, Ouzounis CA. Comparative genomics of transcriptional control in the human malaria parasite *Plasmodium falciparum*. *Genome Res*. 2004;14:1548–54.
34. Zanghi G, Vembar SS, Baumgarten S, Ding S, Guizetti J, Bryant JM, et al. A Specific PfEMP1 is expressed in *P. falciparum* sporozoites and plays a role in hepatocyte infection. *Cell Rep*. 2018;22:2951–63.
35. Chaal BK, Gupta AP, Wastuwidyaningtyas BD, Luah YH, Bozdech Z. Histone deacetylases play a major role in the transcriptional regulation of the *Plasmodium falciparum* life cycle. *PLoS Pathog*. 2010;6: e1000737.
36. Cortés A, Deitsch KW. Malaria epigenetics. *Cold Spring Harb Perspect Med*. 2017;7: a025528.
37. Gómez-Díaz E, Yerbanga RS, Lefèvre T, Cohuet A, Rowley MJ, Ouedraogo JB, et al. Epigenetic regulation of *Plasmodium falciparum* clonally variant gene expression during development in *Anopheles gambiae*. *Sci Rep*. 2017;7:40655.
38. Connacher J, von Grüning H, Birkholtz L. Histone modification landscapes as a roadmap for malaria parasite development. *Front Cell Dev Biol*. 2022;10: 848797.
39. Duraisingh MT, Skillman KM. Epigenetic variation and regulation in malaria parasites. *Annu Rev Microbiol*. 2018;72:355–75.
40. Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. *PLoS Biol*. 2003;1:E5.
41. Goyal M, Heinberg A, Mitesser V, Kandelis-Shalev S, Singh BK, Dzikowski R, et al. Phosphorylation of the canonical histone H2A marks foci of damaged DNA in malaria parasites. *mSphere*. 2021;6:e01131–20.
42. Miao J, Lawrence M, Jeffers V, Zhao F, Parker D, Ge Y, et al. Extensive lysine acetylation occurs in evolutionarily conserved metabolic pathways and parasite-specific functions during *Plasmodium falciparum* intraerythrocytic development. *Mol Microbiol*. 2013;89:660–75.
43. Kafack BFC, Rovira-Graells N, Clark TG, Bancells C, Crowley VM, Campino SG, et al. A transcriptional switch underlies commitment to sexual development in malaria parasites. *Nature*. 2014;507:248–52.
44. Sinha A, Hughes KR, Modrzynska KK, Otto TD, Pfander C, Dickens NJ, et al. A cascade of DNA-binding proteins for sexual commitment and development in *Plasmodium*. *Nature*. 2014;507:253–7.
45. Coleman BI, Skillman KM, Jiang Rays HY, Childs LM, Altenhofen LM, Ganter M, et al. A *Plasmodium falciparum* histone deacetylase regulates antigenic variation and gametocyte conversion. *Cell Host Microbe*. 2014;16:177–86.
46. van Bijljon R, van Wyk R, Painter HJ, Orchard L, Reader J, Niemand J, et al. Hierarchical transcriptional control regulates *Plasmodium falciparum* sexual differentiation. *BMC Genomics*. 2019;20:920.
47. Rovira-Graells N, Gupta AP, Planet E, Crowley VM, Mok S, Ribas de Pouplana L, et al. Transcriptional variation in the malaria parasite *Plasmodium falciparum*. *Genome Res*. 2012;22:925–38.
48. Andrews KT, Walduck A, Kelso MJ, Fairlie DP, Saul A, Parsons PG. Antimalarial effect of histone deacetylation inhibitors and mammalian tumour cytodifferentiating agents. *Int J Parasitol*. 2000;30:761–8.
49. Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, Allocco JJ, et al. Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proc Natl Acad Sci USA*. 1996;93:13143–7.
50. Hammam E, Ananda G, Sinha A, Scheidig-Benatar C, Bohec M, Preiser PR, et al. Discovery of a new predominant cytosine DNA modification that is linked to gene expression in malaria parasites. *Nucl Acids Res*. 2020;48:184–99.
51. Ponts N, Fu L, Harris EY, Zhang J, Chung DW, Cervantes MC, et al. Genome-wide mapping of DNA methylation in the human malaria parasite *Plasmodium falciparum*. *Cell Host Microbe*. 2013;14:696–706.
52. McInroy GR, Beraldi D, Raiber EA, Modrzynska K, van Delft P, Billker O, et al. Enhanced methylation analysis by recovery of unsequenceable fragments. *PLoS ONE*. 2016;11: e0152322.
53. Hammam E, Sinha A, Baumgarten S, Nardella F, Liang J, Miled S, et al. Malaria parasite stress tolerance is regulated by DNMT2-mediated tRNA cytosine methylation. *mBio*. 2021;12:0255821.
54. Baumgarten S, Bryant JM, Sinha A, Reyser T, Preiser PR, Dedon PC, et al. Transcriptome-wide dynamics of extensive m(6)A mRNA methylation

- during *Plasmodium falciparum* blood-stage development. *Nat Microbiol.* 2019;4:2246–59.
55. Vembar SS, Scherf A, Siegel TN. Noncoding RNAs as emerging regulators of *Plasmodium falciparum* virulence gene expression. *Curr Opin Microbiol.* 2014;20:153–61.
 56. Lucky AB, Wang C, Liu M, Liang X, Min H, Fan Q, et al. A type II protein arginine methyltransferase regulates merozoite invasion in *Plasmodium falciparum*. *Commun Biol.* 2023;6:659.
 57. Batugedara G, Lu XM, Hristov B, Abel S, Chahine Z, Hollin T, et al. Novel insights into the role of long non-coding RNA in the human malaria parasite. *Plasmodium falciparum Nat Commun.* 2023;14:5086.
 58. Saraf A, Cervantes S, Bunnik EM, Pons N, Sardu ME, Chung D-WD, et al. Dynamic and combinatorial landscape of histone modifications during the intraerythrocytic developmental cycle of the malaria parasite. *J Proteome Res.* 2016;15:2787–801.
 59. Dastidar EG, Dzeyk K, Krijgsveld J, Malmquist NA, Doerig C, Scherf A, et al. Comprehensive histone phosphorylation analysis and identification of Pf14-3-3 protein as a histone H3 phosphorylation reader in malaria parasites. *PLoS ONE.* 2013;8: e53179.
 60. Trelle MB, Salcedo-Amaya AM, Cohen AM, Stunnenberg HG, Jensen ON. Global histone analysis by mass spectrometry reveals a high content of acetylated lysine residues in the malaria parasite *Plasmodium falciparum*. *J Proteome Res.* 2009;8:3439–50.
 61. von Grüning H, Coradin M, Mendoza MR, Reader J, Sidoli S, Garcia BA, et al. A dynamic and combinatorial histone code drives malaria parasite asexual and sexual development. *Mol Cell Proteomics.* 2022;21: 100199.
 62. Ruiz JL, Tena JJ, Bancells C, Cortés A, Gómez-Skarmeta JL, Gómez-Díaz E. Characterization of the accessible genome in the human malaria parasite *Plasmodium falciparum*. *Nucleic Acids Res.* 2018;46:9414–31.
 63. Bártfai R, Hoeijmakers WA, Salcedo-Amaya AM, Smits AH, Janssen-Megens E, Kaan A, et al. H2AZ demarcates intergenic regions of the *Plasmodium falciparum* epigenome that are dynamically marked by H3K9ac and H3K4me3. *PLoS Pathog.* 2010;6:1001223.
 64. Shrestha S, Lucky AB, Brashear AM, Li X, Cui L, Miao J. Distinct histone post-translational modifications during *Plasmodium falciparum* gametocyte development. *J Proteome Res.* 2022;21:1857–67.
 65. Salcedo-Amaya AM, van Driel MA, Alako BT, Trelle MB, van den Elzen AMG, Cohen AM, et al. Dynamic histone H3 epigenome marking during the intraerythrocytic cycle of *Plasmodium falciparum*. *Proc Natl Acad Sci USA.* 2009;106:9655–60.
 66. Lopez-Rubio J-J, Mancio-Silva L, Scherf A. Genome-wide analysis of heterochromatin associates clonally variant gene regulation with perinucleus repressive centers in malaria parasites. *Cell Host Microbe.* 2009;5:179–90.
 67. Coetzee N, Sidoli S, van Biljon R, Painter H, Llinás M, Garcia BA, et al. Quantitative chromatin proteomics reveals a dynamic histone post-translational modification landscape that defines asexual and sexual *Plasmodium falciparum* parasites. *Sci Rep.* 2017;7:607.
 68. Lucky AB, Wang C, Li X, Chim-Ong A, Adapa Swamy R, Quinlivan EP, et al. Characterization of the dual role of *Plasmodium falciparum* DNA methyltransferase in regulating transcription and translation. *Nucleic Acids Res.* 2023;51:3918–33.
 69. Wei H, Jiang S, Chen L, He C, Wu S, Peng H. Characterization of cytosine methylation and the DNA methyltransferases of *Toxoplasma gondii*. *Int J Biol Sci.* 2017;13:458–70.
 70. Adams R. 1996. DNA methylation. In: Bittar EE, Bittar N (eds). Chapter 3 Principles of Medical Biology University of Wisconsin Madison Molecular and Cellular Genetics. Elsevier. Amsterdam.
 71. Cui L, Miao J. Chromatin-mediated epigenetic regulation in the malaria parasite *Plasmodium falciparum*. *Eukaryot Cell.* 2010;9:1138–49.
 72. Fan Q, An L, Cui L. *Plasmodium falciparum* histone acetyltransferase, a yeast GCN5 homologue involved in chromatin remodeling. *Eukaryot Cell.* 2004;3:264–76.
 73. Srivastava S, Bhowmick K, Chatterjee S, Basha J, Kundu TK, Dhar SK. Histone H3K9 acetylation level modulates gene expression and may affect parasite growth in human malaria parasite *Plasmodium falciparum*. *FEBS J.* 2014;281:5265–78.
 74. Miao J, Wang C, Lucky AB, Liang X, Min H, Adapa SR, et al. A unique GCN5 histone acetyltransferase complex controls erythrocyte invasion and virulence in the malaria parasite *Plasmodium falciparum*. *PLoS Pathog.* 2021;17: e1009351.
 75. Miao J, Fan Q, Cui L, Li X, Wang H, Ning G, et al. The MYST family histone acetyltransferase regulates gene expression and cell cycle in malaria parasite *Plasmodium falciparum*. *Mol Microbiol.* 2010;78:883–902.
 76. Patel V, Mazitschek R, Coleman B, Nguyen C, Urgaonkar S, Cortese J, et al. Identification and characterization of small molecule inhibitors of a class I histone deacetylase from *Plasmodium falciparum*. *J Med Chem.* 2009;52:2185–7.
 77. Kumar A, Dhar SK, Subbarao N. In silico identification of inhibitors against *Plasmodium falciparum* histone deacetylase 1 (PfHDAC-1). *J Mol Model.* 2018;24:232.
 78. Andrews KT, Haque A, Jones MK. HDAC inhibitors in parasitic diseases. *Immunol Cell Biol.* 2012;90:66–77.
 79. Chakrabarty SP, Saikumari YK, Bopanna MP, Balam H. Biochemical characterization of *Plasmodium falciparum* Sir2, a NAD⁺-dependent deacetylase. *Mol Biochem Parasitol.* 2008;158:139–51.
 80. Neugebauer RC, Sippl W, Jung M. Inhibitors of NAD⁺ dependent histone deacetylases (sirtuins). *Curr Pharm Des.* 2008;14:562–73.
 81. Joshi MB, Lin DT, Chiang PH, Goldman ND, Fujioka H, Aikawa M, et al. Molecular cloning and nuclear localization of a histone deacetylase homologue in *Plasmodium falciparum*. *Mol Biochem Parasitol.* 1999;99:11–9.
 82. Horrocks P, Wong E, Russell K, Emes RD. Control of gene expression in *Plasmodium falciparum* – Ten years on. *Mol Biochem Parasitol.* 2009;164:9–25.
 83. Rono MK, Nyonda MA, Simam JJ, Ngoi JM, Mok S, Kortok MM, et al. Adaptation of *Plasmodium falciparum* to its transmission environment. *Nat Ecol Evol.* 2018;2:377–87.
 84. Kanyal A, Rawat M, Gurung P, Choubey D, Anamika K, Karmodiya K. Genome-wide survey and phylogenetic analysis of histone acetyltransferases and histone deacetylases of *Plasmodium falciparum*. *FEBS J.* 2018;285:1767–82.
 85. Duraisingh MT, Voss TS, Marty AJ, Duffy MF, Good RT, Thompson JK, et al. Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in *Plasmodium falciparum*. *Cell.* 2005;121:13–24.
 86. Freitas-Junior LH, Hernandez-Rivas R, Ralph SA, Montiel-Condado D, Ruvalcaba-Salazar OK, Rojas-Meza AP, et al. Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. *Cell.* 2005;121:25–36.
 87. Religa AA, Waters AP. Sirtuins of parasitic protozoa: In search of function(s). *Mol Biochem Parasitol.* 2012;185:71–88.
 88. Tonkin CJ, Carret CK, Duraisingh MT, Voss TS, Ralph SA, Hommel M, et al. Sir2 paralogs cooperate to regulate virulence genes and antigenic variation in *Plasmodium falciparum*. *PLoS Biol.* 2009;7: e84.
 89. Lee IH. Mechanisms and disease implications of sirtuin-mediated autophagic regulation. *Exp Mol Med.* 2019;51:1–11.
 90. Fan Q, Miao J, Cui L, Cui L. Characterization of PRMT1 from *Plasmodium falciparum*. *Biochem J.* 2009;421:107–18.
 91. Cui L, Fan Q, Cui L, Miao J. Histone lysine methyltransferases and demethylases in *Plasmodium falciparum*. *Int J Parasitol.* 2008;38:1083–97.
 92. Volz Jennifer C, Bártfai R, Petter M, Langer C, Josling Gabrielle A, Tsuboi T, et al. PfSET10, a *Plasmodium falciparum* methyltransferase, maintains the active var gene in a poised state during parasite division. *Cell Host Microbe.* 2012;11:7–18.
 93. Ngwa CJ, Gross MR, Musabyimana JP, Pradel G, Deitsch KW. The role of the histone methyltransferase PfSET10 in antigenic variation by malaria parasites: a cautionary tale. *mSphere.* 2021;6:01217–20.
 94. Jiang L, Mu J, Zhang Q, Ni T, Srinivasan P, Rayavara K, et al. PfSETvs methylation of histone H3K36 represses virulence genes in *Plasmodium falciparum*. *Nature.* 2013;499:223–7.
 95. Duffy MF, Selvarajah SA, Josling GA, Petter M. The role of chromatin in *Plasmodium* gene expression. *Cell Microbiol.* 2012;14:819–28.
 96. Matthews KA, Senagbe KM, Nötzel C, Gonzales CA, Tong X, Rijo-Ferreira F, et al. Disruption of the *Plasmodium falciparum* life cycle through transcriptional reprogramming by inhibitors of jumoni demethylases. *ACS Infect Dis.* 2020;6:1058–75.
 97. Cui L, Miao J, Cui L. Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: inhibition of histone acetylation and

- generation of reactive oxygen species. *Antimicrob Agents Chemother.* 2007;51:488–94.
98. Bechtsi DP, Waters AP. Genomics and epigenetics of sexual commitment in *Plasmodium*. *Int J Parasitol.* 2017;47:425–34.
 99. Brancucci NMB, Bertschli NL, Zhu L, Niederwieser I, Chin Wai H, Wampfler R, et al. Heterochromatin protein 1 secures survival and transmission of malaria parasites. *Cell Host Microbe.* 2014;16:165–76.
 100. Flueck C, Bartfai R, Volz J, Niederwieser I, Salcedo-Amaya AM, Alako BT, et al. *Plasmodium falciparum* heterochromatin protein 1 marks genomic loci linked to phenotypic variation of exported virulence factors. *PLoS Pathog.* 2009;5: e1000569.
 101. Govindaraju G, Jabeena CA, Sethumadhavan DV, Rajaram N, Rajavelu A. DNA methyltransferase homologue TRDMT1 in *Plasmodium falciparum* specifically methylates endogenous aspartic acid tRNA. *Biochim Biophys Acta Gene Regul Mech.* 2017;1860:1047–57.
 102. Mira-Martinez S, Rovira-Graells N, Crowley VM, Altenhofen LM, Llinás M, Cortés A. Epigenetic switches in clag3 genes mediate blasticidin S resistance in malaria parasites. *Cell Microbiol.* 2013;15:1913–23.
 103. Sharma P, Wollenberg K, Sellers M, Zainabadi K, Galinsky K, Moss E, et al. An epigenetic antimalarial resistance mechanism involving parasite genes linked to nutrient uptake. *J Biol Chem.* 2013;288:19429–40.
 104. Mira-Martinez S, Pickford AK, Rovira-Graells N, Guetens P, Tinto-Font E, Cortes A, et al. Identification of antimalarial compounds that require CLAG3 for their uptake by *Plasmodium falciparum*-infected erythrocytes. *Antimicrob Agents Chemother.* 2019;63:e00052–e119.
 105. Nguiragool W, Bokhari Abdullah AB, Pillai Ajay D, Rayavara K, Sharma P, Turpin B, et al. Malaria parasite clag3 genes determine channel-mediated nutrient uptake by infected red blood cells. *Cell.* 2011;145:665–77.
 106. Cortés A, Carret C, Kaneko O, Yim Lim BY, Ivens A, Holder AA. Epigenetic silencing of *Plasmodium falciparum* genes linked to erythrocyte invasion. *PLoS Pathog.* 2007;3: e107.
 107. Crowley VM, Rovira-Graells N, de Pouplana LR, Cortés A. Heterochromatin formation in bistable chromatin domains controls the epigenetic repression of clonally variant *Plasmodium falciparum* genes linked to erythrocyte invasion. *Mol Microbiol.* 2011;80:391–406.
 108. Ariei F, Witkowski B, Amarantunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature.* 2014;505:50–5.
 109. Birnbaum J, Scharf S, Schmidt S, Jonscher E, Hoijmakers WAM, Flemming S, et al. A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. *Science.* 2020;367:51–9.
 110. Witkowski B, Lelievre J, Barragan MJ, Laurent V, Su XZ, Berry A, et al. Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. *Antimicrob Agents Chemother.* 2010;54:1872–7.
 111. Yu X, Wang C, Zhao Y, Tang J, Zhu M, Platon L, et al. Ring-stage growth arrest: Metabolic basis of artemisinin tolerance in *Plasmodium falciparum*. *iScience.* 2023;26:105725.
 112. Rawat M, Kanyal A, Sahasrabudhe A, Vembar SS, Lopez-Rubio J-J, Karmodiya K. Histone acetyltransferase PfGCN5 regulates stress responsive and artemisinin resistance related genes in *Plasmodium falciparum*. *Sci Rep.* 2021;11:852.
 113. Lucky AB, Wang C, Shakri AR, Kalamuddin M, Chim-Ong A, Li X, et al. *Plasmodium falciparum* GCN5 plays a key role in regulating artemisinin resistance-related stress responses. *Antimicrob Agents Chemother.* 2023;67: e0057723.
 114. Chan A, Dziedzic A, Kirkman LA, Deitsch KW, Ankarklev J. A histone methyltransferase inhibitor can reverse epigenetically acquired drug resistance in the malaria parasite *Plasmodium falciparum*. *Antimicrob Agents Chemother.* 2020;64:e02021–e2119.
 115. Cui L, Miao J, Furuya T, Fan Q, Li X, Rathod PK, et al. Histone acetyltransferase inhibitor anacardic acid causes changes in global gene expression during in vitro *Plasmodium falciparum* development. *Eukaryot Cell.* 2008;7:1200–10.
 116. Ha TJ, Kubo I. Lipoygenase inhibitory activity of anacardic acids. *J Agric Food Chem.* 2005;53:4350–4.
 117. Kumar A, Bhowmick K, Vikramdeo KS, Mondal N, Subbarao N, Dhar SK. Designing novel inhibitors against histone acetyltransferase (HAT: GCN5) of *Plasmodium falciparum*. *Eur J Med Chem.* 2017;138:26–37.
 118. Sen U, Nayak A, Khurana J, Sharma D, Gupta A. Inhibition of PfMYST histone acetyltransferase activity blocks *Plasmodium falciparum* growth and survival. *Antimicrob Agents Chemother.* 2020;65:e00953–e1020.
 119. Sabnis RW. Novel histone acetyltransferase (HAT) inhibitors for treating diseases. *ACS Med Chem Lett.* 2021;12:1198–9.
 120. Andrews KT, Tran TN, Lucke AJ, Kahnberg P, Le GT, Boyle GM, et al. Potent antimalarial activity of histone deacetylase inhibitor analogues. *Antimicrob Agents Chemother.* 2008;52:1454–61.
 121. Andrews KT, Gupta AP, Tran TN, Fairlie DP, Gobert GN, Bozdech Z. Comparative gene expression profiling of *P falciparum* malaria parasites exposed to three different histone deacetylase inhibitors. *PLoS One.* 2012;7:e31847.
 122. Collins JE, Lee JW, Bohmer MJ, Welden JD, Arshadi AK, Du L, et al. Cyclic tetrapeptide HDAC inhibitors with improved *Plasmodium falciparum* selectivity and killing profile. *ACS Infect Dis.* 2021;7:2889–903.
 123. Trenholme K, Marek L, Duffy S, Pradel G, Fisher G, Hansen FK, et al. Lysine acetylation in sexual stage malaria parasites is a target for antimalarial small molecules. *Antimicrob Agents Chemother.* 2014;58:3666–78.
 124. Potluri V, Shandil RK, Gavara R, Sambasivam G, Campo B, Wittlin S, et al. Discovery of FNDR-20123, a histone deacetylase inhibitor for the treatment of *Plasmodium falciparum* malaria. *Malar J.* 2020;19:365.
 125. Huang Z, Li R, Tang T, Ling D, Wang M, Xu D, et al. A novel multistage antiplasmodial inhibitor targeting *Plasmodium falciparum* histone deacetylase 1. *Cell Discov.* 2020;6:93.
 126. Li R, Ling D, Tang T, Huang Z, Wang M, Ding Y, et al. Discovery of novel *Plasmodium falciparum* HDAC1 inhibitors with dual-stage antimalarial potency and improved safety based on the clinical anticancer drug candidate Quisinosat. *J Med Chem.* 2021;64:2254–71.
 127. Malmquist NA, Moss TA, Mecheri S, Scherf A, Fuchter MJ. Small-molecule histone methyltransferase inhibitors display rapid antimalarial activity against all blood stage forms in *Plasmodium falciparum*. *Proc Natl Acad Sci USA.* 2012;109:16708–13.
 128. Malmquist NA, Sundriyal S, Caron J, Chen P, Witkowski B, Menard D, et al. Histone methyltransferase inhibitors are orally bioavailable, fast-acting molecules with activity against different species causing malaria in humans. *Antimicrob Agents Chemother.* 2015;59:950–9.
 129. Dembélé L, Franetich J-F, Lorthiois A, Gego A, Zeeman A-M, Kocken CHM, et al. Persistence and activation of malaria hypnozoites in long-term primary hepatocyte cultures. *Nat Med.* 2014;20:307–12.
 130. Coetzee N, von Gruning H, Opperman D, van der Watt M, Reader J, Birkholtz LM. Epigenetic inhibitors target multiple stages of *Plasmodium falciparum* parasites. *Sci Rep.* 2020;10:2355.
 131. Bouchut A, Rotili D, Pierrot C, Valente S, Lafitte S, Schultz J, et al. Identification of novel quinazoline derivatives as potent antiplasmodial agents. *Eur J Med Chem.* 2019;161:277–91.
 132. Nardella F, Halby L, Hammam E, Erdmann D, Cadet-Daniel V, Peronet R, et al. DNA methylation bisubstrate inhibitors are fast-acting drugs active against artemisinin-resistant *Plasmodium falciparum* parasites. *ACS Cent Sci.* 2020;6:16–21.
 133. Li J, Hao D, Wang L, Wang H, Wang Y, Zhao Z, et al. Epigenetic targeting drugs potentiate chemotherapeutic effects in solid tumor therapy. *Sci Rep.* 2017;7:4035.
 134. Nepali K, Liou J-P. Recent developments in epigenetic cancer therapeutics: clinical advancement and emerging trends. *J Biomed Sci.* 2021;28:27.
 135. Chua MJ, Arnold MSJ, Xu W, Lancelot J, Lamotte S, Späth GF, et al. Effect of clinically approved HDAC inhibitors on *Plasmodium*, *Leishmania* and *Schistosoma* parasite growth. *Int J Parasitol Drugs Drug Resist.* 2017;7:42–50.
 136. Le Govic Y, Houzé S, Papon N. Repurposing anticancer drugs to tackle malaria. *ChemMedChem.* 2021;16:2192–4.
 137. Hesping E, Chua MJ, Pflieger M, Qian Y, Dong L, Bachu P, et al. QSAR Classification models for prediction of hydroxamate histone deacetylase inhibitor activity against malaria parasites. *ACS Infect Dis.* 2022;8:106–17.
 138. Mackwitz MKW, Hesping E, Antonova-Koch Y, Diedrich D, Woldearegai TG, Skinner-Adams T, et al. Structure-activity and structure-toxicity relationships of peptoid-based histone deacetylase inhibitors with dual-stage antiplasmodial activity. *ChemMedChem.* 2019;14:912–26.

139. Ontoria JM, Paonessa G, Ponzi S, Ferrigno F, Nizi E, Biancofiore I, et al. Discovery of a selective series of inhibitors of *Plasmodium falciparum* HDACs. *ACS Med Chem Lett*. 2016;7:454–9.
140. Koumpoura CL, Robert A, Athanassopoulos CM, Baltas M. Antimalarial inhibitors targeting epigenetics or mitochondria in *Plasmodium falciparum*: recent survey upon synthesis and biological evaluation of potential drugs against malaria. *Molecules*. 2021;26:5711.
141. Nardella F, Halby L, Dobrescu I, Viluma J, Bon C, Claes A, et al. Procainamide-SAHA fused inhibitors of hHDAC6 tackle multidrug-resistant malaria parasites. *J Med Chem*. 2021;64:10403–17.
142. Marfurt J, Chalfein F, Prayoga P, Wabiser F, Kenangalem E, Piera KA, et al. Ex vivo activity of histone deacetylase inhibitors against multidrug-resistant clinical isolates of *Plasmodium falciparum* and *P. vivax*. *Antimicrob Agents Chemother*. 2011;55:961–6.
143. Merrick CJ, Jiang RH, Skillman KM, Samarakoon U, Moore RM, Dzikowski R, et al. Functional analysis of sirtuin genes in multiple *Plasmodium falciparum* strains. *PLoS ONE*. 2015;10: e0118865.
144. Chen PB, Ding S, Zanghi G, Soulard V, DiMaggio PA, Fuchter MJ, et al. *Plasmodium falciparum* PfSET7: enzymatic characterization and cellular localization of a novel protein methyltransferase in sporozoite, liver and erythrocytic stage parasites. *Sci Rep*. 2016;6:21802.
145. Sun Y, Jiang X, Chen S, Price BD. Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Lett*. 2006;580:4353–6.
146. Cui SX, Qu XJ, Xie YY, Zhou L, Nakata M, Makuuchi M, et al. Curcumin inhibits telomerase activity in human cancer cell lines. *Int J Mol Med*. 2006;18:227–31.
147. Reyser T, Paloque L, Nguyen M, Augereau J-M, Fuchter MJ, Lopez M, et al. Epidrugs as promising tools to eliminate *Plasmodium falciparum* artemisinin-resistant and quiescent parasites. *Pharmaceutics*. 2023;15:2440.
148. Huber K, Doyon G, Plaks J, Fyne E, Mellors JW, Sluis-Cremer N. Inhibitors of histone deacetylases. *J Biol Chem*. 2011;286:22211–8.
149. Engel JA, Jones AJ, Avery VM, Sumanadasa SDM, Ng SS, Fairlie DP, et al. Profiling the anti-protozoal activity of anti-cancer HDAC inhibitors against *Plasmodium* and *Trypanosoma* parasites. *Int J Parasitol Drugs Drug Resist*. 2015;5:117–26.
150. Zhou W, Chen X, He KE, Xiao J, Duan X, Huang RUI, et al. Histone deacetylase inhibitor screening identifies HC toxin as the most effective in intrahepatic cholangiocarcinoma cells. *Oncol Rep*. 2016;35:2535–42.
151. Bougdour A, Maubon D, Baldacci P, Ortet P, Bastien O, Bouillon A, et al. Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. *J Exp Med*. 2009;206:953–66.
152. Di M, Bougdour A, Wong Y-S, Brenier-Pinchart MP, Al Curt, Hakimi M-A, et al. Activity of the histone deacetylase inhibitor FR235222 on *Toxoplasma gondii*: inhibition of stage conversion of the parasite cyst form and study of new derivative compounds. *Antimicrob Agents Chemother*. 2010;54:4843–50.
153. You BR, Park WH. Trichostatin A induces apoptotic cell death of HeLa cells in a Bcl-2 and oxidative stress-dependent manner. *Int J Oncol*. 2013;42:359–66.
154. Spencer J, Amin J, Wang M, Packham G, Alwi SS, Tizzard GJ, et al. Synthesis and biological evaluation of JAHA: ferrocene-based histone deacetylase inhibitors. *ACS Med Chem Lett*. 2011;2:358–62.
155. Brinkmann H, Dahler AL, Popa C, Serewko MM, Parsons PG, Gabrielli BG, et al. Histone hyperacetylation induced by histone deacetylase inhibitors is not sufficient to cause growth inhibition in human dermal fibroblasts. *J Biol Chem*. 2001;276:22491–9.
156. Dow GS, Chen Y, Andrews KT, Caridha D, Gerena L, Gettayacamin M, et al. Antimalarial activity of phenylthiazolyl-bearing hydroxamate-based histone deacetylase inhibitors. *Antimicrob Agents Chemother*. 2008;52:3467–77.
157. Lee JH, Nam SH, Seo WT, Yun HD, Hong SY, Kim MK, et al. The production of surfactin during the fermentation of cheonggukjang by potential probiotic *Bacillus subtilis* CSY191 and the resultant growth suppression of MCF-7 human breast cancer cells. *Food Chem*. 2012;131:1347–54.
158. Lai YS, Chen JY, Tsai HJ, Chen TY, Hung WC. The SUV39H1 inhibitor chaetocin induces differentiation and shows synergistic cytotoxicity with other epigenetic drugs in acute myeloid leukemia cells. *Blood Cancer J*. 2015;5: e313.
159. Ukaegbu UE, Zhang X, Heinberg AR, Wele M, Chen Q, Deitsch KW. A unique virulence gene occupies a principal position in immune evasion by the malaria parasite *Plasmodium falciparum*. *PLoS Genet*. 2015;11: e1005234.
160. Vanheer LN, Zhang H, Lin G, Kafsack BFC. Activity of epigenetic inhibitors against *Plasmodium falciparum* asexual and sexual blood stages. *Antimicrob Agents Chemother*. 2020. <https://doi.org/10.1128/AAC.02523-19>.
161. Thinnes CC, England KS, Kawamura A, Chowdhury R, Schofield CJ, Hopkinson RJ. Targeting histone lysine demethylases—Progress, challenges, and the future. *Biochim Biophys Acta Gene Regul Mech*. 2014;1839:1416–32.
162. Ueda R, Suzuki T, Mino K, Tsumoto H, Nakagawa H, Hasegawa M, et al. Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J Am Chem Soc*. 2009;131:17536–7.
163. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*. 2012;488:404–8.
164. King ON, Li XS, Sakurai M, Kawamura A, Rose NR, Ng SS, et al. Quantitative high-throughput screening identifies 8-hydroxyquinolines as cell-active histone demethylase inhibitors. *PLoS ONE*. 2010;5: e15535.
165. Wang L, Chang J, Varghese D, Dellinger M, Kumar S, Best AM, et al. A small molecule modulates Jumonji histone demethylase activity and selectively inhibits cancer growth. *Nat Commun*. 2013;4:2035.
166. Parrish JK, McCann TS, Sechler M, Sobral LM, Ren W, Jones KL, et al. The Jumonji-domain histone demethylase inhibitor JIB-04 deregulates oncogenic programs and increases DNA damage in Ewing Sarcoma, resulting in impaired cell proliferation and survival, and reduced tumor growth. *Oncotarget*. 2018;9:33110–23.
167. Gnyszka A, Jastrzebski Z, Flis S. DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Res*. 2013;33:2989–96.
168. Hollenbach PW, Nguyen AN, Brady H, Williams M, Ning Y, Richard N, et al. A comparison of azacitidine and decitabine activities in acute myeloid leukemia cell lines. *PLoS ONE*. 2010;5: e9001.
169. Datta J, Ghoshal K, Denny WA, Gamage SA, Brooke DG, Phiasivongsa P, et al. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res*. 2009;69:4277–85.

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