

Review

Opportunities and Difficulties in the Repurposing of HDAC Inhibitors as Antiparasitic Agents

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Abstract: Ongoing therapy for human parasite infections has a few known drugs but with serious side effects and the problem of drug resistance, impelling us to discover novel drug candidates with newer mechanisms of action. Universally, this has boosted the research in the design and development of novel medicinal agents as antiparasitic drugs with a novel mode of action. Histone deacetylase inhibitors (HDACis) are used in a vast variety of diseases due to their anti-inflammatory properties. Drug repurposing strategies have already approved HDACis as cancer therapeutics and are now under investigation for many parasitic infections. Along with the expression of the gene, histone deacetylase (HDAC) enzymes also act as a slice of great multi-subunit complexes, targeting many non-histones, changing systemic and cellular levels signaling, and producing different cell-based specified effects. Zinc (Zn²⁺)- and nicotinamide adenine dinucleotide (NAD⁺)-dependent HDACs of parasites play pivotal roles in the alteration of gene expression of parasites. Some of them are already known to be responsible for the survival of several parasites under odd circumstances; thus, targeting them for therapeutic interventions will be novel for potential antiparasitic targets. This point of view outlines the knowledge of both class-I and class-II HDACis and sirtuin inhibitors that emerged to be the key players in the treatment of human parasitic disorders like Leishmaniasis, Schistosomiasis, Malaria, Trypanosomiasis, and Toxoplasmosis. This review also focuses on repurposing opportunities and challenges in HDAC inhibitors that are preceded by their clinical development as potent new antiparasitic drugs.

Keywords: histone deacetylase; parasite; hydroxamate; sirtuin; cyclictetrapeptide; aminoanilide; toxoplasma; human parasite diseases; drug repurposing



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1. Introduction

Parasitic disease globally affects a large number of humans and makes up one of the most concerning health problems, with notable morbidity and mortality. It is more prevalent in underdeveloped regions and developing countries. In 2020, 241 million cases and 627,000 deaths were reported worldwide due to malaria [1]. Similarly, in 2015, there were about 214 million clinical patients of malaria, with a mortality of 438,000 [2]. Apart from this disease, other parasitic diseases such as Leishmaniasis, Schistosomiasis, Lymphatic filariasis, and Trypanosomiasis cause notable health burdens. About 250 million patients have been infected with *Leishmania* and *Schistosoma* parasite infection [1,3,4]. Every year, the death toll resulting from parasitic diseases is increasing. Some research studies have suggested that these parasites may impair efficient immune responses, increasing the morbidity and mortality of COVID-19 patients. Alternatively, it has also been found that no discernible variations in disease severity were observed between co-infected individuals

and naïve COVID-19 cases [5]. However, a Brazilian study indicated that people co-infected with parasite diseases and COVID-19 were less likely to develop severe COVID-19 [6].

RTS,S/AS01 (Mosquirix) and R21/Matrix-M are some of the recently approved vaccines for malaria by the WHO. However, the unavailability of vaccines for various human parasitic diseases makes the problem more urgent. Further, prevention and treatment are also sometimes difficult because of the side effects and developed drug resistance against the currently available therapeutics [7–11]. Thus, identifying novel druggable targets and chemotherapies with newer modes of action is of dire need. Small molecules that react with histones are effective for post-translational modifications of these epigenetic regulatory proteins and are gaining interest as a chemical arsenal as well as probable new leads that could suppress the parasite growth mechanisms [12–14].

Clinically, repurposing of the approved drugs is intriguing due to its efficiency in identifying novel applications. This approach expedites market availability and reduces economic costs compared to de novo drug discovery for parasitic diseases in tropical regions [15]. Based upon this approach, drugs approved as histone deacetylase inhibitors (HDACis) for the treatment of cancer are now being repurposed to target various parasitic diseases. However, the repurposing, especially with HDACis, faces hurdles. Variations in HDACis pharmacological profiles between diseases complicate the translation of efficacy from one condition to another. Regulatory complexities hinder their smooth transition, demanding robust evidence for safety and efficacy in the new therapeutic context. Ensuring safety necessitates understanding long-term effects, potential side effects, and interactions with existing treatments, which is also challenging. The absence of standardized drug testing methods adds complexity to evaluating HDAC inhibitors' effectiveness in novel indications.

Histone and many non-histone proteins are being processed by HDACs that are occasionally called lysine deacetylases (KDACs). Histone/lysine deacetylases remove the acetyl group from histones and other associated proteins, while acetyltransferases append acetyl groups to such proteins. Similarly, the corresponding demethylases and methyltransferases eliminate and attach methyl groups to lysine side chains of proteins, respectively [16]. These post-translational alterations in the eukaryotes synchronize the regulation of transcription, cell cycle, and apoptosis [17–20]. Some human disorders, like cancers, express an altered expression of epigenetic regulatory proteins/enzymes and could be druggable targets [16,21,22]. HDACis are being investigated for their potential as antiparasitic drugs based on their mechanism of action in cancer, which include cell differentiation, cell cycle arrest, apoptosis induction, and immune system regulation. Studies have demonstrated that HDACis can cause parasites to undergo apoptosis, halt their growth, and alter the host immune system's reaction to parasitic diseases. Furthermore, it has been discovered that HDACis promote apoptosis and other cell-damaging processes, which may be pertinent in the context of parasite infections, in order to increase tumor cell death [23]. Similarly, several epigenetic regulatory proteins exhibited a vital role during the evolution and life cycle of parasitic pathogens, with little resemblance or remarkable dissimilarity in principle catalytic domains to human proteins, making them compelling antiparasitic drug targets [12,24–28]. Homologs of HDAC have been identified in most parasitic pathogens of humans. The non-identical category of HDACis have exhibited their action in some of these resistance parasites, such as *Plasmodium*, *Leishmania*, and *Schistosoma* species [12,26,27]. Several HDACis have already received approval for therapeutic applications in various cancer types, making them potential candidates for addressing parasitic diseases. Noteworthy among the approved HDACis used in the management of peripheral or cutaneous T-cell lymphoma and applied in combination therapy for multiple myeloma are Belinostat (1) (PXD101), Panobinostat (2) (LBH-589), Vorinostat (3) (SAHA), and Romidepsin (4) (FK228) [29–32] (Figure 1).

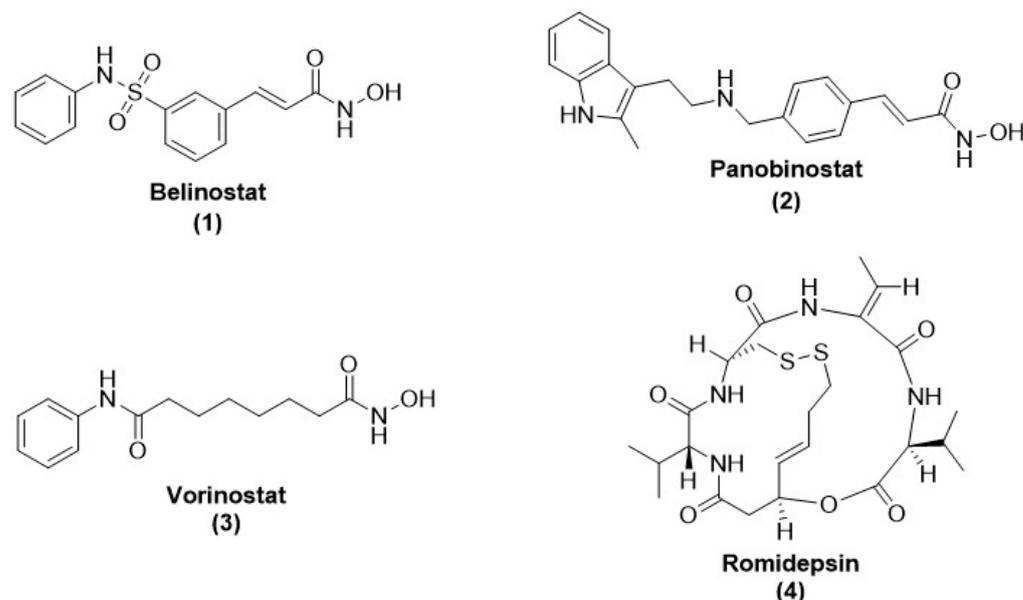


Figure 1. Chemical structure of FDA-approved drugs acting as HDAC inhibitors. These HDACis have anti-cancer properties.

However, the discovery of antiparasitic drugs is challenging due to partial underlying molecular mechanisms for parasite resistance and a slow pace of identification and validation of novel drug targets. The complex life cycles of parasites with more than one host, morphological form, mode of reproduction (asexual and sexual replication), parasites' genetic separation, and lack of easy genetic manipulation and biochemical characterization of many species have also sometimes hampered the process of drug discovery. The review is essential to address the urgent global health concern posed by parasitic diseases, which significantly impact human morbidity and mortality, particularly in underdeveloped and developing regions. Despite recent advancements in malaria vaccine development, many parasitic diseases lack effective vaccines, and the current treatments face challenges such as side effects and drug resistance. The review aims to fill a crucial knowledge gap by exploring the potential of HDACis in repurposing parasitic diseases.

2. Human Parasitic Illness

Several parasitic pathogens are involved in various diseases in humans, and the gravity of the disease is directly connected to the host's immune status. Malaria is a mosquito-borne disease infecting 40% of the population of the African, Southeast Asian, and Western Pacific regions [33]. Six species of *Plasmodium* cause mortality. As per the WHO 2020, six million deaths were reported due to malaria, with more than two hundred million clinical cases of infection each year [34]. Children below the age of 5 years in indigenous regions affected by malaria have underdeveloped immune systems; thus, they are more susceptible to the threat of serious malarial disease and associated mortality [1]. Recently, the WHO approved Mosquirix (also known as RTS and S/AS01), an antimalarial vaccine, which acts through the active immunization process for extremely affected children [35]. Women having first pregnancies are more vulnerable to a serious form of malaria compared to non-pregnant women [36]. Further, immune-compromised native people with HIV/AIDS and travelers who have visited malaria-endemic regions are also at risk of severe malaria. A frightening signal of the development of resistance leading to treatment failure is also being detected. *Plasmodium falciparum* has become progressively resistant against chloroquine, and there is a progressive decline in the efficacy of artemisinin and its derivatives, the last line of defense, which leads to a terrifying condition [9,37,38]. The discovery and advancement of novel antimalarial drugs furnished with a novel mode of action is the rationale for the successful treatment of parasites.

Similarly, the causative agent for toxoplasmosis is *Toxoplasma gondii*, an obligate intracellular protozoan. In healthy persons, toxoplasmosis indications are self-limiting, but it is a more significant threat or is lethal towards women's fetuses during pregnancy. These parasites are also responsible for serious opportunistic infections in immune-compromised people who received chemotherapy or organ transplants and people with AIDS [39]. The restricted ongoing treatment for toxoplasmosis is the combination therapy of pyrimethamine with a sulfonamide, but it suffers from efficacy problems along with serious adverse events [15,39,40].

One of the crucial neglected parasitic diseases across the globe is Schistosomiasis, which creates severe public health problems, mostly in the Middle East, Asia, South America, and Africa [41,42]. Among six species of *Schistosoma*, *S. mansoni*, *S. trematodes*, and *S. haematobium* are the leading causes of the disease in humans [43,44]. Globally, the infection of *Schistosoma trematodes* influences nearly 259 million people, with an annual death rate of 280,000. Further, chronic schistosomiasis develops long-term morbidity in millions of people [45–48]. Praziquantel is the premier drug to treat schistosomiasis [48,49]. It is an amazingly low-cost antischistosomal agent that is effective against all species of *Schistosoma*, although its mode of action is still not properly acknowledged [50,51]. It is administered in a single dose through the per oral (P.O.) route with negligible side effects [52]. Due to the emergence of drug resistance, the extended use of praziquantel has produced intense concerns over its potential [50,51,53–55]. Several reports have shown its reduced efficacy against some *Schistosoma* species and the development of drug resistance in some laboratory strains [56–61]. Based upon this lacuna, there is a need to develop novel antischistosomal agents.

Chagas disease is caused by *Trypanosoma cruzi* and has emerged as endemic in Latin America. This is a chronic infectious sickness that affects 6–7 million people worldwide [62]. The acute phase of Chagas disease is treated through benznidazole and nifurtimox [62,63]. Drug resistance is one of the largest constraints, including cytotoxicity, which causes severe side effects upon exposure to these available drugs [62–65]. Thus, for continuous management and attempts at disease eradication, the development of novel curative molecules with improved modes of action, ensuring increased efficacy and safety, is pivotal.

Another common tropical parasitic disease is Human African Trypanosomiasis (HAT), caused by *Trypanosoma brucei*. This epidemic developed terrible public health problems in sub-Saharan Africa but is now under check, with 10,000 or fewer cases annually reported [63]. The parasite enters the human body through the Tsetse fly, causing African sleeping sickness [66]. In humans, the parasite thrives freely in the bloodstream and can escalate the host's immune reaction via antigenic drift [67]. If the parasite remains unchecked, then it leads to central nervous system infection and might be fatal [68]. Presently, the treatment strategy for this disease is based on a restricted number of drugs, such as eflornithine, pentamidine, nifurtimox, melarsoprol, and suramin.

Similarly, Leishmaniasis is a tropical vector-borne infection caused by the genus *Leishmania*, a protozoan parasite. There are three main manifestations of Leishmaniasis: cutaneous, visceral (kala-azar), and mucocutaneous. Among these, kala-azar is deadly if proper treatment is not undertaken. An estimate suggests 20,000–30,000 annual deaths and 0.9–1.3 million newly reported cases, creating an elevated risk of infection for 350 million people worldwide [69,70]. Leishmaniasis treatment activates a limited number of drug options, like antimony agents, paromomycin, meglumine antimoniate, amphotericin B, sodium stibogluconate, and miltefosine. Nonetheless, these drugs have limited potency and require long-term treatment with parenteral injection, causing severe toxicity [71,72]. The presently available treatments are more problematic in immune-compromised patients who have opportunistic infections like HIV / AIDS [73].

3. Classification and Biological Significance of Human Histone Deacetylases (HDACs) *Biological Significance of Human HDAC Isoforms*

Harmonization of cell chromatin structure and gene expression of eukaryotic organisms is completed by pivotal enzymes through histone protein alteration. Histone

acetyltransferase enzyme does open chromatin for ease in gene transcription, whereas HDACs oppose the acetylation that leads to a 'closed' chromatin state and ultimately suppresses transcription. However, the enhancement of the number of non-histone proteins revealed that HDACs perform several biological functions in eukaryotic cells, such as structural proteins, stability, interactions, and modification of proteins, DNA-binding properties, factors affecting transcription, chaperones, proteins that remodel chromatin, nuclear import proteins, and mediators of signaling [74,75]. Overall, these non-histone proteins play a crucial role in cell invasion, cell cycle regulation, tumor progression, angiogenesis, and apoptosis process [75–77]. The influence of HDACs causes many diseases like cancer, immunological disorders, metabolic disorders, and neurodegenerative complications [78]. This ensures HDACs as a remarkable drug target class.

Based upon similarity in sequence and dependence on cofactors, HDACs are classified into four classes in mammals: class-I HDACs (HDAC 1, 2, 3, and 8), class-II HDACs (HDAC 4, 5, 6, 7, 9, and 10), class-III HDACs, and class-IV HDACs (HDAC11). Class-I HDACs are confined in the nucleus and share greater homology to yeast RPD3 [79–81]. Class-II HDACs run between the cytoplasm and nucleus specific to tissue and share homology with yeast HDA1 [76,82]. Class-I and class-II HDAC proteins have an indistinguishable catalytic core that utilizes zinc ions as a cofactor but vary in size and structural organization. Class-III (Silent Information Regulator-2 (Sir2)-related protein (sirtuin)) comprise seven nicotinamide adenine dinucleotide (NAD⁺)-dependent enzymes (SIRT1-7) that share homology with yeast Sir2-related protein. Class-III HDACs have more specificity towards the tissue in the cytoplasm and nucleus and utilize cofactor NAD⁺. The latest work has spotlighted the role of class-III sirtuins in various functions of the cell, including repression of genes, apoptosis, repair of DNA, and the promotion of durability [53,75,80,83–86]. The mammalian class-IV HDACs, such as HDAC11, are localized in both the cytoplasm and nucleus and are allied with HDAC6, class-II enzyme, instead of class-multi-protein I HDACs complexes [87]. HDACs consistently target class-I/II HDACs that possess the same catalytic core as a cofactor (Zn²⁺). Thus, sirtuin activators and inhibitors (SIRTis) are designated as potential therapeutics for multiple diseases, such as cardiovascular diseases, cancer, diabetes, obesity, inflammation, various ageing-related diseases, and metabolic and neuronal diseases [79,81].

4. Functions and Roles of Human Parasite Histone Deacetylases (HDACs)

4.1. HDACs of *Plasmodium Falciparum*

The genome of *Plasmodium falciparum* comprises five HDAC-encoding genes. Three HDAC genes encode proteins that share mammalian homology with class I or class II (PfHDAC1, PfHDAC2, and PfHDAC3), while two genes are of class-III HDAC homologs (PfSir2A and PfSir2B) [15,25,26,88,89]. PfHDAC1 is confined to the nucleus, which comprises up to 55% amino acid sequences that share similarities to several eukaryotic class-I HDACs. These are transcribed in various stages of the protozoan lifecycle, such as the asexual intraerythrocytic phase, gametocytes, and sporozoites [12,26,88,90,91]. PfHDAC1 plays a key role in post-translational modification of histone and non-histone and gene expression in *Plasmodium*, which appears crucial for its survival [12,26,88,92–95]. Homology modelling analysis revealed that PfHDAC1 has an extremely conserved active site tunnel similar to that of human HDACs, but the differences at the entrance to the tunnel of active site differentiate from human HDACs (hHDACs) [92,93]. This dissimilarity may describe the better inhibitory activity of some HDAC inhibitors for *P. falciparum* as compared to mammalian cells [12,26,88]. Thus, it is also further exploited by new HDACis that could offer selectivity for PfHDAC1 over hHDACs [12,26,88,90–93]. It is foretold that both PfHDAC2 and PfHDAC3 are proteins with high molecular weight, having less than 14% amino acid residues identical to each other and restricted sequence homology with other class-II HDACs [25,89–91]. A knockdown experiment reported that PfHDAC3 inhibits *P. falciparum* parasite growth and survival in the sexual stage and controls its transcription [25]. Both PfSir2A and PfSir2B have 30% and 38% identical sequences in class-III HDAC (*Archaeoglobus fulgidus*) and group IV sirtuins, respectively. PfSir2A possesses both histone deacetylase and ADP-ribosyl transferase

activity and is also effective for the removal of long-chain and medium fatty acyl groups [96]. The catalytic activity of PfSir2A maintains the length of telomeres, sets up heterochromatin in subtelomeric genomic areas, and modulates a subset of genes of *P. falciparum* implied in cytoadhesion, antigenic variation, pathogenesis, and virulence [94,97]. PfSir2 is not essential for in vitro growth of *P. falciparum*, but the regulation of virulence gene expression makes it a powerful target for antidiarrhoeal therapy [97,98]. Similarly, PfSir2A and PfSir2B function as promoters involved in different telomeric-associated var gene subsets expression that encodes for erythrocyte membrane protein 1 (PfEMP1). It is expressed on the surface of erythrocyte *P. falciparum*, avoiding immune surveillance of the host at the time of *P. falciparum* infection [94,97]. PfSir2B also protects the end of the telomere. Knockout studies regarding PfSir2A and PfSir2B suggest non-mortality towards the parasite if any aspect is absent and set up functional dismissal in the parasite. Still, the concurrent knockout of PfSir2A and PfSir2B has not yet been assessed. PfSir2A and PfSir2B not only halt the in vitro growth and development of *P. falciparum* but are also useful for the regulation of var gene expression, which is in turn vital for the determination of in vivo survivability in the parasite inside a host. It has been suggested as a prospective target for antimalarial therapy. Specifically, *P. falciparum*'s sirtuins interrupt infected erythrocyte cellular adhesion towards the host cell receptor that conciliates the severity of the disease or obstructs the avoidance of *Plasmodium* from the innate immune system of the host [94,97]. It has been proposed that the var gene is not identified in other species of *Plasmodium*. Further, sirtuins (PfSir2A and PfSir2B) can express a subtelomeric gene that can divert the antigen through switching [97].

4.2. HDACs of *Toxoplasma*

The genome of *T. gondii* comprises five class-I and class-II HDAC homologs (TgHDAC1-5) besides two homolog subtypes of class-III HDACs, i.e., Sir2, along with the deviation of TgHDAC3 [12]. TgHDAC3 is confined in the nucleus and comprises greater than 60% sequences identical to hHDAC1. The TgHDAC3 sequence is a fragment of a huge multi-protein complex designated as a co-repressor complex of *T. gondii*, similar to the HDAC3-containing complex of humans. HDACs of *T. gondii* co-repressor complex encompasses TgHDAC3, *T. gondii* transducin b-like protein-1 (TgTBL1), two heat-shock protein-70 (HSP70)-like proteins, actin, and *T. gondii* TCP-1 ring complex (TRiC) subunits [59]. *T. gondii* HDACs along with deacetylase inhibitory activity additionally correlated with modulation of the gene in distinct forms of this parasite, such as stages of conversion from tachyzoite to bradyzoite [99]. However, the subtelomeric gene family has not been recognized in *T. gondii*. TgSir2A and TgSir2B proteins are suitably implied in processes other than antigenic variation [97] (Figure 2).

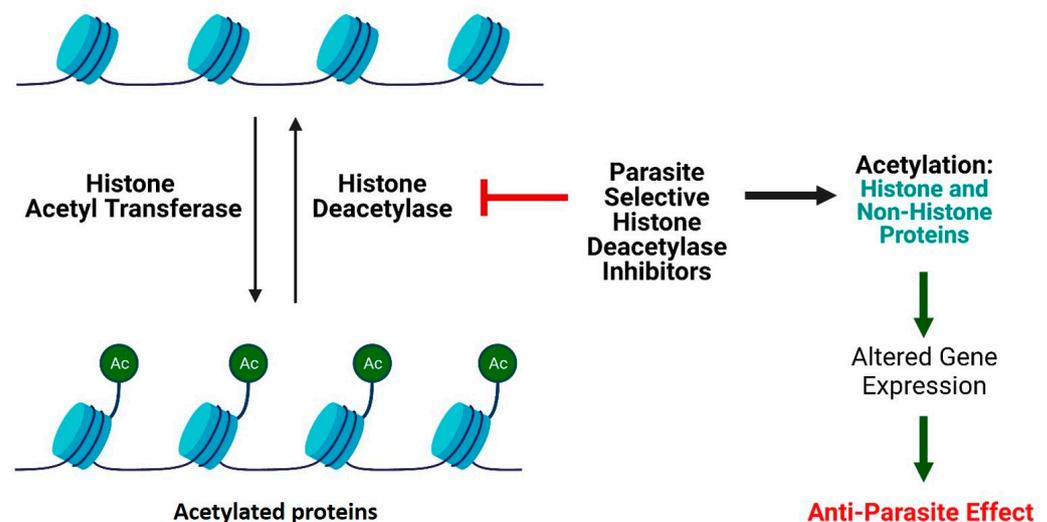


Figure 2. Mechanism of action of HDAC inhibitors (created with BioRender.com). The dysregulation of dynamics of acetylation and deacetylation is responsible for Antiparasitic activity.

4.3. HDACs of *Schistosoma*

Schistosoma mansoni comprises three HDACs that have been identified as mammalian class-I HDACs orthologues [100]. Additionally, it also comprises some presumed class-II HDAC orthologues; nevertheless, no specific one has been functionally identified [101]. SmHDAC1 is a class-I homologue that suppresses transcription factor Gal4 or fusion protein Gal4-NF- κ B in mammalian cells through a co-expression or co-transfection system [100]. This suppression was partly reinstated by the application of HDAC compound 4 (Trichostatin or TSA). Thus, it has been thought that it is HDAC-dependent. Whenever the two histidine residues present in the active site of the SmHDAC1 protein (H176/177A) were altered, simultaneously, Gal4-NF- κ B-dependent transcription was suppressed, specifying the principal role of these residues in the activity of the protein [102]. Still, schistosomes class-III HDACs have not been identified, although expressed sequence tags (ESTs) encoding multiple assumed members are available in the *S. mansoni* genomic datasets [103].

4.4. HDACs of *Trypanosoma*

There are four types of class-I/II HDAC orthologs (TbDAC1-4) available in *Trypanosoma brucei* [12,104,105]. TbDAC1 and TbDAC2 show identical sequences indistinguishable from mammalian class-I HDACs, whereas both TbDAC3 and TbDAC4 are more intimately linked to class-II HDACs [104–107]. Moreover, TbDAC1 and TbDAC3 are confined in the nucleus and crucial for the survival of parasites, while TbDAC2 and TbDAC4 are chiefly confined to the cytoplasm [106,107]. *T. brucei*, when present in the bloodstream of the human host, through the periodic switching of single-variant surface glycoproteins (VSGs), evades immunity. These surface glycoproteins are encoded by a massive range of VSG genes, but, at one time, just one VSG expression site is transcriptionally active [106]. Based upon this consideration, TbDAC3 displayed the need for a VSG expression site in the bloodstream and insect-stage cells through the cycling/silencing phenomenon, thus permitting the parasite to avoid the host immune defenses. In contrast, *T. brucei* carried three distinct homologs of sirtuin (TbSir2rp1-3) [12,108,109]. Nuclear-chromosome-associated protein TbSir2rp1 is not only essential for parasite survival but also for varied antigenicity. Subtelomeric gene suppression occurs in both blood stages and in the insect despite TbSir2rp1 participation in RNA-polymerase-I mediated transcription. Under DNA damaging conditions or during genotoxic stress, TbSir2rp1 is vital for the survival of parasites because, through catalytic reaction, there is an alteration, i.e., deacetylation and ADP-ribosylation, of histone protein, mostly H2A and H2B, responsible for the DNA repair. TbSir2rp1 is overexpressed to provide resistance toward DNA damage in the cell. Based upon this, the selective inhibitors of TbSir2rp1, combined with standard DNA-targeting chemotherapeutics, work to boost antiparasitic efficacy [108,109]. The individual knockouts of TbSir2rp2 and TbSir2rp3 do not affect the differentiation and proliferation forms of the bloodstream as they are mitochondrial proteins [108,109].

TcSir2rp1 genesis cytosolic protein and TcSir2rp3 genesis mitochondrial protein provide code for sirtuins in *Trypanosoma cruzi* [92,93]. Both sirtuins play vital roles in the multiplication of *T. cruzi* reproductive forms, host–parasite interactions, and various stages of the lifecycle [110,111]. TcSir2rp1 overexpression diminishes the growth and differentiation of the parasite, whereas TcSir2rp3 overexpression ameliorates the growth and differentiation of the parasite. A recent report showed that the salermide (SIRTi 3) inhibits TcSir2rp3, leading to the inhibition of growth and differentiation of *T. cruzi* (in vitro and in vivo). This indicates that sirtuin inhibitors are useful for chemotherapy of Chagas disease [111].

4.5. HDACs of *Leishmania*

The genome of *Leishmania* is also composed of four genes encoding homologs of class-I/II HDACs, whereas, for homologs of class-III HDACs (sirtuin), only three encoding genes are present [12]. Still, no one class-I/II HDAC species of *Leishmania* has been practically identified, but only one homolog is upregulated temporarily during differentiation (promastigote–amastigote) conducted without a host model system, signifying a

probable role in regulating transcription or in chromatin structure modification [12,112]. Some studies have explored class-III HDACs' useful roles in *Leishmania* species. Several research studies must look over the biological responsibility of sirtuins in unusual species of *Leishmania* specifically, also called Sir2rp1 (Sir2-related protein 1). The existence of this Sir2rp1 protein is observed in the granules of cytoplasm at distinct developmental stages of parasite *Leishmania* (promastigotes–amastigotes), such as in *L. major*, *L. infantum*, and currently in *L. amazonensis* [12,113,114]. A *Leishmania*, a major protein with considerable homology to yeast SIR2p *Leishmania major* Sir2rp1 (*Leishmania major* SIR2-related protein-1), has been identified, and these protein homologues are demonstrated in different *Leishmania* species' cytoplasm and during developmental stages of the parasite (promastigotes and amastigotes) [113,114]. Both *L. major* Sir2rp1 and *L. infantum* Sir2rp1 represent ADP-ribosyltransferase and NAD⁺-dependent deacetylase activities independent of epigenetic silencing, as designated by gene disruption and overexpression studies. This revealed their crucial role in the in vitro and in vivo survivability of two *Leishmania* species [114–116].

L. infantum Sir2rp1, associated with deacetylation of α -tubulin, bears a resemblance to human Sir2 and HDAC6 cytoskeleton network function [115]. Several studies suggested that parasites' soluble fractions overexpress *L. major* Sir2rp1 (this HDAC), which co-immunoprecipitates with HSP83, the *Leishmania* orthologue of mammalian HSP90 [117]. However, intracellular *L. infantum* Sir2rp1 quantity did not modify the state acetylation of *L. infantum* HSP83, so it is not so far comprehensible that *L. infantum* HSP83 is an HDAC substrate [118]. Sirtuins of *Leishmania* also perform the role of immunomodulation [12,115,118,119]. *L. major* Sir2rp1 brings out effector B-cell function, production of specific antibodies, as well as energizing differentiation of murine B-cell (in vivo). Recombinant *L. major* Sir2rp1 purified and expressed from *Escherichia coli* can activate the function of the B-cell effector, advocating the modulation of the immune system for this HDAC during infection [118]. Likewise, through Toll-like receptor-2 (TLR2)-mediated mode of action, *L. infantum* Sir2rp1 has the capability to activate splenic B-cells, which leads to upregulation of MHC-II, a major histocompatibility complex, and CD₄₀ and CD₈₆ molecules. It is also responsible for the release of tumor necrosis factor in the working mouse model [119].

The host innate immune system and this activation pathway entail the signaling pathway of Toll-like receptors in the initial identification of *Leishmania* parasites and may lead to the development of resistance towards infection and provide help for the development of immunity. During monitoring of TLR2-dependent activation of B-cells, after immunization, TLR2-deficient mice could still produce antibodies to *L. infantum* Sir2rp1, showing that *L. infantum* Sir2rp1 may also link with other types of cells in the absence of TLR2 [119]. The literature studies on pathogens and signaling of host products through TLR2 and TLR4 remain ambiguous.

The *Leishmania* Sir2rp1 immunomodulatory role increases the chance that TLR2 and TLR4 protein might function as a probable adjuvant in a multi-component vaccine. Particularly, the sirtuin HDAC proteins are pivotal for the growth of the *Leishmania* parasite (in vitro and in vivo). Moreover, previously, several researchers have focused on targeting multiple inhibitors with the ambition of recognizing the most effective antileishmanial agents [120–122].

5. Opportunities of HDAC Inhibitors as Antiparasitic Agent

5.1. HDAC Inhibitors as Antimalarial Agent

For decades, HDAC inhibitors have been employed as antimalarials. Apicidin (a fungal-derived HDACi) was initially identified as an effective HDACi against Apicomplexan protozoa and *Plasmodium* parasites [123]. Ever since, many modified HDACi classes have been documented as potent antimalarial agents.

5.1.1. Cyclictetrapeptide HDAC Inhibitors

Darkin-Ratray et al. observed in vitro potent activity of apicidin (5) against *P. falciparum*, *T. gondii* and other protozoan parasites. Hyperacetylation of histones was observed in *P.*

falciparum upon treatment with apicidin. It inhibited the recombinant PfHDAC1 enzymatic activity ($IC_{50} = 1 \text{ nM}$) [95,123]. Notable alterations were observed during the transcriptional phase of the intra-erythrocytic cycle of *P. falciparum*, with altered expression by other genes [124,125]. These findings revealed a similar effect produced by the compound as in HDACis on higher eukaryotic cells [126,127]. Apicidin caused parasitic histone protein hyperacetylation and genome-wide transcriptional alteration in the protozoan [95,124]. The derivatives of a quinolone, but not derivatives of N-substituted indole, showed their selectivity (up to ~200-fold) for *P. falciparum* versus mammalian cells at the whole-cell level [128–130]. In another study, a comparison was made between apicidin and a series of synthetic analogues for *T. cruzi*, *T. brucei*, *P. falciparum*, and *L. donovani* [131]. The cyclic tetrapeptide compound FR235222 (6), isolated from *Acremonium* species in the fermentation broth, showed its potential against tachyzoites of *T. gondii* and drug-sensitive and drug-resistant *P. falciparum*-infected erythrocytes with IC_{50} value of 10 nM [132] (Figure 3).

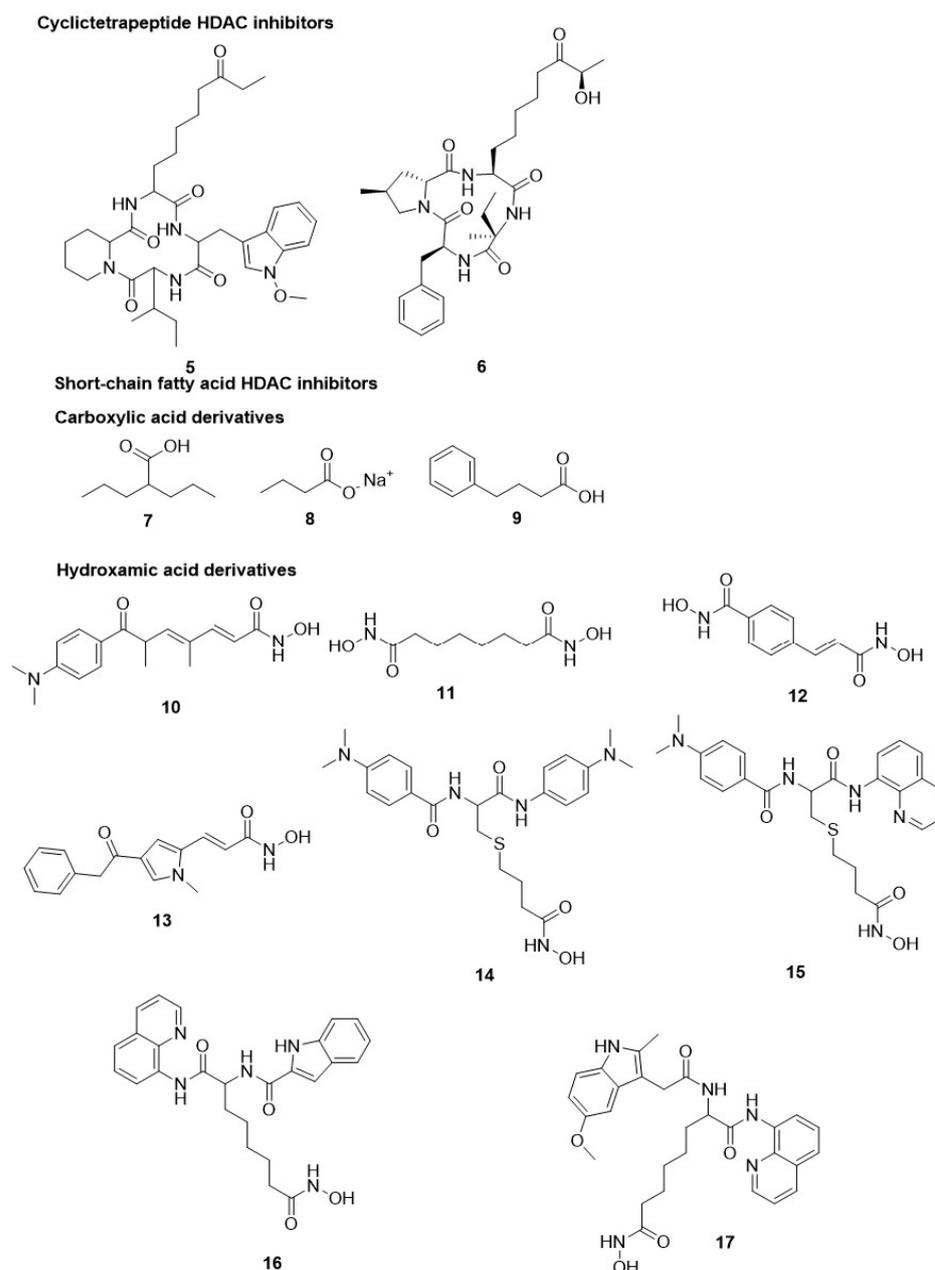


Figure 3. Chemical structure of cyclictetrapeptide and short-chain fatty acid HDAC inhibitors. Cyclictetrapeptide, carboxylic acid hydroxamic acid derivatives have shown HDAC inhibitory activity.

5.1.2. Short-Chain Fatty Acid HDAC Inhibitors

Several drugs, like valproic acid, sodium butyrate, and its derivatives, are short-chain fatty acid HDACis. These HDAC inhibitors possess weak inhibitory activity in opposition to mammalian HDACs but are also active on additional targets, making their effects tough to ascribe beyond HDAC inhibition. However, some of these compounds have manifested clinical efficacy. Valproic acid (7), an antiepileptic drug, has been extensively used as a mood stabilizer and is now investigated in several clinical trials for HDAC-pertinent diseases [133]. All compounds, valproic acid, sodium butyrate (8), and 4-phenyl butyrate (9), displayed weak selectivity towards the parasite in comparison to HS69 mammalian cells and produced relatively weak activity against tachyzoites of *T. gondii*, with IC₅₀ values of 1.0, 1.6, and 5.4 mM, respectively [134,135]. Similarly, valproic acid only displayed moderate activity against *P. falciparum* and *S. mansoni*. Particularly, with *S. mansoni*, an ~80% reduction in miracidia development was observed upon treatment with 50 mM valproic acid after 4 h. Nonetheless, there is a loss of viability of schistosomula up to 30% at 5 mM concentration after 7-day continued exposure [104]. Still, valproic acid has so far not been evaluated in animal models for any parasitic disease [104,135] (Figure 3).

5.1.3. Hydroxamate-Based HDAC Inhibitors

Several natural and modified HDACis for class I/II, such as trichostatin A (TSA, 10), vorinostat or suberoylanilide hydroxamic acid (SAHA, 3), suberoyl bis-hydroxamate (SBHA, 11), MW2796 (12), and aroyl-pyrrolyl hydroxyamide (APHAs, 13), have been considered for their antimalarial potential [136]. These compounds displayed inhibitory potential against *P. falciparum* but exhibited a lack of selectivity affecting the mammalian host cells. However, compound 11 showed effective sensitivity for the parasite compared to cytotoxicity against mammals [12,26,88,95,137–139].

TSA hampered PfHDAC1 activity, with an IC₅₀ value of 0.6 nM in parasites. It is more potent than SAHA against *P. falciparum*, with IC₅₀ values of 0.008–0.120 μM compared to SAHA 0.025–2.2 μM. However, SAHA showed improvement in the selectivity up to 200-fold toward the parasite [12,26,137]. In contrast, compound 11 is less potent with IC₅₀ 0.8–2.3 μM but showed higher selectivity towards the parasite than compound 3. Further, this comparative study was conducted in *P. berghei*-infected BALB/c mice; the administration of compound 11 at an i.p. dose of 200 mg/kg twice a day for 3 days showed significant inhibition of peripheral parasitemia, indicating a cytostatic effect, but the efficacy was not observed in the mice [137]. The early report about compound 11 suggested that hydroxamate-based HDACis would probably be powerful antimalarial agents [12,26,139]. Hence, encouraged by such outcomes, various HDACis displayed in vitro activity with better selectivity against *P. falciparum* than compounds 3 and 11.

Over the years, the screening of zinc-binding hydroxamic acid compounds revealed varying levels of inhibition of HDACs. These structural patterns resulted in the identification of extensive pharmacophoric groups binding to other important sites for HDACs. The CAP group acts as an enzyme surface identification moiety of protein and binds at the catalytic tunnel entrance. The linker region connects the CAP with the zinc-binding group site. This site is an expandable polar connection unit (CU) that lies between the CAP protein and the linker [12,26,77,88] (Figure 3).

Some L-cysteine-based compounds with thioether linker regions, such as compound 14 and 2-aminobutyric-acid-based compound 15 (2-ASA-9), displayed indistinguishable in vitro antiparasitic activity. Compound 14 displayed an IC₅₀ value of 48 nM against chloroquine-resistant (Dd2), and compound 15 showed IC₅₀ values of 71 and 19 nM against both Dd2 and chloroquine-sensitive (3D7) strains, respectively, of *P. falciparum*. Compound 15 displayed improved selectivity over the mammalian cells. Among them, compounds 15 and 11 produced a noticeable hyperacetylation of histones in *P. falciparum*, which led to the inhibition of the growth of *P. falciparum* in erythrocytes at early and later stages in the parasite life cycle [93]. One reported study revealed that compounds 10, 5, and 15 were explored for their gene expression effects, indicating genome-based alteration of

transcription in a range of 2–21% following HDAC inhibition in *P. falciparum*. However, these three inhibitors overall displayed dissimilar effects on gene expression profile. However, tubulin II was found to be one of the sets of genes that were upregulated after treatment with three HDACis. Hence, they could be recognized as markers of transcription in *P. falciparum* induced by structurally variable HDACis. Thus, this marker might be used for the development of HDACis identified as antimalarial candidates [140].

Compounds **15**, **16**, and **3** were also the earliest HDACis tested against the second most crucial human-infecting malaria parasite *P. vivax*. It causes significant morbidity linked to malaria due to relapses as the parasite remains dormant in the liver [26]. In an ex vivo study, all three hydroxamates inhibited the growth of multidrug-resistant *P. falciparum* and *P. vivax* directly isolated from infected patients [141]. Furthermore, a similar activity profile was observed for compounds **3**, **15**, and **16** against *P. falciparum* with IC₅₀ values of 310, 533, and 266 nM, respectively, as well as *P. vivax* with IC₅₀ values of 170, 503, and 278 nM, respectively. HDACis target multiple species of malaria parasites that infect humans; hence, they are highly favorable for further clinical studies. Several series of 2-aminosuberic acid compounds were tested, including compound **17** carrying non-steroidal anti-inflammatory drug (NSAID) components near CAP against *P. falciparum*, for their inhibitory potential. Compound **17** showed the utmost potential against *P. falciparum*, with an IC₅₀ value of 0.013 μM, but the selectivity towards the parasite compared to compound **15** did not prevail (Table 1) [142].

Table 1. In vitro activity of antischistosomal, antimalarial class-I, class-II, class-III, antitrypanosomal class-I/II, antileishmanial class-I/II, and antitoxoplasmal class-I/II/IV HDAC (sirtuin) inhibitors.

Compound ID	Class/Inhibitor	Parasite Growth Inhibition (IC ₅₀) and Effective Concentration (EC ₅₀)	Mammalian Cell, Class-I hHDACs in HeLa Cells, and Sirtuin Inhibition IC ₅₀	References
5	Cyclic Tetrapeptide	<i>P. falciparum</i> IC ₅₀ ~0.200 μM	Mammalian Cell IC ₅₀ 0.05–0.1 μM	[117,124]
6	Class-I/II/IV HDAC inhibitors	<i>T. gondii</i> IC ₅₀ ~10 nM	-	[132]
7	Short-Chain Fatty Acids	<i>P. falciparum</i> IC ₅₀ > 100 μM	Mammalian Cell IC ₅₀ ~1350 μM	[133]
10	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.008–0.120 μM <i>T. brucei</i> IC ₅₀ ~7 μM <i>T. gondi</i> IC ₅₀ ~41 nM	Mammalian Cell IC ₅₀ 0.2–0.3 μM	[86,92,128,131,134]
3	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.025–2.2 μM <i>T. gondi</i> IC ₅₀ 83 nM	Mammalian Cell IC ₅₀ 0.26–20 μM	[128,130,133,135]
11	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.8–2.3 μM <i>T. gondi</i> IC ₅₀ 213 nM	Mammalian Cell IC ₅₀ 50–300 μM	[128,131,139]
12	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.9–1.1 μM	-	[131]
13	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 1.2–4 μM	Mammalian Cell IC ₅₀ > 20 μM	[132]
14	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.048 μM	Mammalian Cell IC ₅₀ ~0.6 μM	[93]
15	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.015–0.039 μM	Mammalian Cell IC ₅₀ ~1.24 μM	[93,134,135]
15	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.013–0.033 μM	Mammalian Cell IC ₅₀ ~0.34 μM	[93,135]

Table 1. Cont.

Compound ID	Class/Inhibitor	Parasite Growth Inhibition (IC ₅₀) and Effective Concentration (EC ₅₀)	Mammalian Cell, Class-I hHDACs in HeLa Cells, and Sirutin Inhibition IC ₅₀	References
17	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.013 μM	Mammalian Cell IC ₅₀ ~0.26 μM	[136]
18	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.015 μM	-	[89]
19	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.017–0.035 μM	Mammalian Cell IC ₅₀ 0.02–0.8 μM	[137]
20	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.0006–0.0016 μM	Mammalian Cell IC ₅₀ ~0.6 μM	[133,138]
21	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.069 μM	-	[139]
22	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.074–0.107 μM <i>L. donovani</i> IC ₅₀ ~32.1 μM	-	[139]
23	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.144–0.148 μM	Mammalian Cell IC ₅₀ > 5.2 μM	[140]
24	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.93–1.24 μM <i>L. donovani</i> IC ₅₀ ~5 μM	Mammalian Cell IC ₅₀ > 5 μM	[140]
25	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.094–0.226 μM	-	[141]
26	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.76–1.32 μM <i>L. donovani</i> IC ₅₀ 3.2–4.7 μM	-	[141]
27	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.08–0.15 μM	Mammalian Cell IC ₅₀ 0.8–>100 μM	[142]
28	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.09 μM	Mammalian Cell IC ₅₀ ~12.47 μM	[143]
29	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.12 μM	Mammalian Cell IC ₅₀ ~3.24 μM	[143]
30	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.17 μM	Mammalian Cell IC ₅₀ > 50 μM	[143]
30	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.16 (3.45) μM	Mammalian Cell IC ₅₀ ~4.9 μM	[144]
31	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.25–0.32 (2.12–2.25) μM	Mammalian Cell IC ₅₀ ~19.5 μM	[145]
32	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ ~0.019 μM	Mammalian Cell IC ₅₀ ~75.1 μM	[146]
33	Cyclic Tetrapeptide	<i>P. falciparum</i> IC ₅₀ 0.09–0.13 μM <i>T. brucei</i> IC ₅₀ 35 nM	Mammalian Cell IC ₅₀ 0.001–<0.005 μM	[130]
34	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.06–0.13 μM <i>T. brucei</i> IC ₅₀ 2.6 μM	Mammalian Cell IC ₅₀ 1.4–2.4 μM	[130]
35	Hydroxamic Acids	<i>P. falciparum</i> IC ₅₀ 0.01–0.03 μM <i>T. brucei</i> IC ₅₀ 1.69 μM	Mammalian Cell IC ₅₀ 0.07–0.18 μM	[130]
37	Thiols	<i>P. falciparum</i> IC ₅₀ 15.2–19.9 μM	-	[23,26]

Table 1. Cont.

Compound ID	Class/Inhibitor	Parasite Growth Inhibition (IC ₅₀) and Effective Concentration (EC ₅₀)	Mammalian Cell, Class-I hHDACs in HeLa Cells, and Sirutin Inhibition IC ₅₀	References
38	Ortho-Aminoanilide	<i>P. falciparum</i> IC ₅₀ 7.8–8.3 μM	Mammalian Cell IC ₅₀ > 20 μM	[23,26,89]
39	N-Methyl Carboxamide	<i>P. falciparum</i> EC ₅₀ ~0.45 μM	Class-I hHDACs in HeLa cells IC ₅₀ ~16.6 μM	[147]
40	N-Methyl Carboxamide	<i>P. falciparum</i> EC ₅₀ ~0.90 μM	Class-I hHDACs in HeLa cells IC ₅₀ ~20.5 μM	[147]
41	N-Methyl Carboxamide	<i>P. falciparum</i> EC ₅₀ ~0.5 μM	Class-I hHDACs in HeLa cells IC ₅₀ > 25 μM	[147]
42	Class-I/II HDAC inhibitor	<i>P. falciparum</i> IC ₅₀ 9–13 μM <i>L. infantum</i> IC ₅₀ 30 μM	IC ₅₀ (PfSir2A) > 50 μM IC ₅₀ (LiSir2rp1) 194 μM IC ₅₀ (hSIRT1) = 245 μM	[89,148,149]
43	class-III HDAC (Sirutin) inhibitor	<i>P. falciparum</i> IC ₅₀ > 10 μM	IC ₅₀ (PfSir2A) > 400 μM	[89,148,149]
44	class-III HDAC (Sirutin) inhibitor	<i>P. falciparum</i> IC ₅₀ 9 μM	IC ₅₀ (PfSir2A) 35 μM IC ₅₀ (hSIRT1) > 600 μM	[148]
45	class-III HDAC (Sirutin) inhibitor	<i>P. falciparum</i> IC ₅₀ 1.5–2 μM	-	[150,151]
46	class-III HDAC (Sirutin) inhibitor	<i>P. falciparum</i> IC ₅₀ 9.9 mM <i>T. cruzi</i> EC ₅₀ 25.8 mM	IC ₅₀ (PfSir2A) 51 μM IC ₅₀ (hSIRT1) 88–250 μM	[148,149,152]
47	class-III HDAC (Sirutin) inhibitor	<i>P. falciparum</i> IC ₅₀ 9.8 μM	IC ₅₀ (PfSir2A) 23 μM	[148]
50	class-III HDAC (Sirutin) inhibitor	<i>T. brucei</i> IC ₅₀ 0.034 μM	-	[153]
51	class-III HDAC (Sirutin) inhibitor	<i>T. brucei</i> EC ₅₀ 0.029 μM	-	[154]
52	Class-I/II HDAC and sirutin inhibitor	<i>T. cruzi</i> EC ₅₀ 10.6 μM	IC ₅₀ (TcSir2rP3) 1 μM	[24]
53	Class-I/II HDAC and sirutin inhibitor	<i>L. donovani</i> IC ₅₀ 4.4–8.8 μg/mL	IC ₅₀ (hHDAC6) 35 nM IC ₅₀ (hHDAC8) 854 nM	[114]
54	Class-I/II HDAC and sirutin inhibitor	<i>L. donovani</i> IC ₅₀ 0.1–6.5 μg/mL	IC ₅₀ (hHDAC6) 457 nM IC ₅₀ (hHDAC8) 1272 nM	[114]
55	Class-I/II HDAC and sirutin inhibitor	<i>L. donovani</i> IC ₅₀ 0.1–6.3 μg/mL	IC ₅₀ (hHDAC6) 847 nM IC ₅₀ (hHDAC8) 4283 nM	[114]
56	Class-I/II HDAC and sirutin inhibitor	<i>L. infantum</i> IC ₅₀ 1.49 mM	-	[115]
57	Class-I/II HDAC and sirutin inhibitor	<i>L. infantum</i> IC ₅₀ 6.03 μM	IC ₅₀ (LiSir2rp1) 5.7 μM IC ₅₀ (hSIRT1) 97.4 μM	[116]
58	class-I/II/IV HDAC inhibitors	<i>T. gondii</i> IC ₅₀ 39 nM	-	[128]

Patel et al. performed a high-throughput assay to study the antimalarial efficacy of approximately 2000 HDACis obtained from the compound library. The library was characterized by a moiety of acyl hydrazone as CU and with diverse moieties for CAP protein and zinc-binding group along with the hydrophobic linker length such as 4–6 methylene units [81]. The study revealed that numerous compounds strongly inhibited the growth of *P. falciparum* and also hampered recombinant PfHDAC1 enzymatic activity. Among them, seventeen derivatives displayed a low range of nanomolar antiparasitic activity for acetylation of histone along with minimal perturbation of human myeloma MM1S cells as

an indicator of selectivity [95]. Within this series, the selective inhibition of *P. falciparum* growth was highly enhanced by the existence of ortho-substituents, such as bromine and hydroxyl, in the aromatic group of CAP protein along with the presence of metal chelator or hydroxamic acid and five methylene units as a linker (compound **18**, Table 1) [95]. Another study by Kozikowski et al. on two series of suberoyl amide hydroxamates substituted moieties of triazolyl phenyl and phenyl thiazolyl as CAP protein groups [137,143]. The triazolyl phenyl-based compound **19** showed high efficacy against the multidrug-resistant strains of *P. falciparum* (C2A and C235), with IC_{50} values in a range of 0.017–0.035 μ M. It was 10-fold more potent compared to its counter congeners such as chloroquine and mefloquine. Compound **19** was 23-fold highly selective for C235 over mammalian cells [143]. In a group of 50 phenyl, thiazolyl hydroxamate-based HDACi, three compounds were highly potent with IC_{50} values < 3 nM and showed 600-fold selectivity against *P. falciparum* compared to mammalian cells. Compound **20** (WR301801) was the most favorable HDACi derivative, with IC_{50} values 0.6–16 nM for several drug-resistant strains, D6, W2, C235, and C2A, of *P. falciparum*. It displayed notable inhibition of HDAC activity in nuclear extracts of *P. falciparum* with IC_{50} ~10 nM along with a strong in situ parasitic histones hyperacetylation [137]. In another report, compound **20**, upon oral administration as monotherapy, at doses of up to 640 mg/kg demonstrated a notable suppression of parasitemia but did not rehabilitate *P. berghei*-infected mice. However, some mice, but not all mice, were rehabilitated upon treatment with compound **20** (52 mg/kg) when co-administered with a subcurative dose of chloroquine (64 mg/kg) [137]. Similarly, compound **20** on oral administration at a dose of 32 mg/kg/day for 3 days to Aotus monkeys infected with *P. falciparum* resulted in suppression of the parasite but not complete elimination [137]. Another reported study showed that compound **20** (50 mg/kg/day for 4 days) on i.p. injection with an experimental follow-up period of 6 weeks enhanced survival of infected mice infected with *P. berghei* and irreversibly downregulated the parasitemia [144]. However, the optimization of the pharmacokinetic properties of compound **20** would show advantageous results as it is quickly hydrolyzed to the corresponding inactive carboxylic acid [137]. These findings suggest the potential of HDACi in mono/combination therapy for malaria treatment [12,26,137,144]. Oyelere et al., reported a series of aryl triazolyl hydroxamate-based HDACis that were screened for their inhibitory activity against *L. donovani* promastigote stages and asexual blood stage of *P. falciparum* [145]. Several compounds displayed improved inhibitory activity and selectivity than compound **3** against D6 and W2 strains of *P. falciparum*. Although several compounds are less active against *P. falciparum*, they showed a modest potency of inhibition against *L. donovani*, with 2- to 4-fold better IC_{50} values than compound **3** and equivalent to the standard drug miltefosine, used to treat visceral Leishmaniasis. Antiparasitic activity depends on polymethylene linker length and the nature of the CAP group. The CAP moiety showed maximum activity against both parasites when 5 and 6 methylene units were present in the spacer region between the CAP and the ZBG. Compounds **21** and **22**, with a CAP protein (3'-biphenyltriazolyl moiety) and linker (6 and 5 methylene units), showed the highest selectivity and activity against *P. falciparum*. Compound **22** also showed its activity against *L. donovani* with an IC_{50} of 32 μ M [145]. Based upon this concept, Oyelere et al., tested five tricyclic ketolide-based phenyltriazolyl HDACi for antimalarial and antileishmanial activities [146]. During the study, it was noticed that compound **23**, with six methylene units as the optimal linker present between the CAP and hydroxamic acid, displayed optimal antimalarial activity. Similarly, compound **24**, hydroxamic acid with nine methylene units, displayed the best antileishmanial activity, but the study does not correlate with the PfHDAC1 inhibition [146]. Particularly, compound **23** displayed IC_{50} in the range of 0.144–0.148 μ M against both chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* strains. However, the compound displayed 7 to 10-fold lesser activity than compound **3** and 10-fold high selectivity against mammalian Vero cells without antileishmanial activity. Further, compound **24** showed anti-*L. donovani* activity with an IC_{50} of 5 μ M, which was 16-fold stronger than compound **3** with an IC_{50} of 81 μ M [146] (Figure 4).

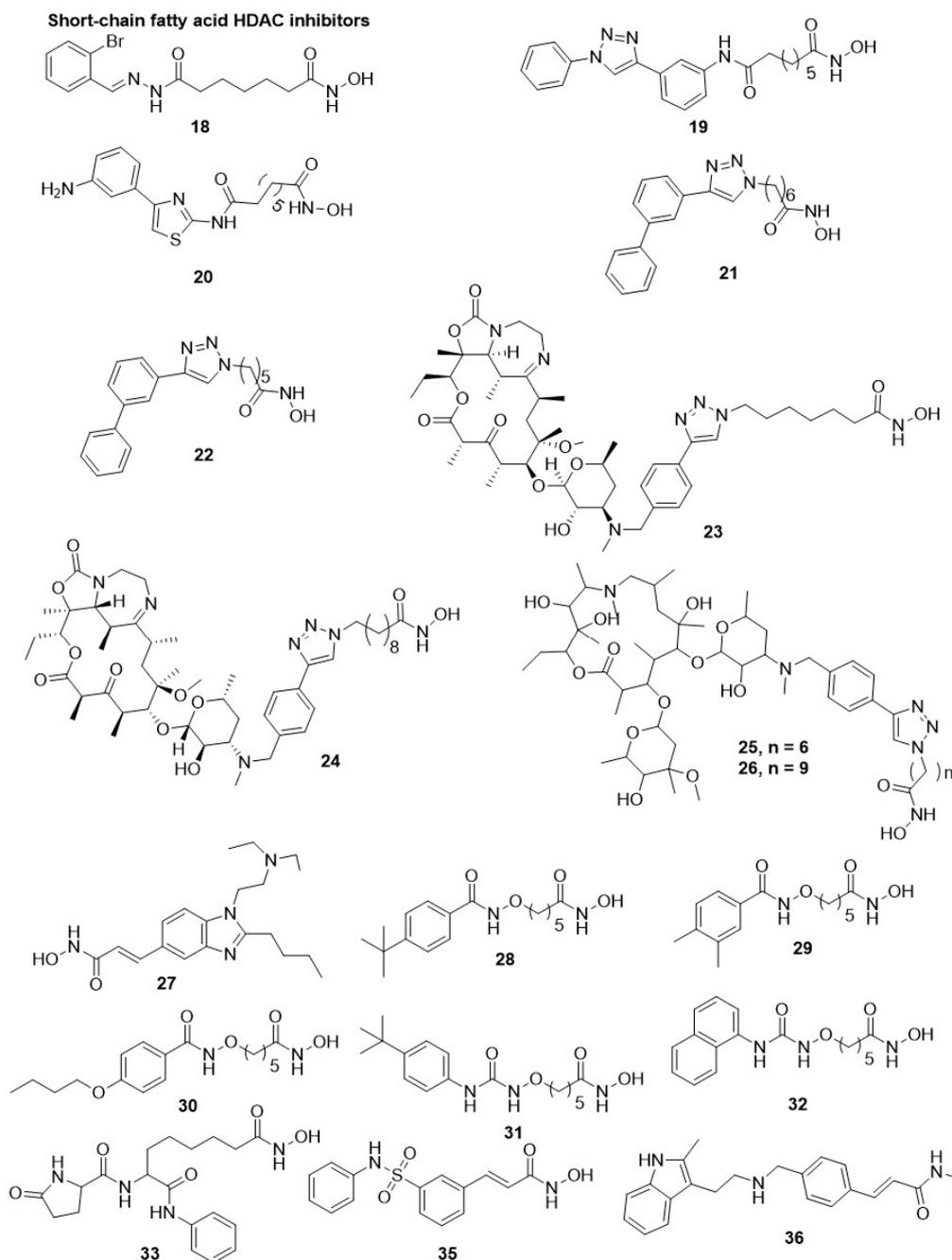


Figure 4. Chemical structure of hydroxamic acid HDAC inhibitors. The short chain fatty acid displays diverse HDAC inhibition.

Oyelere et al., further investigated and described the antimalarial and antileishmanial potential of 14 and 15-membered nonpeptide macrocyclics connected to a moiety of phenyl-triazolyl, a CAP protein group, as HDACis. All compounds inhibited the proliferation of chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* [155]. However, the best activity and selectivity against *P. falciparum* were attained for the six methylene units linker between CAP (triazole ring) and ZBG group (hydroxamate). Among them, compound 25, a skeleton of 15-membered macrolide with an IC₅₀ value of 29 nM against PfHDAC1 enzyme, manifested 11-fold higher antiplasmodial activity with 14-fold enhanced selectivity over mammalian Vero cells compared to compound 3. Surprisingly, the linker group having five to seven methylene units was lacking anti-*L. donovani* activity on the promastigote stage for both skeletons of a macrolide, whereas the compound having

eight or nine methylene units as linker displayed the highest activity similar to ketolide-based HDACis, but distinct from the structure–activity relationship was noticed in aryl triazolyl hydroxamates [145].

Specifically, compound **26** with a skeleton of 14-membered macrolide and methylene units linker was 25-fold more active than compound **3** with an IC₅₀ value of 81 μM, showing the best antileishmanial activity, with IC₅₀ values of 3.2 and 4.7 μM against promastigote and amastigote stages of the parasite, respectively [155]. Andrews et al., identified compound **27** (pracinostat or SB939), an orally active anticancer HDACi, having antiplasmodial activities [156]. Furthermore, compound **27** strongly hampered the proliferation of the asexual-stage of *P. falciparum* in mammalian erythrocytes, with IC₅₀ values in the range of 0.08–0.15 μM. Compound **27** also caused hyperacetylation on histone and non-histone proteins in the parasite and displayed selectivity over mammalian cells ranging 4 to >1250-fold over tested cell lines [156]. Additionally, an additive effect was first observed for its combination with lopinavir, a protease inhibitor, as antimalarial targeting a liver stage. Compounds **27** strongly inhibit the exo-erythrocytic stage of *P. berghei* in human HepG2 hepatocytes, with an IC₅₀ value of 150 nM [156]. The oral administration of compound **27** up to a dose of 100 mg/kg/day decreased peripheral parasitemia and total parasite load in the ANKA mouse model infected with *P. berghei*. However, the administration of compound **27** to mice fended off the occurrence of cerebral malaria-like symptoms up to 2–3 weeks after the intervention but did not affect hyperparasitemia in the treated animals [156]. Oyelere et al. also explored a group of 21 HDACi with Pentylloxamide as a linker and substituted benzene ring as CAP for their effect against different stages of *Plasmodium*, such as *P. falciparum* (a sexual blood stage (3D7) cell line), *P. berghei* (tissue schizontocidal stage), and late stage of *P. falciparum* gametocyte (IV and V). All compounds exhibited antimalarial activity against *P. falciparum* asexual form with potency and selectivity enhanced as the bulkiness of alkyl/alkoxy substituents in the phenyl ring at the *para* position were increased regarding compound **28**. Three derivatives, compounds **28**, **29**, and **30**, showed their activity against all three stages of *P. falciparum* with IC₅₀ values of 0.09, 0.12, and 0.17 μM, respectively. Several compounds of this series displayed better selectivity against the parasite compared to compound **3** for the asexual and exo-erythrocytic life cycle stages of the parasite [157]. Further, Oyelere et al., described the structure–activity relationship and antimalarial activity of a group of HDACi based on alkoxyurea. Some compounds actively inhibited *P. falciparum* (3D7 cell line) and showed gametocytocidal activities at early and late-developmental stages with IC₅₀ values ranging 1.68–6.65 μM [147,148]. Structure–activity relationship study revealed that the hydroxamic acid as a zinc-binding group and 5 methylene units as a linker were important for antiplasmodial activity. N-methyl hydroxamic acid, o-hydroxyanilide and o-amino anilide were inactive as a zinc-binding group, while the short-chain analogues with linker less than 5 methylene units displayed lower potency [147]. Furthermore, the 4th position is substituted with bulky alkyl/alkoxy substituent of the phenyl ring of the CAP group. When this group is replaced with bulky aromatic rings leads to the development of more potent and effective compounds against asexual and gametocyte forms of *P. falciparum*, as denoted by the 4-tert-butyl derivative (**31**) and 1-naphthyl derivative (**32**). However, compounds **31** and **32** did not display better potency than compound **3**, but, under the test condition, **32** displayed higher selectivity than compound **3** along with gametocidal activity in the asexual and later stages [148].

In 2015, Giannini et al., evaluated twelve analogues for their antiparasitic activity against *P. falciparum*, *T. cruzi*, *L. donovani*, *G. lamblia*, and *T. brucei*. These twelve analogues were differentiated at the α position of the anilide (CU) and meta position of the phenyl ring (CAP) and substituted with β-lactam-carboxamides and trifluoro methyl groups, respectively. Additionally, a thiol or hydroxamic acid is present as a zinc-binding group. Compound **33** of this series displayed a significant IC₅₀ of 0.019 μM against *P. falciparum* [149]. In 2015, Andrews et al., described the potential of four clinically approved HDACi, compounds **34** (romidepsin or FK228), **35** (belinostat), **36** (panobinostat), and **3**, for cancer

treatment against *P. falciparum* and *T. brucei* parasites. All compounds inhibited the growth of *P. falciparum* parasite, with IC_{50} values ranging from 0.09–0.13, 0.025–2.2, 0.06–0.13, and 0.01–0.03 μ M, respectively. Interestingly only compound **34** was active against the bloodstream form of *T. brucei*, with an IC_{50} value of 35 nM, despite lacking mammalian cell selectivity. These four HDACi inhibited the *P. falciparum* nuclear extract deacetylase activity and recombinant PfHDAC1 due to hyperacetylation of non-histone protein and contrastingly affecting histones (H3 and H4) acetylation. Compounds **3** and **35** did not show selectivity towards malaria parasites over mammalian HEK29 and NFF 3 cells [148] (Figure 5).

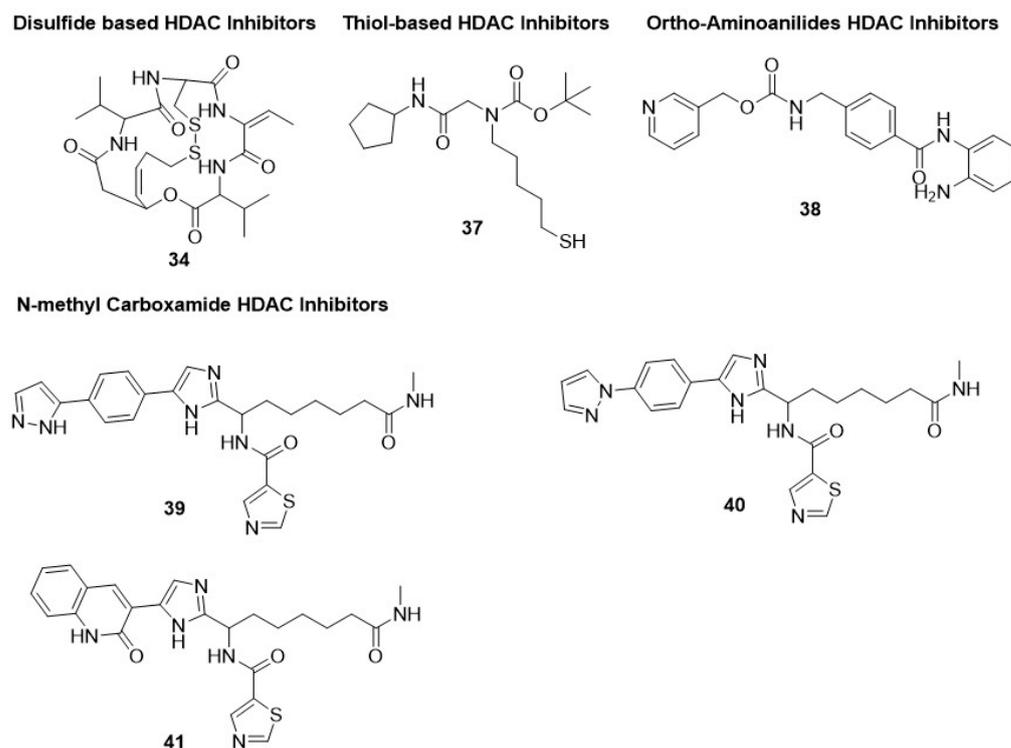


Figure 5. Chemical structure of disulfide-based, thiol-based, orthoamino-anilide-based, and N-methylcarboxamide-based HDAC inhibitors. These inhibitors displayed anti-malarial activity.

5.1.4. Thiol-Based HDAC Inhibitors

Thiol-based HDAC6-selective inhibitor **37** displayed poor inhibitory potential against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum*, with IC_{50} values in the range of 15.2–19.9 μ M [150]. Thus, further verification is needed to validate the antimalarial potential of hydroxamate-based pan-HDACi [93] (Figure 5).

5.1.5. Ortho-Aminoanilides HDAC Inhibitors

A similar inference can be made by choosing an *ortho*-amino anilide group as a zinc-binding group. The precursor of these HDACi in the class-I-HDAC-selective inhibitor is denoted as compound **38** (MS275 or entinostat) [154–156]. Multiple trials have been completed on compound **38**, revealing its potential as a PfHDAC1 inhibitor and parasitic proliferation inhibitor, with IC_{50} values of 0.94 and 8 μ M, respectively [12,26,88,93,95]. Compounds **39**, **40**, and **41** are N-methyl carboxamide derivatives that displayed antimalarial potential against *P. falciparum* [158] (Figure 5).

5.2. Antimalarial Class-III HDAC (Sirtuin) Inhibitors

Fewer Sir2 inhibitors have been tested against *P. falciparum*-infected erythrocytes for their antiproliferative activity and inhibition of the recombinant PfSir2A protein [12,26]. Generally, the Sir2 inhibitors showed moderate activity against the growth of *P. falciparum*. A poor homology has been observed between the parasite and other eukaryotic Sir2

proteins [97,98]. Several known natural and synthetic sirtuin inhibitors that have been investigated over the years for growth inhibition of *P. falciparum*. Compound **42** (sirtinol), **43** (splitomycin), **44** (surfactin), and **45** (hyperforin) displayed IC₅₀ values in the range of 9–13, >10, 9, and 1.5–2 µM, respectively, against *P. falciparum* [153,154,159,160]. Compound **46** (nicotinamide) is a Sir2-catalysed reaction product and causes non-competitive inhibition of both acetylated peptide and NAD⁺ but less active at the whole-cell level, with an IC₅₀ value of 9.9 mM for retarding parasite growth [161]. Nicotinic acid, also a product of Sir2-dependent enzymatic pathways, did not show this effect [161].

Compounds **44** and **46** displayed different activity against PfSir2A, with IC₅₀ values of 35 and 51 µM, respectively. Meanwhile, compounds **43** and **44** showed less active activity, with IC₅₀ values of >400 and >50 µM, respectively. Chakrabarty et al., synthesized analogues of lysine-based tripeptide based on a mechanism of competition with the binding pocket of PfSir2 [162]. From the four analogues, three analogues showed equivalent or improved activity, with IC₅₀ values in the range of 23–34 µM against PfSir2A in contrast to compounds **44** and **46** [162]. Compound **47** was highly potent against PfSir2A and also tested against *P. falciparum*-infected erythrocytes where it displayed parasitic inhibitory potential similar to compounds **34** and **36** with an IC₅₀ value of 9.8 µM [162]. Resveratrol (**48**) and isonicotinic acid (**49**) are activators of hSIRT1 that moderately inhibit *P. falciparum* growth, but during in vitro testing against recombinant PfSir2A, no enzymatic activation or inhibition was reported [79,160]. However, this is fully anticipated as PfSir2A and PfSir2B are not required for the growth and development of the parasite and share a few homological sequences with Sir2 proteins of other eukaryotes [97,98]. In addition, several compounds, **42**, **43**, **46**, and, to a lesser extent, compound **44**, displayed lower inhibitory potential against PfSir2A and the in vitro antiproliferative effects due to their multiple unrelated characters at a single gene locus. Thus, the identification and development of inhibitors with a notable enhancement of potency against PfSir2 and the selectivity towards sirtuins of humans could be a functional gadget considering *P. falciparum* sirtuins' biology and their pharmacological validation as a target of drugs that either directly or indirectly produce antiparasitic activity through obstructing the parasite avoidance from the host innate immune system [97,98] (Figure 5).

5.3. Antitrypanosomal HDAC Inhibitors

A few compounds were tested against the *Trypanosoma* species. Compound **10** appeared to prevent the spread of the bloodstream form of *T. brucei* at a concentration of 7 µM but without affecting a silent variant surface glycoprotein expression site [163]. Another study was conducted where the similar compound **10** hampered recombinant TbDAC3 and TbDAC1 activity by >50% at 5 µM concentration. However, this compound cannot alter the acetylation of the histone protein of the parasite at a concentration of 0.3 µM [146]. Further, four HDACis, compounds **34**, **3**, **35**, and **36**, were also tested by Engel et al., for the inhibition of trypanosomal and malaria parasites [164]. The result of the study revealed that all inhibitors effectively inhibit *T. brucei* but all of them displayed cytotoxicity towards the human NFF and HEK 293 cells at lower concentrations, with a selectivity index less than 1 [136]. However, compound **34** is highly potent against *T. brucei* with an IC₅₀ value of 35 µM.

In 2012, several clinically approved hydroxymates-based HDACis like long-chain amides, sulphonamides, heterocycle-containing acrylamides, and sulphonylpiperazines were tested against a cultured bloodstream form of *T. brucei* for their inhibitory effect [165]. The result of the study indicated that long-chain amides with IC₅₀ values > 10 µM displayed a notable inhibitory property on the growth in the range of IC₅₀ values 0.034–1.54 µM. Among them, sulphonylpiperazines with heteroaryl ring attached to the piperazine moiety displayed potent inhibition. Compound **50** displayed an IC₅₀ value of 34 µM and was able to induce parasite death at 2 µg/mL within 4 h after treatment. All four compounds displayed powerful inhibition of the hHDAC activity, with IC₅₀ values in the range of 0.010–0.212 µM [165] (Figure 6).

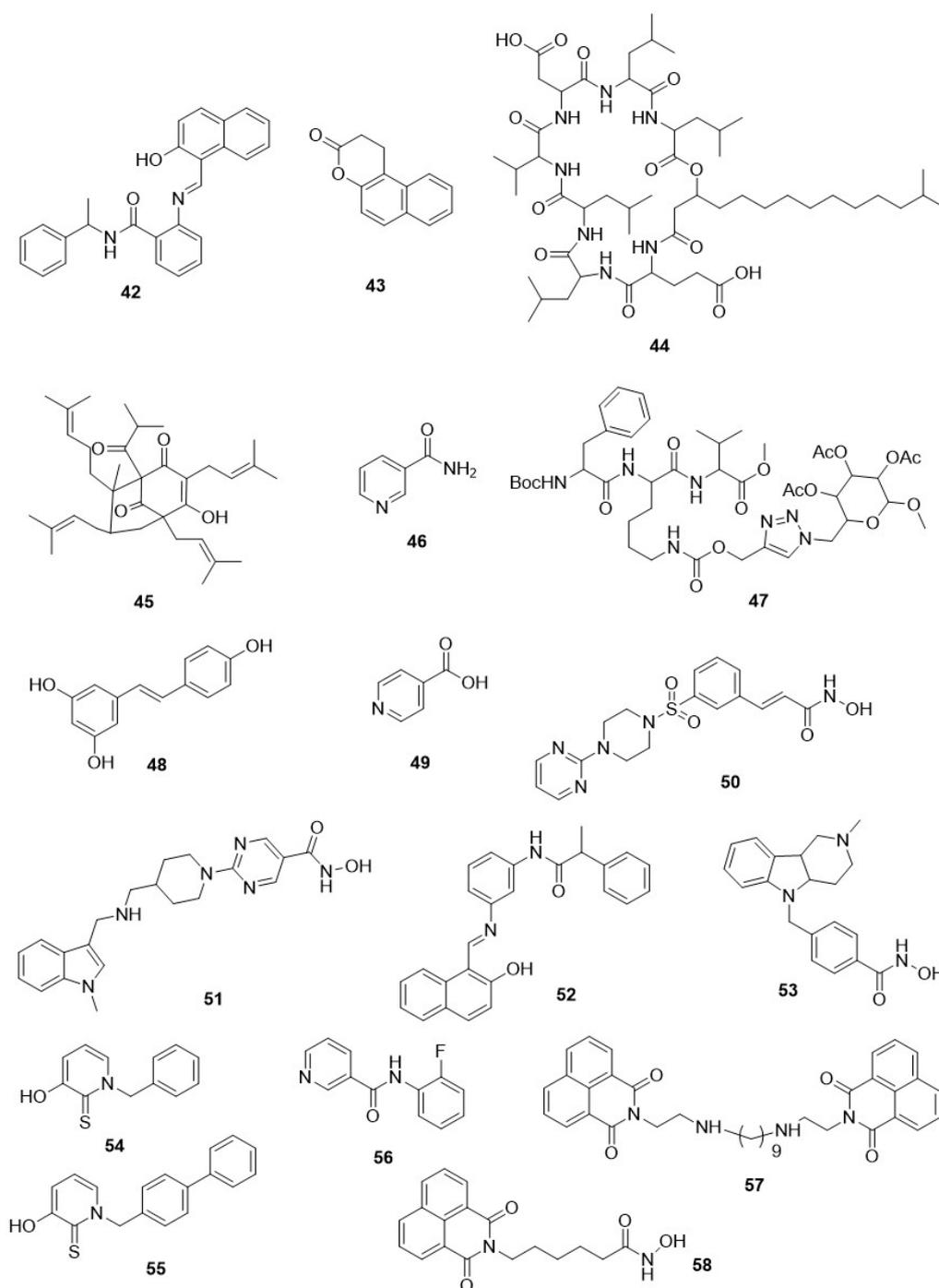


Figure 6. Chemical structure of other HDAC inhibitors. Diverse chemical structure shows varying range of parasitic HDAC inhibition.

Lately, two recently approved hydroxamate-based HDACis (36, 51) and four hydroxamate-based HDACis that are already available as anticancer agents under clinical trial were investigated to determine their inhibitory capacity on the proliferation of *T. brucei* in the cultured bloodstream. The result of the study revealed that all compounds moderately inhibited the parasite, with EC_{50} ranging 0.029 to 11 μ M. The most effective compound was 51 (quisinostat) [166]. Compounds 35 and 36, anticancer drugs, were unable to destroy the cultured parasites at the human-tolerated doses after a single dose administration. Furthermore, compound 36, a sustained-acting HDACi when administered with Human African Trypanosomiasis (HAT) drugs, like pentamidine, suramin, melarsoprol,

and nifurtimox, did not display a synergistic effect [166]. Accordingly, the application of these two HDACis as single agents or in combination for repurposing in HAT treatment is ruled out. Overall, there is lack of associations between the potency against any isoform of hHDAC and the prevention of *T. brucei* proliferation. From this idea, the author proposed that HDAC of trypanosome might have a distinctive specificity that could be used for the development of powerful HDACis having selectivity towards the parasite over hHDACs [166].

5.4. Antitrypanosomal Class-III HDAC (Sirtuin) Inhibitors

Compound **46** has been described as a putative inhibitor of *T. cruzi* sirtuin TcSir2rp1 and a growth inhibitor of the parasite at its developmental stages, including trypomastigote and epimastigote. It is evident by a notable inhibition in the number of amastigotes in the *T. cruzi*-infected macrophages [167]. However, besides SIRT inhibition, it also possesses a pleiotropic response; i.e., one gene influences many phenotypic expressions [167]. Similarly, Moretti et al., characterized two sirtuins, TcSir2rp1 and TcSir2rp3, of *T. cruzi* and described that compound **52**, an antitumor agent in a different model of cancer, could be a potential SIRTi and inhibit growth and differentiation of *T. cruzi* [79,111,168,169]. Compound **52** diminished the proliferation of epimastigote, with an EC₅₀ value of 10.6 µM, and prevented its transformation into infective forms. Furthermore, the activity of the recombinant TcSir2rp3 was hampered by compound **52**, resulting in an IC₅₀ value of <1 µM. Hence, it could be proposed that the antiparasitic activity of the compound occurred via TcSir2rp3 inhibition. Experimental evidence holds up this finding that, with the addition of compound **52**, the overexpression of TcSir2rp3 and all phenotypic effects were reduced. Appreciably, compound **52** reduced parasitemia in a *T. cruzi*-infected BALB/c mouse model at a lower dose in comparison to a higher in vivo dose that is used for cancer treatment. Nonetheless, compound **52** was not able to prevent the mortality of mice at this dose. Thus, rationally, it can be used to develop a more potent and selective isoform-based SIRTi of the parasite as a potential agent against *T. cruzi* infection [111].

Furthermore, a molecular docking study of pan-SIRTi regarding compounds **42**, **46**, and SIRTi (thiobarbiturate derivatives) of class-III inhibitors revealed that there was strong correlation between human mitochondrial SIRT5 and TcSir2rp3 with respect to strength and binding mode [170]. Although the comparison of TcSir2rp3 catalytic pocket with human protein SIRT5 homologous through in silico surface and structural analysis has permitted the recognition of insignificant but notable structural differences at specific inhibitory catalytic domains, that would probably be used for the development of preferable TcSir2rp3 inhibitors with higher specificity towards *T. cruzi* [170].

5.5. Antileishmanial HDAC Inhibitors

The response of *Leishmania* parasite towards HDACi indicates the HDACs' specificity and importance for survivability [12,120,145,146,155]. Both antimalarial and antileishmanial properties of hydroxamate-based class-I/II HDACis were discussed by Oyelere et al. [155]. Furthermore, they studied the effect of 3-hydroxypyridine-2-thiones (3HPTs) on the *L. donovani* survivability in amastigote and promastigote forms, which were earlier described as inhibitors of hHDAC6/hHDAC8 [120]. Compound **3**, the pan-HDACi, along with compound **53** (tubastatin A), another hydroxamate-based HDACi, and the reference compound PCI-34051, a selective inhibitor of hHDAC6 and hHDAC8, was taken [120]. Compound **53**, an hHDAC6-selective inhibitor, produced equal antileishmanial activity at both the developmental stages of *L. donovani*. This effect was not observed with compound **3** and PCI-34051, a selective hHDAC8 inhibitor. The result also revealed that compounds **54** and **55**, 3-HPT-derived HDACi, produced cytotoxicity both at intracellular and extracellular species of *L. donovani*, with IC₅₀ values ranging from 0.1 to 6.5 µg/mL. Thus, the investigator suggested that the antileishmanial efficacy resulted in *L. donovani* due to inhibition of HDAC6. This paves a new way for protozoan HDAC6-like activity besides selective isoform inhibitors that might develop a more attentive therapeutic primary plan for Leishmaniasis treatment [120].

5.6. Antileishmanial Class-III HDAC (Sirtuin) Inhibitors

Still, a smaller number of SIRTis furnished with antileishmanial activity have been described [12]. Compound **44** demonstrated developmental-stage-specific antiproliferative properties against *L. infantum* parasites [171]. The compound led to apoptotic cell death due to DNA double helix breakage and inhibited the multiplication of axenic amastigotes but did not alter the growth of parasite promastigotes with an IC_{50} value of $>60 \mu\text{M}$ [171]. Overexpression of LmSir2rp1 of parasite results in less susceptibility towards the fragmentation of DNA on the treatment of compound **12** [171].

Similarly, compound **46** can inhibit the growth of *L. major*; even LmSir2rp1 overexpression did not protect the parasite against this compound [172]. Several in silico and biochemical studies disclosed significant differences between the LmSir2rp1 and catalytic domains of human SIRTs [102]. In another in vitro investigation, four compounds effectively inhibited the axenic amastigote growth of *L. infantum*, but compound **56**, a nicotinamide derivative, inhibited LmSir2rp1 [121]. Later, an investigation revealed that compounds **42** and **46** can also inhibit the recombinant LiSir2rp1, with IC_{50} values of 194, and 40 μM , respectively, but similar results were also observed against the human SIRT1 enzyme. Thus, selectivity towards the parasite was lacking [122]. Furthermore, Tavares et al., described antileishmanial activity and structure–activity relationship study of twelve compounds belonging to bis-naphthalimidopropyl (BNIP) derivatives that varied in the central alkyl chains length, with 2, 3, or 4 nitrogen atoms, connecting two moieties of BNIP [122]. All derivatives of BNIP could suppress LiSir2rp1, with IC_{50} values ranging from 7 to 54.7 μM , and displayed selectivity for hSIRT1 in certain cases. The inhibition and selectivity of LiSir2rp1 seem to rely on linker group length and total charge. Diamine BNIP derivatives having linker 4–7 methylene units were less effective than 8–12 methylene linker units. Further, the additions of the positive amino group to the linker do not affect the selectivity or efficacy.

Compound **57** (BNIP9), having nine methylene units in the linker group, displayed IC_{50} value of 5.7 μM against parasitic LiSir2rp1 with 17-fold enhancement in specificity over hSIRT1 [122]. Furthermore, the derivatives of BNIP could inhibit the intracellular development of *L. infantum* amastigotes, with IC_{50} values ranging from 1–10 μM . Still, a linear interaction between LiSir2rp1 inhibitory property and antiproliferative effects in the amastigote stage could not be established. Certain derivatives are committed to have favorable antileishmanial efficacy, as shown in *L. infantum*-infected BALB/c mouse model [173].

5.7. Antitoxoplasma HDAC Inhibitors

Several HDACs belonging to class I/II with the cyclic tetrapeptides displayed inhibitory activity on the growth of *Toxoplasma gondii*. Compound **5** with IC_{50} values ranging from 3 to 15 nM showed its potential but did not possess selectivity over the mammalian cells [123]. The cyclic tetrapeptide compound **6** obtained from the fermentation broth of *Acremonium* species showed rapid growth inhibitory effects against tachyzoites of *T. gondii*, with IC_{50} value of 10 nM and 13-fold specificity over human foreskin fibroblasts [132]. These compounds are effective against drug-sensitive and resistant *P. falciparum*-infected erythrocytes. Compound **6** caused hyperacetylation of H4 histone of *T. gondii* through specific inhibition of TgHDAC3 enzyme. The sensitivity for compounds **5** and **6** was reduced due to single-point mutations within TgHDAC3 (T99A and T99I). Hence, these compounds targeted TgHDAC3, providing genetic proof. Intriguingly, compound **6** displayed turning off the enzyme inhibition due to the insertion of two residues (T99 and A98) within the TgHDAC3 catalytic site. This indicated the presence of a simple safeguard in the HDAC3 family of Apicomplexan, which is not available in any other HDACs of eukaryotes [132].

In addition, altered gene expression and stage-specific transformation in *T. gondii* were completed by TgHDAC3. The treatment with compound **6** converts the replicative tachyzoite to the non-replicative bradyzoite [174]. A successive study narrates that compound **6** was effective in *T. gondii* cysts treated ex vivo and also affected converted cysts and bradyzoites. Particularly, compound **6** diminished the capacity of bradyzoites for conversion to tachyzoites beyond cyst wall injury; thus, isolated free bradyzoites after cell

wall lysis were not able to multiply. Ultimately, the cysts formerly treated with compound 6 injected in mice did not produce infection.

Similar studies were conducted for compound 6 cognate (W363 and W399), which is highly active against tachyzoites and afforded better selectivity of 48–62 fold over human foreskin fibroblast cells. Hence, it could be preferable for in vivo studies in the near future [175]. However, compounds 7, 8, and 9 displayed poor activity against tachyzoites of *T. gondii* and showed lower selectivity over HS69 mammalian cells [135]. Hydroxamate-based HDACis have also been assessed against *T. gondii* parasites in the tachyzoite stage [134]. Among them, compounds 10, 58 (scriptaid), and 3 hampered tachyzoites of *T. gondii*, showing IC₅₀ values of 41, 39, and 83 nM, respectively. However, compound 11 displayed 2–5 times lower potential than others, with IC₅₀ of 213 nM [134]. Likewise, compounds 3 and 58 displayed improved selectivity towards the *P. falciparum* parasite than compound 10 over mammalian cells [134]. Surprisingly, compounds 10, 3, and 48 at low concentrations of 1–50 nM reduced the infectivity of *T. gondii* tachyzoite through suppression of proliferation and survival [134]. Very few facts are available on the effectiveness of HDACis belonging to class III against several species of *Toxoplasma*. A single report suggested that compound 46 is inactive against tachyzoites of *T. gondii*, with an IC₅₀ value of 50 mM [134].

6. Challenges Regarding HDAC Inhibitors as Antiparasitic Agents

HDACis are a new class of favorable compounds that would function as potential antiparasitic agents. Some of them, such as Vorinostat, Romidepsin, Belinostat, Panobinostat, and many others, show notable in vitro and ex vivo potential [61,176]. A few of them have shown their in vivo potential against particular parasites. Despite the increasing focus on their antiparasitic effects, many censorious points are yet to be explored before taking SIRTi/HDACi towards the clinical phase as antiparasitic candidates [12,26].

The clinical therapy of parasitic infections is quite different compared to other diseases, like cancer. Most morbidities and mortalities are linked with parasitic diseases that happen in underdeveloped areas of the world. Immune-compromised people are at utmost risk. Concomitant infection with dissimilar infective agents is quite a common contributory factor to the development of parasitic disease and needs to be observed because of potential drug–drug interactions. The salient features in prior consideration regarding HDACis for clinical application include: (i) compounds should have high potency with in vivo selectivity towards the parasite compared to normal host cells; (ii) highly active against organisms that are resistant to present drugs; (iii) having an elevated level of safety profile for application in high-risk groups like pregnant women and children; (iv) possess high oral bioavailability and efficacious pharmacokinetic profiles necessitating a single-dose/day; (v) cost-effective to allow potential treatment in hundreds of millions of underprivileged people; and (vi) supportive pharmacokinetics with powerful partner drug to fend off the occurrence of drug resistance.

To take HDACi belonging to class I/II towards clinical trial for parasitic illness, an elevated level of potency and selectivity for parasites against host cells is required. There is an opportunity for optimization of the chemical structure of presently available HDACis belonging to class I/II for therapeutic application against parasites by alteration of the zinc-binding group, CAP protein, and linker. This paves the way for medicinal chemistry programs to specifically target isoforms of parasitic HDAC. Such approaches are still challenging; however, they require cloning and expression of recombinant parasitic HDAC isoforms for enzyme assays and crystallization studies as well as information on the molecular function of these enzymes. This in turn paves the way to specifically pick out HDAC isoforms of a parasite.

The zinc-binding site in HDACs and the presently available tube-shaped pocket between zinc and the HDAC enzyme surface are well-preserved between the enzymes of humans and parasites. The linker moieties for HDACis are not abundant as they are confined by the size and shape of the tunnel to the active site [92,93]. Nonetheless,

this region can still be utilized for the development of selective HDACis of the parasite. Heimburg et al., reported selective smHDAC8 inhibitors that displayed a heteroaromatic ring as a linker group [175]. In addition, the linker group length has been initiated to be a major structural decisive factor for both antimalarial and antileishmanial activities of two categories of nonpeptides. Macrocyclic HDACi shows higher antileishmanial activity, with linkers consisting of eight or nine methylene units, while, on the contrary, the foremost antimalarial potency was noticed for spacers with five or six methylene units [146]. The CAP protein group implied in the interplay with the rim at the catalytic tunnel entrance provides a greater extent of dissimilarity and provides room to enhance the activity and selectivity for future antiparasitic HDACis. Furthermore, for increased clinical effectiveness, replacement of the connecting unit could also be launched, such as with Giannini et al., who reported the addition of a γ -lactam carboxamide in α position to the anilide CU of the compound **3** [149]. The zinc-binding hydroxamate, available in several of the most effective HDACis, confers potency and affinity for HDAC enzymes and is not an absolute zinc-binding group. The hydroxamate moiety is also a possible site for metabolic attack and has no selectivity for chelating the zinc ion of HDACs. However, it can tie up to other proteins containing zinc or other metal ions, leading to such probable toxicity issues as those noticed for hydroxamate-carrying inhibitors of matrix metalloprotease [177,178]. However, the alteration of the zinc-binding group was not successful in most cases. Recently, Ontoria et al. were able to use the secondary amide moiety along with the hydroxamate group as a selective HDACi of *P. falciparum*. This proposed a method for the development of novel HDACis with enhanced metabolic stability and selectivity [158].

To restrict the toxicity potential, subsequent studies were carried out to identify compounds with parasite sensitivity for HDACs/SIRT and their isoform selectivity. The development of certain isoform-specific HDACis has begun to spare hHDACs and is described as the first blueprint of HDACis having selectivity towards the parasite regarding smHDAC8-selective inhibitors. The crystal structures of mHDAC8 form a network with the 3-amido benzohydroxamate group of HDACis. It was established that the two residues, His292 and Lys20, are independently connected by hydrogen bonds to the amide group of the previously mentioned inhibitors. This recommends a scheme for enhancing the activity against smHDAC8 and providing specificity over hHDAC6 and hHDAC1. Nonetheless, the exploration of HDACis with selectivity towards the parasite remains demanding since it requires the cloning and expression of each isoform of recombinant parasite HDAC. To affirm the designing of preferred HDACis, 3D models have been generated for HDACs of parasites and their human homologs through homology modelling [91,92,121,167,170,175,179–181]. In silico homology modelling studies of PfHDAC1 have displayed similarity with hHDACs; however, some discrepancies could be utilized for designing novel selective potential inhibitors [92].

7. Discussion

Melesina et al., studied recent homology models of *Leishmania*, *Trypanosoma*, and *Plasmodium* HDACs and differentiated them from *S. mansoni* and human HDACs. Molecular dynamics and docking studies have identified the existence of various asymmetrical binding cavities and topologies aside from the preserved amino acid residue in the catalytic site that could help in the structure-based design of parasite-specific HDACis [156]. Still, no three-dimensional models have been available for any of the SIRT enzymes of the parasite. However, various three-dimensional models have been developed for TcSir2rp1, LiSir2rp1, TbSir2rp1, and LmSir2rp1 through homology modelling using the crystal structure of hSIRT2 as a template [102,157]. Overall, these studies focused on the nicotinamide adenine dinucleotide (NAD⁺) binding site of the SIRT active site. The studies revealed notable distinctions regarding compound **46** binding to human and parasite SIRTs and might pave the way for developing selective inhibitors towards the SIRTs of the parasites [121,170,180,182,183]. In addition, the previously discussed selective LiSir2rp1 inhibitor compound **57** binds with the binding area of NAD⁺ at the LiSir2rp1 in contrast

to hSIRT1. Thus, the binding location of NAD⁺ is utilizable to develop selective SIRT inhibitors for parasites and spare human enzymes [122].

The activity against drug-resistant strains is crucial for any novel antiparasitic agent. The preference for an HDACi for in vivo testing use must be supported by the early observation of prevention of cross-resistance for numerous strains of the parasite. Some antimalarial HDACis have displayed indistinguishable in vitro activities against the strains of *P. falciparum* that are resistant and sensitive to presently available drugs like mefloquine and chloroquine [93,136–138]. Intriguingly, the study of compound 3 and two other 2-aminosuberic acid HDACis showed that they have potential against multidrug-resistant *P. vivax* and *P. falciparum* clinical isolates. The vulnerability towards compounds 15 and 16, 2-aminosuberic acid derivative, was linked to the identical traditional antimalarial drug in *P. vivax* but not in *P. falciparum*, recommending the probability of variation in the sensitivity profiles of distinct species of *Plasmodium* [141]. A convenient approach to evaluate the drug resistance potential of novel antiparasitic drugs is in vitro resistant laboratory strains generation or by mutagenesis. While these tolerant or resistant cell lines have not been available for antimalarial HDACi so far, as discussed above, compound 6 and HDACis have been generated that are resistant towards the parasites of *T. gondii* [132]. Based upon that, the cell line of *T. gondii* with mutation to the residue of amino acid in the gene of TgHDAC3 showed decreased sensitivity to compound 2 (FR235222). PfHDAC1 homology modelling with bound HDACis displayed that amino acid T76 is prognosticated to be one of the lead binding area residues in PfHDAC1 [92,93]. Additional studies are needed to elucidate the molecular mode of action of HDACi resistance by considering the role of this amino acid on HDAC inhibitory activity along with an in vitro selection of HDACis that are resistant/tolerant towards the parasitic cell lines. However, HDACi resistance mechanisms have recently been initiated to be identified in mammalian cells [184,185]. Particularly, both P-glycoprotein-dependent and -independent mechanisms are connected to the resistance of compound 34 in leukemia, and tolerance to compounds 7 and 3 in cells of adenocarcinoma appears to be self-sufficient by the countenance of multidrug resistance (MDR) [184,185]. This mechanism of resistance developed by two drugs is displayed in numerous parasites [12,26]. Still, now, few studies have been executed to characterize probable companion drugs for HDACis [12,26]. Nonetheless, in rare cases during experiments, favorable additive effects were found, like a combination between compound 20 and chloroquine in mice infected with *P. berghei* and also in one more combination between compound 27 and lopinavir [156]. In several cases, introductory experimental proofs suggested HDACi potential application in combination therapy of parasitic diseases [12,26,182]. Furthermore, the investigational proof narrates that sirtuin TbSir2rp1 of *T. brucei* is crucial for the survival of the parasite via DNA repair. In regard to genotoxicity, it has been proposed that TbSir2rp1-selective inhibitors could be used in the form of co-treatment with standard DNA-targeting chemotherapeutic agents to enhance their antiparasitic efficacy [108,111]. Additional studies like considerable in vivo interactivity studies are now needed to assess the potential benefit of combining SIRTi and HDACi therapy. Based upon this phenomenon, the immune-modulatory potentials of SIRTis and HDACis should be considered. Distinct SIRT and HDAC enzymes are displayed to play crucial roles in the antigenic alteration of the parasite, bypassing the host-immune supervision, causing interplay between innate as well as adaptive immunity. HDACis/SIRTis can powerfully affect T-cells, dendritic cells, and macrophages as many cellular components of the immune system [12,76,77,79,80]. Cellular components like dendritic cells, T-cells, and macrophages are key to immunity towards human parasite infections. Further, in vivo therapy with SIRTis and HDACis on host immune reactions and antiparasitic effects should be considered simultaneously. Dendritic cells play a crucial role in the setup of T- and B-cell adaptive responses and can also express co-stimulatory molecules CD40, CD86, and CD80. After HDACi application, CD40, CD86, and CD80 are decreased, followed by enhancement of immunosuppressive molecule indoleamine-2,3-dioxygenase [111]. Several reports have also suggested that HDAC inhibitor treatment

increased viral, bacterial, and fungal pathogen susceptibility [110]. HDACis safeguard mice against immune-mediated diseases such as symptoms of septic shock, lupus, and graft-versus-host disease [110,111].

8. Conclusions

The ongoing research is expanding our understanding of the molecular structures and mechanisms of action of HDAC inhibitors in parasitic diseases. The development of selective inhibitors targeting specific parasite HDACs shows promise, although challenges such as drug resistance and variations in sensitivity across parasite species necessitate further investigation. The potential application of HDAC inhibitors in combination therapy and their immune-modulatory effects on host responses highlight the complexity of their roles in antiparasitic strategies. Future studies should focus on elucidating resistance mechanisms, conducting in vivo interactivity studies, and exploring the synergistic effects of HDAC inhibitors with other therapeutic agents to enhance their efficacy against parasitic diseases.

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