Chapter

A Double Line of Defense: Heat Shock Proteins and Polyamines Act as Contributing Factors to Drug Resistance of some *Plasmodium* Parasites

Xolani Henry Makhoba

Abstract

Malaria remains a threat to human life worldwide with children under the age of 5 being the most vulnerable. *Plasmodium falciparum*, known as the causative agent of the deadliest malaria, survives both in the mosquito vector and human host. The sudden temperature change seems to not affect the parasite's cellular system. Heat shock proteins and polyamines are the major house-keepers of the parasite's cellular system to remain viable, despite the temperature changes that the parasite gets exposed to. While heat shock proteins protect newly synthesized proteins until they are properly folded polyamines are needed for cell differentiation, proliferation, and cell growth. In plants for example, polyamines have been reported to act as molecular chaperones when cells are exposed to unfavorable conditions that could be detrimental to cells. In this review, the role of heat shock proteins and polyamines in plasmodium parasite drug resistance and their role in parasite survival are discussed. The current drugs against malaria as well as the alternative future approach towards malarial drug development are reviewed.

Keywords: heat shock proteins, polyamines, drug resistance, *Plasmodium falciparum*, drug development

1. Introduction

According to the world health organization, malarial cases are expected to double in recent times due to the much global focus to fight the covid-19 pandemic [1]. The global fight against the covid-19 pandemic slows down efforts to control or eliminate malaria as one of the life-threatening diseases worldwide. In 2018, 228 million cases of malaria were reported with 405 000 people died, and in Sub-Saharan Africa, 67% of children under the age of 5 prematurely succumbed to the disease [1, 2]. The currently available drugs in the market are not effective enough and there is a growing concern of reported cases of drug resistance in some parts of the world. These drugs include artemisinin combination therapy which was a promising treatment for malaria. Therefore, there is an urgent need for alternative drugs or vaccines for malaria. Among the six species of malaria causative agents namely,

Plasmodium vivax (*P. vivax*), *P. ovolae*, *P. malarae*, *P. yoelli*, and *P. falciparum*, *P. falciparum* is the causative agent of the deadliest form of malaria. *Plasmodium falciparum* parasites survive in the female anopheles mosquito and the human host. When the Anopheles mosquito bites a human during its blood meal, the parasite is then transferred to a human host. It is reported that the temperature in the mosquito vector is about 22°C, whereas in the human host the normal body temperature is 37°C, but when the malaria symptoms kick in, the temperature goes as high as 38°C and above [3–5]. However, these sudden temperature changes do not affect the parasite viability. The temperature changes put pressure on the physiology of the parasite because there is a group of proteins whose production increases when the parasite is exposed to human host temperature. These molecules or proteins are believed to be the housekeepers of the cellular system of the *P. falciparum* parasite. When the parasite enters the human host, it produces schizonts into the liver, they then matured into merozoite and rapture in the red blood cells, therefore invade red blood cells. This stage of the parasite life cycle is very important because this is when the parasite would then multiply, differentiate and produce more merozoite to invade red blood cells and malaria symptoms would then start to show and the human host temperature would increase above 38°C [6]. Therefore, the production of proteins called heat shock proteins steadily increases as means for parasite protection under harsh conditions or sudden change of temperature.

Heat shock proteins are ubiquitous, highly conserved molecules that occur in all recognized life forms. The constitutively expressed heat shock proteins are generally designated as 'heat shock cognate' (Hsc) forms to differentiate them from the inducible heat shock protein (Hsp) forms. The constitutively expressed forms play a housekeeping role, while the inducible forms are normally expressed in response to stress. The role of heat shock proteins is to protect the newly synthesized proteins from misfolding, which could result in the formation of inclusion bodies or truncated proteins that can be toxic to the cellular system of the parasite. On the other hand, a group of proteins known as polyamines is produced in the parasite for proliferation, differentiation, and growth. When merozoite invades red blood cells, polyamines are believed to be at the center of the parasite multiplication process and act as molecular chaperones. For example, when cells that lack polyamines are added with polyamines and exposed to a temperature above 37°C, the cells do survive [7], signifying that polyamines display chaperone activities. Wide studies conducted in plant biochemistry demonstrated that when plant cells are exposed to abiotic temperature, polyamines protect plant cells and improve growth and production [3]. In P. falciparum, it could be that polyamines do cooperate with heat shock proteins as means for the parasite to survive under harsh conditions. For example, Polyamines protected plasmid DNA strand breaks in vitro and aided the cell survival against irradiation in polyamine deficient *Escherichia coli* mutant strain [8]. It is shown that DNA strand breaks were prohibited 4–6 fold more by polyamines such as spermidine and spermine compared to putrescine and cadaverine in the dithiothreitol/Fe (III)/O_2 system [9]. After UV-irradiation, the protection of DNA strand disruptions by spermine and spermidine was twofold as effective as that by putrescine and cadaverine. To measure the viability of *Escherichia coli* cells lacking polyamines, they were grown in the medium containing putrescine and spermidine. They displayed increased survivability compared to polyamine-depleted medium at a dose of 60 and 40 J/m^2. After γ-irradiation to a dose of 80 Gy, cell survivals of a mutant strain were significantly increased to 7.7- and 23.8-fold by putrescine and spermidine, respectively. Taken together these results suggest the probability that polyamines play a powerful role in the protection of DNA or cell damage by radiation. Polyamines can play an essential role in cell growth and differentiation and are also involved in the protection of cell structures [10].

In addition, reports suggest that when polyamines metabolism is disrupted, several cellular processes are affected, including transcription, translation, gene expression regulation, autophagy, and stress resistance. Some studies reported that in fact, polyamines influence the production or synthesis of heat shock proteins, even though it is not clear how this process takes place. Heat shock proteins come in different sizes and activities, whereas polyamines include putrescine, spermidine, and spermine. With *P. falciparum* having a unique biosynthesis of polyamines, for example, S-Adenosylmethionine decarboxylase is connected to Ornithine decarboxylase (AdoMetDC/ODC) has been regarded as an ideal drug target [11]. Therefore, drug development starts at the protein level, where characteristics of proteins are examined, this unique arrangement of P. falciparum AdoMetDC/ODC is regarded as an ideal drug target for malaria due to its role in the biosynthesis of polyamines in the parasite [9, 12, 13]. On the other hand, heat shock proteins such as Hsp70/Hsp40 partnership, Hsp90, Hsp110, small heat shock proteins, have been extensively studied and they have been well documented on how they keep the parasite viable, but what we do not know is how the parasite cellular system brings together polyamines and heat shock proteins as its double line of defense as a survival strategy. This is protective system is especially vital during red blood cell merozoite invasion, which is a crucial stage for the parasite's survival. This, therefore, justifies the need to understand this mysterious partnership between these two molecules towards multidrug development. In our laboratory, we are currently interested in getting answers in that direction, intending to develop alternative drugs against malaria [8, 14–16].

2. The life cycle of *plasmodium falciparum* parasite

The parasite *Plasmodium falciparum* has a complex life cycle which includes anopheles' female mosquito and the human host. After parasites have been sucked up, Oocysts develop in the gut wall of the mosquito. Sporozoites then develop in the oocyte and the Sporozoites migrate to the salivary glands. When the mosquito bites, Sporozoites are injected into the human body, who then becomes the second host to the parasites (**Figure 1**). The Sporozoites enter the liver cells where they multiply for about 7 to 14 days, producing between 10,000 and 30,000 daughter cells called merozoites. These daughter cells then burst and invade the red blood cells. In the red blood cells, further multiplication occurs by asexual reproduction [9, 10]. Between 8 and 16 merozoites are produced every 48 or 72 hours, depending on the species of *Plasmodium*. Merozoites are then released through the bursting of red blood cells. This release of toxic substances causes febrile attacks of the disease. After several such cycles, male and female gametocytes are produced (the sexual stage) and taken up by a feeding mosquito. The *Plasmodium* life cycle is completed by sexual reproduction, resulting in new sporozoites.

Some of the symptoms of malaria include but not all, fever and headache, these normally display when merozoite invade red blood cells and this stage is essential for the parasite survival. Fever is shown by elevated temperature above 38°C in the human host system. This therefore, puts stress on the cellular system of the parasite thus results in increased production of heat shock proteins for cellular system protection (protein folding). On the other hand, the parasite proliferates when the merozoite invades the red blood cells. The primary role of polyamines includes cell proliferation, differentiation, and growth of which are what the parasite needs at the red blood cell stage in a human host. Therefore, both heat shock proteins and polyamines serve as a shield of the parasite in the human host when exposed to stress conditions [17–20]. A study reported that the chaperone activities of Hsp70

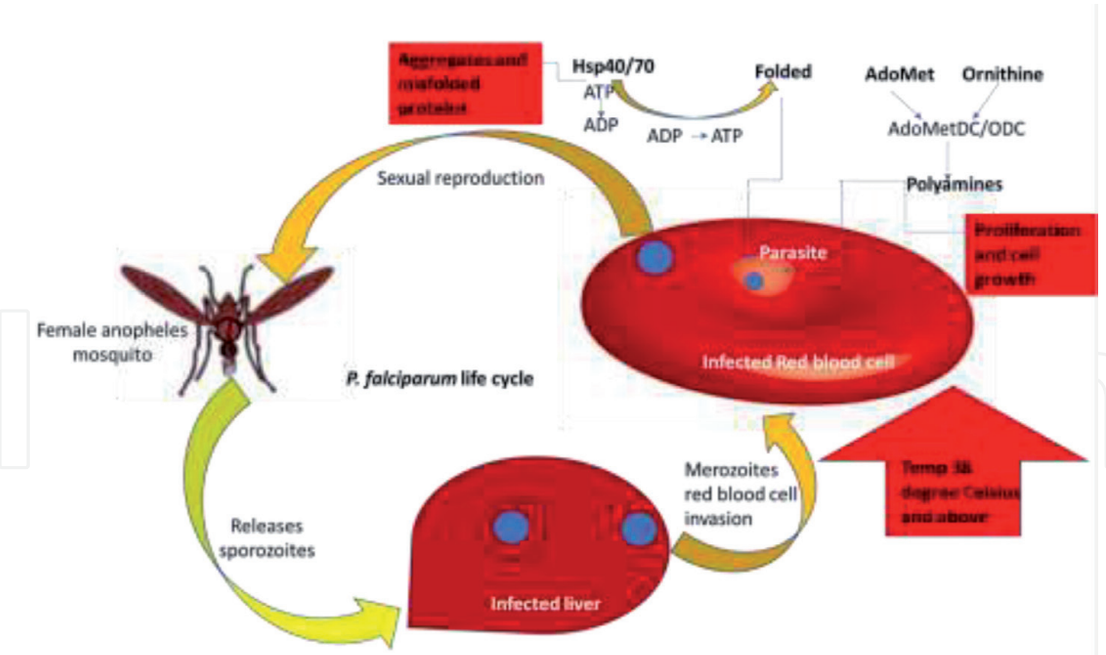

Figure 1.
Highlights of the plasmodium falciparum *life cycle and the synthesis of heat shock proteins and polyamines under stressful conditions for the parasite survival. Female mosquito bites during the blood meal release sporozoites to liver cells, where they undergo maturation stage and rapture to release matured merozoites to invade red blood cells. At this stage, the parasite undergoes heat shock and release stress proteins such as a heat shock proteins and polyamines so that the parasite proliferates and grow.*

sequester protein aggregates accumulated in bacteria during antibiotic treatment, therefore reducing the effect of the cure. Also, polyamines such as putrescine and spermidine have been suggested to exhibit chaperone activities when cells are exposed to stressful environments such as antibiotic therapies [21]. Taken together, the role polyamines and heat shock proteins play in a cellular system suggests that *Plasmodium falciparum* could apply similar methods to render current drugs ineffective by keeping the system's proteins in good shape (properly folded) during drug treatment [22]. In general, obligate human parasites depend upon a robust protein quality control system to ensure their survival, and hence, both employ a competent heat shock machinery and polyamines to this end.

3. Heat shock proteins

The outside milieu affects the in-house activity of the cellular system. If cells are exposed to stressful conditions, several molecular functions could be upset. For cells to remain functional active, the interior system should remain in good condition and if that is not the case, this could lead to cell death. Therefore, heat shock proteins of different sizes perform various functional activities to keep the cellular system in good condition. Molecular chaperone or heat shock proteins perform some activities as housekeepers of the cell, such as foldase, holdase, protein transportation, removal of inclusion bodies, modulation, and stabilization (**Table 1**). Whereas others are responsible for bringing the substrate for binding to reach a 3-dimensional structure. In *Plasmodium falciparum*, heat shock proteins have been regarded as ideal drug targets due to the aforementioned activities. Even though the role played by heat shock proteins favors the parasite viability, it is believed that their role in the parasite contributes to drug resistance. For example, when *E. coli* cells were exposed to some antibiotics, the production of Hsp70 chaperones was observed to have increased. It was then concluded that the cells developed resistance against antibiotics due to the

2HSPs	Role	Drugs	Refs
Hsp 40	Co-chaperone	Geldanamycin, radicicol, celastrol	[15, 23]
Hsp 70	Foldase, holdase, transportation	Geldanamycin	[24]
Hsp 90	Foldade and holdase	Geldanamycin, benzoquinone	[25, 26]
Hsp 110	Foldase	Geldanamycin	[16, 27]
Small Hsps	Modulation and stabilization		[19, 20, 22, 26]

Table 1.
Heat shock proteins and some compounds tested against them.

increased production of Hsp70 [16]. *Plasmodium falciparum* parasite is believed to use the same techniques when exposed to various drugs by increasing the production of heat shock proteins as a strategy to protect its internal environment, thus developing resistance to many drugs available in the market [28, 29].

Different kinds of compounds have been synthesized and their effectiveness against heat shock proteins was tested [17]. The complex nature of the *P. falciparum* makes it very difficult to develop effective drugs or vaccines. It is therefore, this reason why drug design and development against malaria has drawn so much research interest as matter of urgency.

4. The functional activities of Hsp70 in partnership with Hsp40

Both heat shock protein 70 and heat shock protein 40 were first discovered in bacteria that were exposed to stressful conditions, thus these proteins were overexpressed in response to the challenging conditions the bacteria organism was faced with [30]. As a result, the cellular protein structure and functional activities were

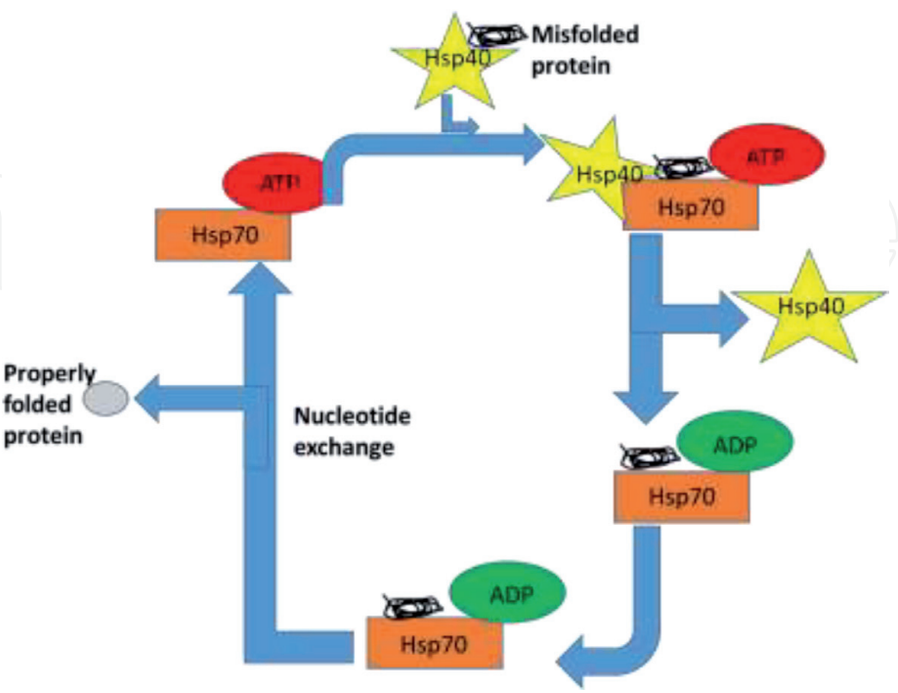

Figure 2.
Hsp70/Hsp40 partnership in folding process of newly synthesized proteins. Hsp40 hands over a newly synthesized protein to Hsp70 for proper folding with APT driven activity with help of nucleic exchange factor being at the Centre of the action. Unsuccessful protein folding are dealt with proteasome for degradation.

affected, Hsp70 was able to rescue aggregated and misfolded proteins (**Figure 2**). The partnership between Hsp70 and Hsp40 plays a major role in helping misfolded proteins to fold and gain their functional activities [31, 32]. To successfully assist misfolded proteins or substrates to fold properly, Hsp70 recognized and bind into the hydrophobic patches. The major role played by Hsp40 is to recognized and present misfolded proteins into an ATP Hsp70 for folding. The ATP is hydrolyzed to ADP, which then allows the substrate to bind to the ADP Hsp70 for folding. Once properly folded, the ADP is then converted to ATP thus releases the properly folded protein. Taken together, newly synthesized protein requires the assistance of heat shock proteins to fold properly, otherwise, they can be toxic to the cells if they are not properly folded.

5. Biosynthesis of polyamines

The synthesis of Polyamines such as putrescine, spermidine, and spermine is driven by *S-Adenosylmethionine decarboxylase* (AdoMetDC) and *Ornithine decarboxylase* (ODC). Both Adenosylmethionine and Ornithine function as precursors of polyamine biosynthesis [33, 34]. Unlike other species, *Plasmodium falciparum* AdoMetDC is connected to ODC which makes it an ideal drug target (**Figure 3**) [27]. These positively charged molecules are involved in various activities in the cellular system such as proliferation, differentiation, cell growth, protein synthesis, and RNA and DNA packaging [35]. In plants, polyamines have been reported to act as molecular chaperones or respond to heat shock to prevent plants. In addition to that, polyamines also prevent DNA damage of the cells exposed to UV radiation which could lead to cell death [35–37]. Taken together, this suggests that both polyamines and heat shock proteins are used by the parasite *Plasmodium falciparum*

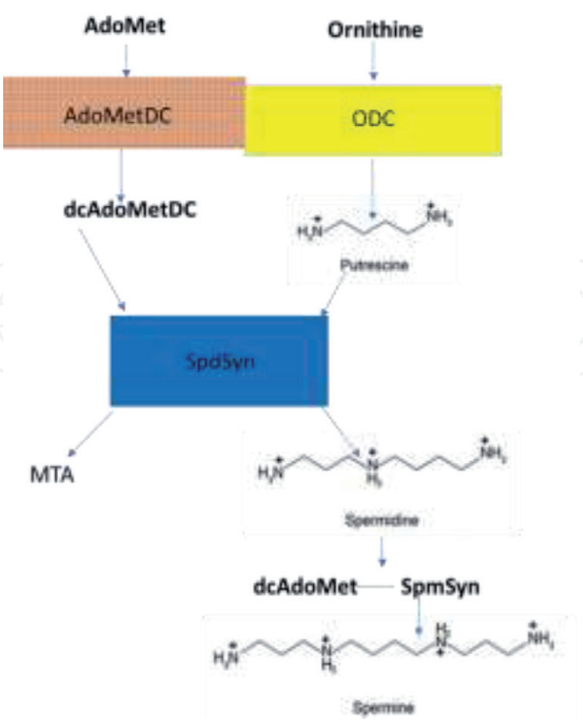

Figure 3.
Polyamines biosynthesis in plasmodium falciparum *parasite. Biosynthesis of polyamines in* P. falciparum *is driven by linked S-adenosylmethionine decarboxylase (AdoMetDC) and ornithine decarboxylase (ODC). Whereas in mammalian cells AdoMetDC and ODC are not joined together. Abreviations: SpdSyn, spermidine synthase; Spd, spermidine; dcAdoMet, decarboxylated S-adenosylmethionine; SpmSyn, spermidine synthase; Spm, spermine.*

as a strategy to survive under unfavorable conditions when it enters the human host (where it experiences a sudden change of temperature) from the mosquito [38–45]. It could be that both polyamines and heat shock proteins contribute a lot to drug resistance that the parasite has demonstrated to current drugs available in the market. Therefore, understanding how these two molecules cooperate in the parasite could lead to the right direction in the development of alternative malarial treatment [28, 29, 32].

6. Obligate parasites have many "talents" of survival

During their growth in the vertebrate host or mosquito vector, *Plasmodium* parasites undertake quick proliferation to yield a huge amount of offspring parasites. This speedy development depends sincerely on the effective achievement of vital nutrients such as purine nucleosides and nucleobases, amino acids, sugars, and vitamins from the host [46]. One of these vitamins, pantothenic acid (Vitamin B5), is a precursor of the important enzyme cofactor, CoA (**Figure 4**). As *Plasmodium* parasites cannot produce pantothenate *de novo*, the uptake and consumption of this precursor from the host are critical for existence. Studies in *P. falciparum* and *P. lophurae* have shown two diverse likely tactics used by malaria parasites in host erythrocytes to synthesize CoA [46]. While *P. falciparum* seems to use endogenous vitamin transporters to take up pantothenate from human plasma and a parasite-encoded transporter on the parasite plasma membrane to transport it from the erythrocyte cytoplasm into the parasite for the following employment, *P. lophurae* consumptions preformed CoA in its nucleated erythrocyte cytoplasm. The CoA transporter used by *P. lophurae* on its plasma membrane has not yet been identified.

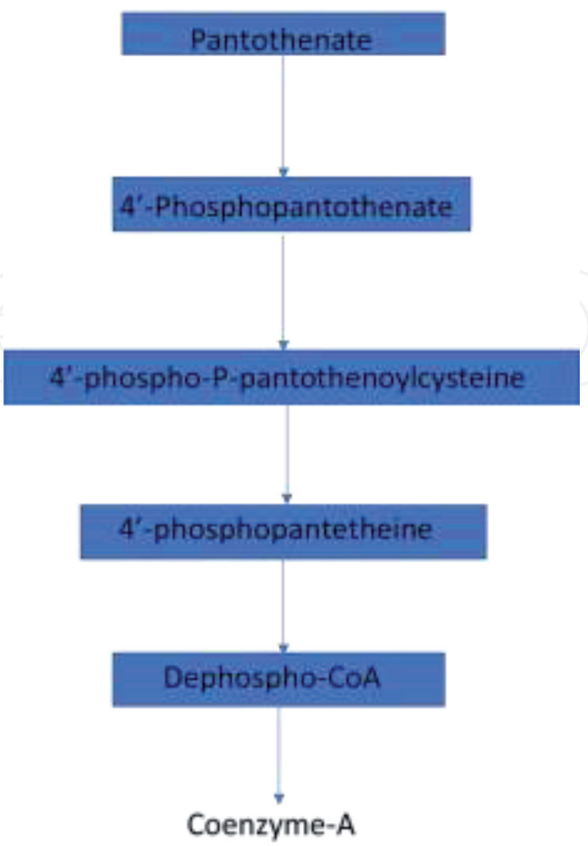

Figure 4.
Highlighting the role of panothenate in the synthesis of coenzyme-a in obligate parasites.

7. Current drugs are available in the market for malaria

Utmost of the antimalarial drugs aim at the asexual erythrocytic stages of the parasite, therefore named blood schizonticidal drugs. Tissue schizonticidal drugs mark the hypnozoites (dormant stage of the parasite) in the liver, while gametocytocidal drugs destroy sexual erythrocytic formulae of the parasite in the bloodstream and thus inhibit transmission of malaria to mosquito. Sporontocides stop or inhibit the formation of malarial oocysts and sporozoites in an infected mosquito. Chloroquine, quinine, and mefloquine are typically fast-acting schizonticidal drugs. Pyrimethamine, sulphonamides, and sulphone also possess schizonticidal activities, nevertheless, their action is dawdling (**Table 2**). Primaquine, Tafenoquine, and other novel kinase inhibitors have gametocidal activities. The main sporontocidal drugs are primaquine and praguanil. These antimalarial drugs were considered based on major metabolic differences of the malaria parasite with its host. Nucleic acid metabolism, heme toxification, oxidative stress, and fatty acid biosynthesis are some of the major pathways that were targeted mostly for antimalarial drug design. However, in the chemotherapy of malaria, the emergence of resistance to the available drugs is the major obstacle.

Furthermost of the existing antimalarial drugs have been used for decades and now their use is restricted by the emergence of drug resistance. According to various literature, there are no existing anti-malarial drugs that were developed in a fully rational manner, with a focused attempt to inhibit a known drug target [35, 36, 47]. Instead, in all cases, anti-malarial potency has been identified in animal or *in vitro* model studies. Consequently, the target of action for most existing agents inside the malaria parasite remains indeterminate.

8. Proposed drug candidate

There is an urgent need to develop new chemotherapeutic agents which display schizontocidal activity, thereby overcoming the making of merozoites from erythrocytes. The rise of drug resistance can be overcome by aiming the parasite transmission at the blood stage. Additionally, powerful drug candidates are vital to be explored. These should prove to be potent enough at a single dose to

Structural name	Types of compounds
(1) Aryl aminoalcohol compounds	• Quinine
	• Metfloquine
	• Halofantine
	• Lumafantine
(2) Antifolate compounds	• Proguanil
	• Pyrmethamine
	• Trimethopin
(3) Artemisinin compounds	• Artemisinin
	• Artesunate
	• Arthemether
	• Arteether

Table 2.
List of current malaria drugs in the market.

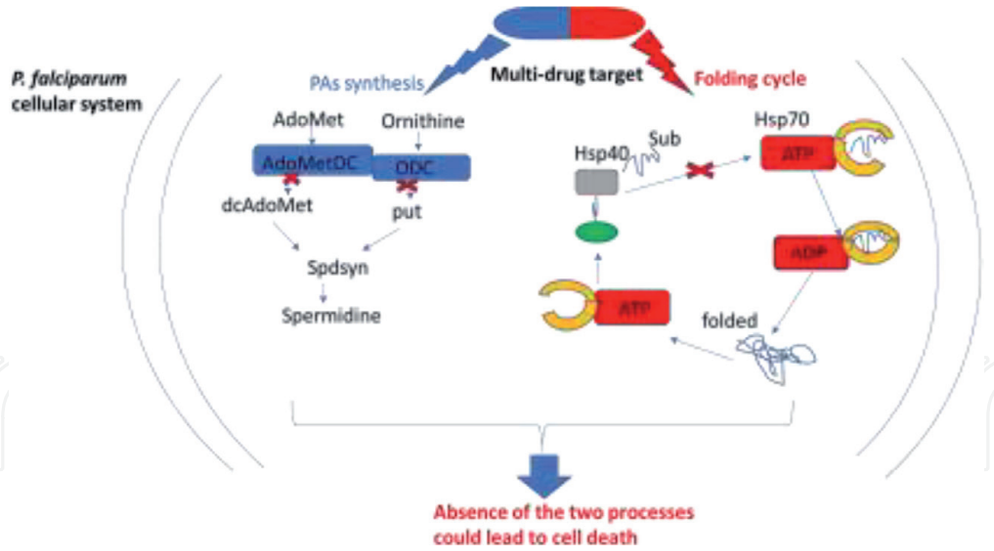

Figure 5.
Proposed drug candidate for plasmodium falciparum*. A multidrug target candidate that will inhibit polyamine biosynthesis and heat shock proteins could lead to dysfunctionality of the* plasmodium falciparum *parasite cellular system, which could lead to cell death.*

block the parasite transmission at the erythrocytic stage. Both safety and efficacy aspects of novel drug candidates also need distinct consideration to be a matter of great concern for antimalarial drug discovery. The medicinal chemist along with the pharmacologist necessities to work hard for the antimalarial drug development to achieve the desired safety, efficacy, and potency in a single dose molecule [30, 48, 49]. General, to decrease the present malaria load competently, more support is necessary in the long run. If the *P. falciparum* parasite uses polyamines and heat shock proteins as its shield under stressful conditions, these molecules could serve as an ideal drug target for malarial drug development [27]. The study to understand the mechanism behind this interplay is currently investigated in our laboratory and how this contributes to drug resistance. Below, we propose a drug candidate that will inhibit the biosynthesis of polyamines which could lead to reduced production of heat shock proteins if indeed polyamines influence the production of the former (**Figure 5**). Their essential role for pathogenic microorganisms growing in a host is of particular interest for drug discovery.

9. Conclusion and future perspectives

There has been regular work over the years for the radical treatment of malaria. Improvement of drug confrontation, existence the most problematic obstacle for the achievement of antimalarial therapy, most of the research is oriented towards overcoming the emergence and spread of resistance to existing drugs by one or the other means. Notwithstanding the pressing need, fewer energies have been absorbed in developing new drugs with new mechanism(s) of action. Now for the last periods, pharmaceutical consideration has on receiving more understandings into numerous metabolic or biochemical pathways of the parasite with the expectation to classify and exploit novel drug targets. The study is also underway to establish the mechanism of action of polyamines being influential in the synthesis of heat shock proteins and the role of polyamines being regarded or acting as chaperones in *P. falciparum* parasite during red blood cells merozoite invasion. This will lead to the development of new chemical compounds or specific inhibitors to act on these new targets.

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Author details

Xolani Henry Makhoba
Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa

*Address all correspondence to: xmakhoba@ufh.ac.za

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