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MINIREVIEW

Plasmodial heat shock proteins: targets for chemotherapy

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Abstract

Heat shock proteins act as molecular chaperones, facilitating protein folding in cells of living organisms. Their role is particularly important in parasites because environmental changes associated with their life cycles place a strain on protein homoeostasis. Not surprisingly, some heat shock proteins are essential for the survival of the most virulent malaria parasite, *Plasmodium falciparum*. This justifies the need for a greater understanding of the specific roles and regulation of malarial heat shock proteins. Furthermore, heat shock proteins play a major role during invasion of the host by the parasite and mediate in malaria pathogenesis. The identification and development of inhibitor compounds of heat shock proteins has recently attracted attention. This is important, given the fact that traditional antimalarial drugs are increasingly failing, as a consequence of parasite increasing drug resistance. Heat shock protein 90 (Hsp90), Hsp70/Hsp40 partnerships and small heat shock proteins are major malaria drug targets. This review examines the structural and functional features of these proteins that render them ideal drug targets and the challenges of targeting these proteins towards malaria drug design. The major antimalarial compounds that have been used to inhibit heat shock proteins include the antibiotic, geldanamycin, deoxyspergualin and pyrimidinones. The proposed mechanisms of action of these molecules and the pathways they inhibit are discussed.

Introduction

Malaria remains a major killer disease, accounting for at least one million deaths annually. Malaria is caused by parasites of the genus *Plasmodium*, of which *Plasmodium falciparum* is responsible for the most virulent form of the disease. The parasite has become increasingly resistant to traditionally used antimalarial drugs such as chloroquine. This has led to the search for alternative antimalarial drugs. Artemisin in based combination therapy (ACT) has been the latest most effective form of malaria treatment, but it is alarming that resistance to ACT by the parasite has been reported (Afonso *et al.*, 2006). This justifies the need to continue looking for effective antimalarial drugs alongside antimalarial vaccine research initiatives.

In order to survive, organisms need to maintain physiologically favourable homeostatic conditions in the event of changes in their environment. One of the challenges that face parasitic organisms is to deal with the physiological changes that they face upon invading the host and during their tenure in this system. This change creates stressful conditions that the parasite has to deal with in order to survive. This stress impacts on many physiological processes in the parasite, among them protein function, as proteins tend to denature in response to stress. In order to facilitate protein folding, prevent and reverse protein misfolding, cells use molecular chaperones. Heat shock proteins are ubiquitous, highly conserved molecules that exist in all known life forms. Heat shock proteins form a major fraction of the cell's molecular chaperone machinery. For this reason, most, but not all, heat shock proteins are stress-inducible. 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 major families of heat shock proteins include small heat shock proteins (sHsp), Hsp40, Hsp60, Hsp70, Hsp90 and Hsp110 (the numbers designate the average size of the proteins in that group in

kDa). As molecular chaperones, heat shock proteins bind to non-native forms of proteins to facilitate their folding to native conformations. In addition, heat shock proteins are implicated in a wide range of other cellular processes such as protein translocation, degradation, protein complex-assembly and disassembly.

The fact that malaria parasites have a life cycle that occurs in two physiologically divergent habitats (cold-blooded invertebrate mosquito vector and the warm-blooded human vertebrate host) indicates that they must have a robust adaptation mechanism to manage change. Furthermore, the fact that during its tenure in the human system the parasite needs to ensure survival in two cellular environments (hepatocytes and erythrocytes) is further evidence that the parasite boasts a physiologically adaptive machinery. To this end, parasitic organisms produce heat shock proteins in response to stress as well as for normal developmental purposes (Silva *et al.*, 1998; Feder & Hofmann, 1999). Not surprisingly, at least 2% of the *P. falciparum* genome is devoted to molecular chaperones (Acharya *et al.*, 2007). Furthermore, malaria pathogenesis is manifested by the parasite's ability to shuttle its own proteins to the erythrocyte surface, leading to invaded erythrocytes sticking to blood vessels. While parasites express heat shock proteins when they invade their host, this creates a paradox as host cells express heat shock proteins in response as a defensive mechanism (Schlesinger, 1990). Ironically, it seems that the parasite hijacks the host chaperone machinery to meet its own ends as host chaperones have been reported to occur in association with proteins of parasitic origin at the erythrocyte membrane (Banumathy *et al.*, 2002). This suggests that host chaperones may facilitate trafficking of proteins of parasitic origin to the erythrocyte surface.

Heat shock proteins are thought to mediate, among other roles, the following processes central to parasite survival: thermo-protection through their chaperone function (Kumar *et al.*, 1991; Sharma, 1992; Shonhai *et al.*, 2005, 2008), export of parasite proteins to the erythrocyte, parasite interorganelle protein trafficking and regulation of parasite infectivity and pathogenesis (Tardieux *et al.*, 1998; Foth *et al.*, 2003; Shonhai *et al.*, 2007; Maier *et al.*, 2008). It is against this background, that research focused on identifying and developing small molecule inhibitors targeting heat shock proteins is receiving attention (Banumathy *et al.*, 2003; Kumar *et al.*, 2003; Ramya *et al.*, 2006, 2007; Chiang *et al.*, 2009). Heat shock proteins possess structural and functional features that make them attractive drug targets. Generally, heat shock proteins interact with a number of regulatory partners (co-chaperones), and in addition, they form functional partnerships in which protein substrates are partially folded by one group of heat shock proteins and then handed over to another group of chaperones before they reach their full functional conformation. This implies that inhibitors targeting one family of heat shock proteins could have far-reaching consequences down their functional cascade. Conversely, this presents a down-side too as some heat shock proteins possess overlapping functions; consequently, inhibition of a particular family of heat shock proteins may not lead to the desired effects as their role may be taken over by another group of heat shock proteins with which they share functional specificity. Structural conservation also presents challenges to drug design efforts as drug selectivity between the parasite protein and the human equivalence may not be adequately established. Some heat shock proteins share conserved functional motifs across otherwise nonhomologous groups. For example, 15-deoxyspergualin (DSG), an antimalarial and immunosuppressant, interacts with both Hsp70 and Hsp90 via the chaperone's respective EEVD motifs (Nadeau *et al.*, 1994; Ramya *et al.*, 2006). So far, the sHsp, Hsp40, Hsp70 and Hsp90, have emerged as major malaria drug targets. For this reason, this review mainly focuses on work conducted on these groups of heat shock proteins. The mechanisms of action of the inhibitor compounds and the pathways that they influence are discussed. In addition, the prospects, merits and challenges of developing potential antimalarial drugs targeting heat shock proteins are highlighted based on the structural and functional features of these proteins.

Heat shock proteins as drug targets for malaria

Antimalarial drugs that are currently in use are becoming increasingly ineffective due to drug resistance by the parasite. Therefore, it is imperative to continue the search for alternative, affordable and effective antimalarial drugs. Heat shock proteins have been proposed as cancer drug targets (Calderwood *et al.*, 2006). Some small molecule inhibitors targeting heat shock proteins are capable of selecting for these proteins in cancer cells, without major effects on normal cells (Calderwood *et al.*, 2006). Geldanamycin, a benzoquinone ansamycin antibiotic (Fig. 1), abrogates ATP binding by Hsp90 (Bedin *et al.*, 2004). Thus, as an antimalarial agent, geldanamycin is proposed to interfere with the function of *P. falciparum* Hsp90 (PfHsp90), with fatal consequences to the parasite (Banumathy *et al.*, 2003; Kumar *et al.*, 2003). Other heat shock protein inhibitors that have been proposed as possessing antimalarial activity include DSG and pyrimidinones (Fig. 2a and b; Ramya *et al.*, 2006; Chiang *et al.*, 2009). Both DSG and pyrimidinones are thought to modulate the activity of the cytosol- and nuclear-localized *P. falciparum* Hsp70 (PfHsp70-1; Table 1; Biswas & Sharma, 1994; Shonhai *et al.*, 2007). The sHsp from *Plasmodium* species generally possess protease activity (Fakruddin *et al.*, 2000; Horrocks & Newbold, 2000), and for this reason, they present an interesting class of potential drug

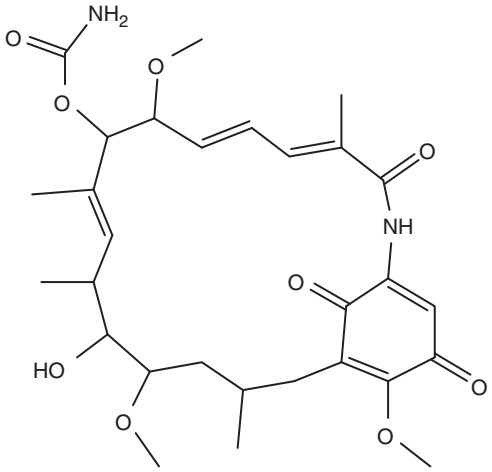

Fig. 1. Chemical structure of geldanamycin.

targets as malarial proteases have been proposed as drug targets (Rosenthal, 1998).

Hsp90 as an antimalarial drug target

This is a highly conserved group of proteins that occur in all organisms. Initial work on the crystal structure of the N-terminal domain of Hsp90 revealed a dimeric structure anchored on a highly twisted 16-stranded β-sheet (Prodromou *et al*., 1997). Hsp90 is made up of three subdomains (25-kDa N-terminal ATP-binding domain, 35-kDa middle domain and a 12-kDa C-terminal domain), all of which are highly conserved and important for its interaction with a substrate (Terasawa *et al*., 2005). The C-terminal domain is also crucial for the dimerization of Hsp90 (Nemoto *et al*., 1995). It is believed that the transient dimerization of Hsp90

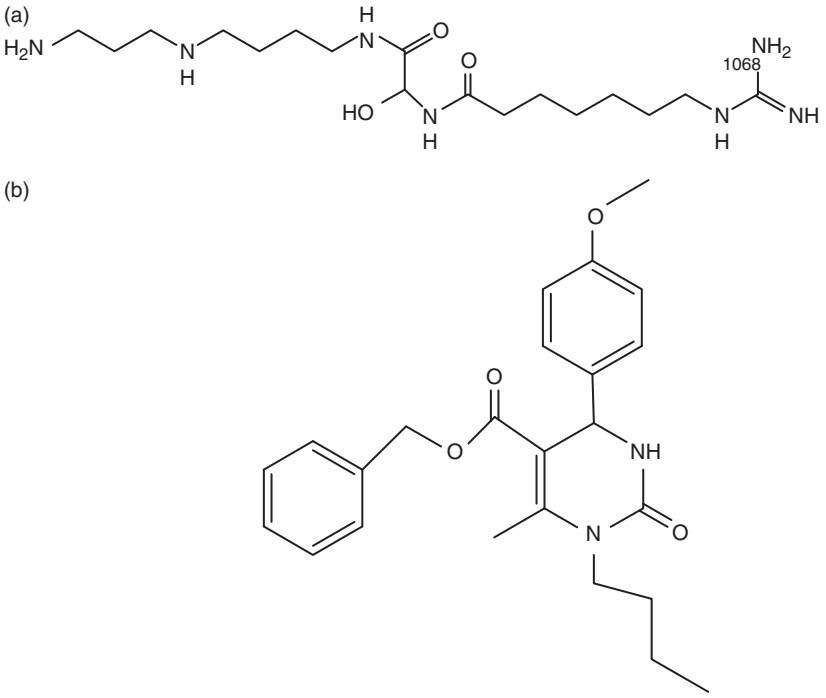

Fig. 2. Chemical structure of DSG (a) and the primidinone, MAL2-39 (b).

Table 1. *Plasmodium falciparum* Hsp70s

Name	PlasmoDB annotation	Cellular location	References
PfHsp70-1	PF08_0054	Nucleus and cytosol	Kumar *et al*. (1991), Sharma (1992), Biswas & Sharma (1994), Shonhai *et al*. (2007)
PfHsp70-x	MAL7P1.228	Nucleus and cytosol	Sargeant *et al*. (2006), Shonhai *et al*. (2007)
PfHsp70-2	PFI0875w	Endoplasmic reticulum	Kumar *et al*. (1991), Kumar & Zheng (1992)
PfHsp70-3	PF11_0351	Mitochondrion	Sargeant *et al*. (2006), Shonhai *et al*. (2007)
PfHsp70-z	PF07_0033	Cytoplasm	Sargeant *et al*. (2006), Shonhai *et al*. (2007)
PfHsp70-y	MAL13P1.540	Endoplasmic reticulum	Sargeant *et al*. (2006), Shonhai *et al*. (2007)

through the middle and the N-terminal domains is essential for the ATPase activity of this protein (Prodromou *et al.*, 2000; Meyer *et al.*, 2003). This has led to speculation that Hsp90 acts as a molecular 'clamp', undergoing transient open and closed conformations assumed in response to the bound nucleotide (Prodromou *et al.*, 2000). Furthermore, the closing and opening cycles are regulated by a cohort of co-chaperones that form multicomponent systems (Pratt & Toft, 2003). Whereas the human genome encodes two cytosolic Hsp90 proteins, both of which have an terminal EEVD motif, *P. falciparum* possesses two *HSP90* genes, but only one of the genes encodes for an Hsp90 protein possessing the EEVD motif (Pavithra *et al.*, 2007). PfHsp90 shares 64% identity with its human counterpart and both proteins possess conserved ATP-binding motifs (Acharya *et al.*, 2007). Like its human counterpart, substrates of Hsp90 include proteins involved in cell cycle events, kinases and transcription factors.

Although both Hsp70 and Hsp90 play independent chaperone roles, it is known that Hsp70 and Hsp90 exist in a functional partnership, ensuring that some peptide substrates are passed from Hsp70 to Hsp90 (Wegele *et al.*, 2004). An 'adaptor', Hsp70–Hsp90 organizing protein (Hop), functionally links Hsp70 to Hsp90 (Smith *et al.*, 1993). PfHsp90 function is thought to be regulated by several co-chaperones (Kumar *et al.*, 2007). Functional partners of Hsp90 include Hsp70, Hip (Hsp70-interacting protein), Hop and immunophillins (Morishima *et al.*, 2003). Cofractionation of PfHsp90 with both PfHsp70-1 and a *P. falciparum* phosphatase (PfPP5), expressed at the erythrocytic stage (Dobson *et al.*, 2001), has been confirmed experimentally (Pavithra *et al.*, 2004).The diverse range of PfHsp90 interactors reflects its essential role in the survival of the parasite, making it a potential drug target. A similar phenomenon is observed in cancer cells, where the pool of Hsp90 protein interactors (including cell signalling factors) is upregulated during tumour development; consequently, Hsp90 inhibitors manifest a more profound effect in cancer cells than in normal cells (Calderwood *et al.*, 2006). PfHsp90 function is also regulated through phosphorylation, and known inhibitors of Hsp90 such as ansamycin drugs have been shown to inhibit the phosphorylation of PfHsp90 (Banumathy *et al.*, 2003).

Findings by Sollars *et al.* (2003) suggest that Hsp90 regulates morphological evolution through epigenetic and genetic mechanisms. In this study, it was observed that inhibition of the activity of Hsp90 effected changes in the chromatin state that were accompanied by abnormal phenotypic changes in *Drosophila melanogaster*. The phenotypic changes persisted in successive generations as heritable traits even after the restoration of Hsp90 activity. Queltsch *et al.* (2002) made similar observations and proposed that Hsp90 serves as a regulatory 'capacitor' acting at the genotype–environment interface. The 'capacitor' role of Hsp90 is based on chaperone function, which allows it to modulate the activity of its substrates, including cell cycle signalling factors. The role of Hsp90 at the crossroads between development and the environment has also been observed in the parasite, *Leishmania donovani* (Wiesgigl & Clos, 2001). In this study, the development of *L. donovani* was regulated by inhibiting the activity of Hsp90, consequently interfering with the chaperone's role in facilitating the cell cycle of the parasite. In *P. falciparum*, Hsp90 plays an important role in the development and pathogenesis of malaria. For example, during the development of febrile malaria, the body temperature rises to 41 °C due to the release of proinflammatory cytokine tumour necrosis factor (Karnumaweera *et al.*, 1992) and the manifestation of this fever promotes malaria pathogenesis (Udomsangpetch *et al.*, 2002). Furthermore, it has been observed that initial heat shock prepares the malaria parasite for better thermal resilience against successive heat shock (Pavithra *et al.*, 2004). It has been proposed that febrile malaria episodes associated with malaria induce PfHsp90, which plays an important role during the intraerythrocytic phase of the parasite; consequently, this enhances parasite circulation counts (Pavithra *et al.*, 2004). The expression of Hsp90 is enhanced during the invasion of host cells by *Eimeria tenella*, an apicomplexan species that causes avaian caecal coccidiosis (Péroval *et al.*, 2006). Inhibition of Hsp90 activity led to a significant reduction in the invasion capacity of the *E. tenella* (Péroval *et al.*, 2006). This is further evidence that Hsp90 may play an important role at the host–parasite interface.

PfHsp90 is stress-inducible and is particularly expressed during the erythrocytic stage of the parasite life cycle (Su & Wellems, 1994). Not surprisingly, PfHsp90 is essential for the survival of *P. falciparum* (Banumathy *et al.*, 2003; Kumar *et al.*, 2003). For this reason, PfHsp90 has become a chemotherapeutic target. A three-dimensional model of PfHsp90 has been proposed towards facilitating drug design efforts (Kumar *et al.*, 2007). In addition, the *PFHSP90* gene is localized to a segment of chromosome 7 on the parasite genome that is associated with chloroquine resistance (Su & Wellems, 1994). This suggests that PfHsp90 could play a part in conferring drug resistance to the parasite. The antimalarial agent, geldanamycin (Fig. 1), is thought to act by inhibiting the ATP-binding capacity of PfHsp90 (Kumar *et al.*, 2003). Geldanamycin displayed antiplasmodial activity at an IC_{50} value of 20 nM comparable to chloroquine's IC_{50}, reported to be around 12 nM under the same experimental conditions (Kumar *et al.*, 2003). Furthermore, the same study reported that the antimalarial activity of geldanamycin was relatively rapid, enabling it to act on a single cell cycle of *P. falciparum* (Kumar *et al.*, 2003). A calcium-dependent phosphatase, calcineurin, is known to be involved in signalling (Klee *et al.*, 1979). The *P. falciparum* calcineurin associates with PfHsp90 and requires the

chaperone for it to attain functional conformation (Kumar *et al.*, 2005). Interestingly, Kumar *et al.* (2005) observed that geldanamycin was able to synergistically support the antiplasmodial activity of cyclosporin A, a known inhibitor of eukaryotic calcineurin (Milan *et al.*, 1994). Activation of calcineurin is known to promote resistance to stress in yeast (Cruz *et al.*, 2002), and by modulating calcineurin function, Hsp90 was able to enhance resistance to azole antifungal drugs in *Saccharomyces cerevisiae* (Cowen *et al.*, 2006). Consequently, geldanamycin along with its derivatives, 17-(allyllyamino)-17-demethoxy-geldanamycin (17-AAG) and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), both of which are undergoing clinical trials as anticancer drugs, were able to effectively suppress drug resistance in fungal species (Cowen *et al.*, 2006, 2009). It is encouraging to note that both 17-AAG and 17-DMAG were effective at concentrations that are nontoxic to humans (Cowen *et al.*, 2009). Thus, it would be interesting to investigate whether inhibitors of Hsp90 and/or calcineurin would reverse resistance to some of the malaria drugs currently in use. It should be noted that although inhibition of the parasite *L. donovani* by geldanamycin was largely a success, some parasite mutants evaded the effects of geldanamycin, presumably through episomal amplification of the *HSP90* gene (Wiesgigl & Clos, 2001). Therefore, it cannot be ruled out that geldanamycin could face resistance from the malaria parasite in the same way some of the *L. donovani* cells survived exposure to geldanamycin (Wiesgigl & Clos, 2001). However, by combining geldanamycin with another antimalarial drug targeting a different pathway, it may be possible to achieve a favourable outcome. Indeed, geldanamycin and chloroquine were found to act synergistically against parasites in the erythrocytic phase of growth (Kumar *et al.*, 2003). Other known Hsp90 inhibitors such as the ansamycin family of antibiotics (Whitesell *et al.*, 1994) have been proposed as possible antimalarial agents (Kumar *et al.*, 2003), although this still has to be experimentally confirmed.

Plasmodial Hsp70s as potential antimalarial drug targets

Hsp70 is a group of highly ubiquitous proteins, localized in the *Escherichia coli* cytosol and some compartments of eukaryotic cells such as the endoplasmic reticulum (ER) lumen, mitochondrial matrix and the cytosol. The compartmentalization of Hsp70s in eukaryotic cells ensures that these proteins participate in specialized cellular processes. The average molecular weight of these proteins is around 70 kDa. Hsp70s consist of two distinct domains: the 45-kDa N-terminal domain that binds ATP and the 25-kDa peptide-binding domain (Flaherty *et al.*, 1990; Wang *et al.*, 1993). Hsp70 has a high affinity for peptides that are eight residues long, possessing an interior hydrophobic core, which is surrounded by basic residues (Gragerov *et al.*, 1994; Jordan & McMacken, 1995).The Hsp70 protein interacts with the peptide substrate in ATP-expending cycles. Hsp40 acts as a co-chaperone of Hsp70, and is capable of stimulating ATP hydrolysis of the chaperone (Laufen *et al.*, 1999). Hsp40 primarily interacts with the ATPase domain of Hsp70 through a channel that is located in the ATPase segment of the protein (Gässler *et al.*, 1998). However, there is evidence that Hsp40 also interacts with the peptide-binding domain of Hsp70 (Demand *et al.*, 1998). Further evidence suggesting that Hsp40 interacts with the peptide-binding domain of Hsp70 stemmed from structural studies suggesting that the positively charged J domain of Hsp40 is within the influence of negatively charged regions of the Hsc70 peptide-binding domain, allowing the Hsp40 J domain to physically interact with Hsc70 through its C-terminal domain (Jiang *et al.*, 2005). It has further been proposed that both Hsp40 and Hop recognize cytosolic Hsp70 through interaction with its C-terminal EEVD motif (Freeman *et al.*, 1995; Demand *et al.*, 1998). Binding of substrate through the C-terminal peptide-binding domain also increases the ATPase activity of Hsp70 (Slepenkov & Witt, 2003). Thus, maximum activation of Hsp70 is attained by two closely coupled events – Hsp40 stimulation and substrate binding. The advantage of this mechanism for stimulation of Hsp70 is that it ensures that Hsp70 is activated only by Hsp40-supplied substrates, thereby avoiding unnecessary energy-consuming cycles (Kelley, 1999).

The *P. falciparum* genome exhibits six *HSP70* genes encoding for Hsp70 species whose structural and functional properties have been characterized previously (Table 1; Sargeant *et al.*, 2006; Shonhai *et al.*, 2007). Of these proteins, PfHsp70-1, which largely occurs in the cytosol and nucleus of the parasite (Kumar *et al.*, 1991), has been extensively characterized. PfHsp70-1 has been linked to a number of pathways, placing it at the intersection of several metabolic pathways (LaCount *et al.*, 2005; Shonhai *et al.*, 2007). PfHsp70-1 is also implicated in interorganelle trafficking as well as in the export of proteins of parasitic origin to the erythrocyte (Foth *et al.*, 2003; Sargeant *et al.*, 2006). PfHsp70-1 possesses the terminal EEVD motif present in eukaryotic cytosolic Hsp70s, and thus PfHsp70-1 potentially interacts with Hop and Hsp40 through this motif (Freeman *et al.*, 1995; Demand *et al.*, 1998). PfHsp70-x (MAL7P1.228; Table 1), which shares close homology and phylogenetic links with PfHsp70-1, has a terminal EEVN sequence, suggesting that it could also be a cytosolic chaperone (Sargeant *et al.*, 2006; Shonhai *et al.*, 2007). Both PfHsp70-1 and PfHsp70-x have a highly conserved bipartite nuclear localization signal (Shonhai *et al.*, 2007). The nuclear localization signals for both PfHsp70-2 (PFI0875w) and PfHsp70-3 (PF11_0351), the endoplasmic and mitochondrial counterparts, respectively, are relatively conserved (Shonhai *et al.*, 2007). However, this signal sequence is weakly conserved in PfHsp70-y (MAL13P.540; Table 1). PfHsp70-z (PF07_0033;

Table 1; Shonhai *et al.*, 2007) has the least conserved residues for this signal compared with other *P. falciparum* Hsp70s. PfHsp70-3 contains a host cell targeting signal (Lanzer *et al.*, 2006), and PfHsp70-2 possesses an ER N-terminal leader sequence and a C-terminal ER-retention signal, both of which seem to be responsible for its occurrence in ER-like structures of *P. falciparum* (Kumar *et al.*, 1991; Shonhai *et al.*, 2007). Although both PfHsp70-y and PfHsp70-z (Table 1) possess conserved ATPase domains, their peptide binding domains are highly divergent from that of the canonical Hsp70 (Shonhai *et al.*, 2007). The two proteins belong to the functionally distinct Hsp110/Grp170 group of molecular chaperones (Shonhai *et al.*, 2007). Another distinct group of molecular chaperones, the Hsp100, possess ATPase activity and are involved in protein disaggregation as well as protein degradation. A recent study proposed that a *P. falciparum* Hsp100 (Q8IIJ8) is localized in the parasitophorous vacuole (PV) membrane, where it forms a channel that facilitates trafficking of proteins of parasitic origin to the erythrocyte cytoplasm (de Koning-Ward *et al.*, 2009). Indeed, given the importance of such a pathway in the development and pathogenicity of the malaria parasite, it is not surprising that this export channel offers a promising drug target.

For a protein to be considered an ideal drug target, it must possess sufficiently distinct structural features to allow for its inhibition at the exclusion of its counterpart where this exists in humans. Hsp70s are generally conserved proteins; hence, it is important to explore whether these proteins possess unique structural and functional features. Human Hsp70 and PfHsp70-1 display 80% sequence similarity (Acharya *et al.*, 2007). Despite this high homology, it is interesting to note that, based on a recent study by Chiang *et al.* (2009), PfHsp70-1 and its human counterpart were modulated variably by selected small molecule inhibitor compounds. Furthermore, Hsp70s from *Trypanosoma cruzi* and *P. falciparum* have been reported to have generally high basal ATPase activities (Olson *et al.*, 1994; Edkins *et al.*, 2004; Matambo *et al.*, 2004). Perhaps this suggests that Hsp70s of parasitic organisms have divergent functional features compared with their counterparts from other free-living eukaryotic organisms. This is important for drug design purposes as the high ATPase activity of PfHsp70-1 could compromise its function under limiting ATP conditions (Matambo *et al.*, 2004). Hsp70 function is regulated through phosphorylation (McCarty & Walker, 1991; Sherman & Goldberg, 1993). Under stressful conditions, Hsp70 undergoes increased phosphorylation, which influences its physical (oligomeric status) and chaperone features, such as its interaction with the substrate and co-chaperone (Sherman & Goldberg, 1993). Threonine-199 is a key target of phosphorylation of *E. coli* DnaK, and for this reason, this residue is important for the regulation of the ATPase activity of DnaK (McCarty & Walker, 1991). The modulation of the activity of DnaK through phosphorylation led to speculation that DnaK is a physiological thermosensor (McCarty & Walker, 1991). Hsp70s from *P. falciparum* display unique phosphorylation capacities, suggesting that their phosphorylation regulates function (Kappes *et al.*, 1993; Shonhai *et al.*, 2007). It is conceivable that the phosphorylation profiles of *P. falciparum* Hsp70s differ from that of the human orthologues, thus further widening structural conformations, rendering selective inhibition possible.

Further evidence showing that Hsp70s are capable of attaining distinct functional capacities despite their high homology comes from a study based on mammalian Hsp70 and Hsc70 that exhibited divergent capabilities to facilitate liposome aggregation (Arispe *et al.*, 2002). Based on this study, it was suggested that Hsc70 was more efficient than Hsp70 in facilitating liposome aggregation and that Hsc70 mediated the process through two steps as opposed to the proposed single rate-limiting step for Hsp70. A study on Hsp70s from polar and temperate species suggested that the environment, and in particular, temperatures of habitats have an influence on the functional features of Hsp70s (Place & Hofmann, 2005). In this study, the Hsc70 purified from species of fish originating from the Antarctic region exhibited maximum substrate refolding capability at lower temperatures (− 2–10 °C); in contrast, an Hsc70 from a temperate New Zealand fish species had maximum substrate refolding activity at 30–40 °C (Place & Hofmann, 2005). It was proposed that the reduced functional capability of Hsc70 from the Antarctic fish species at higher temperatures was due to adverse structural changes (Place & Hofmann, 2005). In agreement with this proposal, it has been observed that human Hsc70 loses functional capability at 41 °C (Freeman & Morimoto, 1996), only 4 °C above the human body temperature. A similar scenario has been reported for Hsc70 from eurythermal goby *Gillichthys mirabilis*, whose refolding capability was lost just above the ambient temperature associated with its habitat (Zippay *et al.*, 2004). We recently observed that purified recombinant PfHsp70-1 was able to suppress heat-induced aggregation of a model substrate, malate dehydrogenase, at 48 °C *in vitro* (Shonhai *et al.*, 2008). This suggests that PfHsp70-1 may have optimal functional temperatures above that of human Hsc70, and indeed its temperature optimum is reportedly around 50 °C, considerably above the human optimum temperature (Misra & Ramachandran, 2009). A study by Oakley *et al.* (2007) illustrated that at high temperatures, expression of parasite proteins especially those associated with protein folding, RNA metabolism as well as secretome undergo significant changes. In addition, the same study observed that elevated temperatures promoted significant trafficking of proteins of parasite origin into the host cytoplasm and membrane. Not surprisingly, it has been reported that high temperature increased the cyto-adherence properties of ring-stage parasites through enhanced extrusion of proteins of parasite origin to the erythrocyte surface (Udomsangpetch *et al.*,

2002). Assuming that chaperones are involved in the export of proteins from the parasite to the erythrocyte, it would be logical to suppose that at elevated temperatures, heat stress may not only improve the expression of key parasite chaperones but activates their function. Indeed, elevated temperatures enhance the chaperoning capacity of Hsp70 (McCarty & Walker, 1991; Sherman & Goldberg, 1993).

Hsp40 proteins from *P. falciparum* are promising drug targets

The Hsp40 (called DnaJ in prokaryotes) family consists of several unique members distinguished by the presence of a highly conserved J domain of approximately 70 residues that constitutes the minimum structure required for the stimulation of Hsp70 activity (Laufen *et al.*, 1999). Hsp40 couples the stimulation of Hsp70 ATPase activity with the concomitant delivery of substrate to Hsp70. The subsequent hydrolysis of ATP by Hsp70 leads to stabilization of the Hsp70–substrate protein complex (Laufen *et al.*, 1999).

A classification system for Hsp40-like proteins has been established based on structure (Cheetham & Caplan, 1998). In this classification system, all Hsp40-like proteins belong to one of the following three groups: type I Hsp40-like proteins have the same domain structure as the standard *E. coli* DnaJ over their full length, containing a J domain, a glycine-phenylalanine (G/F)-rich region, cysteine repeat domain and a C-terminal domain. Type II proteins are similar to DnaJ over the J domain and the G/F-rich region. Type III proteins are those with only a J domain in common with DnaJ, present anywhere along their structure. Generally, type I and II Hsp40-like proteins are functionally similar; both groups bind to nonnative substrates, which they hand over to Hsp70 (Walsh *et al.*, 2004). However, type III Hsp40-like proteins are functionally distinct, and do not seem to bind nonnative peptides. Instead, their role is to recruit Hsp70s to specific cellular locations, where they fold specific substrates (Walsh *et al.*, 2004). Type III Hsp40 proteins with a variation in the otherwise highly conserved HPD motif located in the J domain have recently been reclassified as type IV Hsp40s (Botha *et al.*, 2007). Generally, the number of Hsp40 species in a cell tends to outnumber that of Hsp70s, particularly in eukaryotes, where several Hsp40 homologues occur in a single cell. Thus, one Hsp70 protein is capable of interacting with more than one Hsp40 co-chaperone, and thus the large pool of Hsp40s in eukaryotic cells ensures a diversity of functional partnerships between the few Hsp70s and the several Hsp40 proteins (Walsh *et al.*, 2004). Furthermore, because eukaryotic cells possess several subcellular organelles, the requirement for specific Hsp40/Hsp70 functional partners in distinct cell organelles such as the ER and mitochondria provides specialized protein folding pathways in these compartments.

Altogether, *P. falciparum* possesses 43 genes encoding for Hsp40-like (PfHsp40) proteins, which have been characterized through a bioinformatics-based study (Botha *et al.*, 2007). Work by Watanabe (1997) is one of the pioneering efforts to characterize Hsp40 proteins from *P. falciparum* and this study focused on PfJ1-4. According to the DnaJ canonical classification system (Cheetham & Caplan, 1998), PfJ1 is a type I Hsp40 and PfJ4 is a type II Hsp40, while PfJ2 and PfJ3 are type III Hsp40s. While heat shock induced a sharp increase in steady-state PfJ3 mRNA levels, a reduced response was observed for PfJ1 and PfJ4. However, a more recent study proposed that PfJ4 is heat-inducible and that the protein colocalizes with PfHsp70-1 (Pesce *et al.*, 2008). PfJ4 may potentially act as a co-chaperone of PfHsp70-1. On the other hand, PfJ2 induction was reduced upon heat shock (Watanabe, 1997). A type IV Hsp40-like protein, ring-infected erythrocyte surface antigen (RESA; Favaloro *et al.*, 1986), which is expressed by the parasite just before the merozoite stage of development, associates with the membrane of the newly invaded red blood cell bound to the erythrocyte protein spectrin (Foley *et al.*, 1991). Based on a gene disruption-based study, RESA has been found to play an important role in reinforcing the resistance of the parasite to heat stress (Silva *et al.*, 2005).

A sequence known as the *Plasmodium* export element (PEXEL; Marti *et al.*, 2004) motif or the vacuolar transport signal (VTS; Hiller *et al.*, 2004) has been identified as being responsible for routing parasite proteins to the erythrocyte (Hiller *et al.*, 2004). Based on the presence of the export signal, three type II and 16 type III and type IV Hsp40-like proteins (including RESA) are predicated to be exported (Templeton & Deitsch, 2005; Sargeant *et al.*, 2006; Botha *et al.*, 2007). Type I PfHsp40-like proteins lack the export signal motif, and hence are proposed to be resident in the parasite (Sargeant *et al.*, 2006). In their model, Templeton & Deitsch (2005), suggested that based on the presence of the signal peptide sequence encoded by an upstream exon 1 (Marti *et al.*, 2004; Sargeant *et al.*, 2006), proteins meant for export are channelled to the PV from the ER/Golgi apparatus system of the cell. Transport across the PV membrane to erythrocyte would depend on the presence of the PEXEL/VTS motif, which is encoded by exon II (Marti *et al.*, 2004; Templeton & Deitsch, 2005; Sargeant *et al.*, 2006). Because most of the PfHsp40s that have export motifs do not have a conserved HPD motif, which is crucial for the co-chaperone function of Hsp40 proteins, the role of these proteins is not clear. Nevertheless, it is speculated that these proteins are possibly exported to the erythrocytes to modulate the function of host chaperones (Sargeant *et al.*, 2006). The export of *P. falciparum* Hsp40 into the erythrocyte has been demonstrated using a green fluorescent protein-tagged PfHsp40 protein fused to an export predictive motif, which appeared to direct the protein into an infected erythrocyte

(Hiller *et al.*, 2004). Indeed, ATP-dependent associations between host chaperones (Hsp70, Hsp60 and Hsp90) with some *P. falciparum* proteins that are exported to the erythrocyte have been observed (Banumathy *et al.*, 2002). Furthermore, Maier *et al.* (2008) described PF10_0381 as a type IV Hsp40 that plays a key role in the assembly of knob-associated histidine-rich protein, a protein that is important in the formation of knobs on the erythrocyte surface. Because PF10_0381 possesses the PEXEL motif, it is predicted to be exported and is thought to be important for the recruitment of Hsp70 during knob assembly (Maier *et al.*, 2008).

Disruption studies of several genes encoding PEXEL containing Hsp40s from *P. falciparum* revealed that some, but not all, the proteins were essential for survival of the parasite (Maier *et al.*, 2008). *Plasmodium falciparum* displays a unique profile of Hsp40 co-chaperones compared with other plasmodial species, in particular, and the rest of the apicomplexan species in general. Only 19 of its 43 Hsp40-like proteins were found to share homology with their counterparts from other apicomplexan species, with most of the divergence observed in the PEXEL-containing group (Botha *et al.*, 2007). The high variation observed between Hsp40s from *P. falciparum* and the less virulent parasite, *Plasmodium vivax*, probably highlights a key role for these proteins in the extreme pathogenicity associated with *P. falciparum* compared with its less virulent forms (Botha *et al.*, 2007). Actin polymerization is a phenomenon that proposes a role for actin filaments in facilitating host cell invasion by parasites (Dobrowolski *et al.*, 1997). Interestingly, Hsp70 from a malaria agent, *Plasmodium Knowlesi*, is thought to facilitate actin polymerization, indirectly regulating parasite movement during host cell invasion (Tardieux *et al.*, 1998). It is logical that the role of Hsp40 in regulating this process is important and it is possible that Hsp70–Hsp40 partnerships in *P. falciparum* could be more effective in modulating actin polymerization than other plasmodial species. Another important group of chaperones that are important in the folding of actin is the Hsp60 family. The eukaryotic form of this group is localized in the mitochondrion and is known as chaperonin or t-complex polypeptide-1 (TCP-1 complex; McCallum *et al.*, 2000). Eukaryotic TCP-1 is a hetero-oligomeric complex, which facilitates folding of different peptides, of which tubulin and actin are most distinct (Melki & Cowan, 1994). Genes encoding for Hsp60 proteins from both *P. falciparum* and *Plasmodium yoelii* have been identified and are known to be stress inducible (Das *et al.*, 1997; Sanchez *et al.*, 1999). Perhaps these proteins could also modulate actin polymerization, thereby regulating the infectivity of the parasites.

In order to target the Hsp70/Hsp40 functional partnerships in *P. falciparum* for rational drug design, these partnerships need to be established. Although the parasite possesses at least 43 Hsp40-like proteins, the specific Hsp70/Hsp40 partnerships in *P. falciparum* are yet to be mapped out. Unlike some of the Hsp40s that are predictably exported, none of the six Hsp70s from *P. falciparum* exhibit export signals. However, some Hsp70s from the parasite, such as the cytosolic homologue, PfHsp70-1, and its mitochondrial equivalence, PfHsp70-3, have been found in the PV (Nyalwidhe & Lingelbach, 2006). In addition, PfHsp70-1, PfHsp70-2 and PfHsp70-3 have been reported to be present in Maurer's clefts (Vincensini *et al.*, 2005; Lanzer *et al.*, 2006). It is possible that some Hsp70s of parasitic origin may be routed to the erythrocyte as it is known that some proteins lacking the parasite export signal are exported by other pathways (Horrocks & Muhia, 2005; Templeton & Deitsch, 2005). However, due to the abundance of Hsp70, the purification protocols and the sensitivity of the methods, it is conceivable that the reported occurrence of PfHsp70 proteins in Maurer's clefts is an experimental artefact. Hsp70s are generally modulated by type I and type II Hsp40, which stimulate not only their ATPase activity, but also facilitate their interaction with substrates (Walsh *et al.*, 2004). PfHsp70-1 would most likely interact with type I and type II Hsp40s. Botha *et al.* (2007) identified only two type I proteins from *P. falciparum*: the putative protein, NP_7022481 (locus PF14_0359) and PfJ1 (Watanabe, 1997). Both of these proteins are predictably confined to the parasite cytosol and hence could regulate PfHsp70-1. Indeed, a recent study confirmed that PfJ1 modulates the ATPase activity of PfHsp70-1 (Misra & Ramachandran, 2009). We previously observed that PfHsp70-1 and PfJ4, a type II Hsp40, are closely associated, but their functional interaction has not been confirmed 1 (Pesce *et al.*, 2008). Perhaps a major drawback in the identification of PfHsp40 partners of *P. falciparum* Hsp70s is the fact that these interactions are largely confirmed through the ability of Hsp40s to stimulate the ATPase activity of Hsp70 partners. This could be problematic and unreliable as Hsp70s from parasites tend to have high basal ATPase activities (Olson *et al.*, 1994; Edkins *et al.*, 2004; Matambo *et al.*, 2004). Consequently, it is possible that attempts to identify PfHsp70-1/PfHsp40 partnerships may have failed due to the high basal ATPase activity of the PfHsp70-1 masking the effect of Hsp40 modulation. This seems to be the case for a recent study by Misra & Ramachandran (2009), who reported that PfJ1 was capable of stimulating the ATPase activity of PfHsp70-1, albeit they only observed a small signal for the ATPase activity of PfHsp70-1 that was due to the effect of PfJ1 on the chaperone.

15-deoxyspergualin and pyrimidinone interfere with plasmodial Hsp70 function

Hsp70s from *P. falciparum* interact with a wide range of partners (LaCount *et al.*, 2005; Shonhai *et al.*, 2007). One of

the most prominent potential functional partners of PfHsp70-1 is PfHsp90 because it is essential for the parasite's survival (Banumathy *et al.*, 2003). Because PfHsp70-1 possesses the EEVD motif, essential for the interaction between Hsp70 with Hsp90 (Smith *et al.*, 1993; Demand *et al.*, 1998), it seems logical that this partnership exists in the parasite. The EEVD motif of Hsp70 binds to co-chaperones including Hsp40 and Hop, whose role is to facilitate the interaction between Hsp70 and Hsp90 (Freeman *et al.*, 1995; Demand *et al.*, 1998). Indeed, a Hop homologue exists in *P. falciparum* (PF14_0324), although no study has confirmed its role in mediating PfHsp70–1/PfHsp90 partnership (Kumar *et al.*, 2007).

The immunosuppressant, DSG, and its derivatives have been shown to modulate the basal ATPase- and Hsp40 mediated-activity of Hsp70 (Fewell *et al.*, 2004). In their study, Ramya *et al.* (2006) suggested that DSG recognizes and binds the EEVD motif of PfHsp70-1. Consequently, DSG is thought to kill the parasite by titrating PfHsp70-1, impairing its chaperone function, thus compromising its role in mediating the trafficking of nuclear-encoded proteins to the apicoplast (Ramya *et al.*, 2007). Presumably, by binding the EEVD motif, DSG is able to compromise the interaction of PfHsp70-1 with its substrates (Ramya *et al.*, 2007). However, it is not quite clear how DSG is able to modulate the activity of Hsp70 both in the presence and in the absence of Hsp40 (Fewell *et al.*, 2004; Ramya *et al.*, 2006) if it binds at the C-terminal EEVD motif of the chaperone. Several proposals have been put forward to suggest the possible mechanism by which DSG operates (Ramya *et al.*, 2006, 2007). It is possible that binding of DSG to the C-terminal EEVD motif of Hsp70 abrogates Hsp40-mediated modulation of Hsp70 (Fewell *et al.*, 2004). DSG is thought to modulate the ATPase activity of PfHsp70-1 through its interaction with the EEVD motif (Ramya *et al.*, 2006); this occurs possibly through interference with interdomain coupling as it is known that chemical shifts are transmitted from the peptide-binding domain to the ATPase domain of Hsp70 (Slepenkov & Witt, 2003). It is thus possible that DSG could influence the interaction of Hsp40 via both the ATPase and the C-terminal domains of PfHsp70-1. We recently observed possible ionic contacts that seem to bring the ATPase and peptide-binding domains of PfHsp70-1 in close spatial orientation (Shonhai *et al.*, 2008). Our three-dimensional model is in consensus with a recent study that suggested that the C-terminal domain of PfHsp70-1 confers the protein with stability against heat stress (Misra & Ramachandran, 2009). It is therefore conceivable that by binding to the EEVD motif, DSG may compromise the stability of PfHsp70-1 against thermal stress. This in turn would compromise the functional capability of PfHsp70-1 during the physiologically dynamic life cycle of the malaria parasite. In addition, molecules that bind to the C-terminal domain of Hsp70 could interfere with the proposed role of the lid in ensuring Hsp70 stability (Fernandez-Sáiz *et al.*, 2006). It is likely that the proposed Hop-mediated interaction between PfHsp70-1 and PfHsp90 would also be affected when DSG is present, presumably bound to both chaperones. Thus, rather than having an effect on a single pathway, DSG may possibly interfere with a wide range of processes in which PfHsp70-1 and possibly PfHsp90 are involved. This would have deleterious effects on the parasite as PfHsp70-1 and PfHsp90 are implicated in several metabolic pathways in which they interact with several functional partners (LaCount *et al.*, 2005; Pavithra *et al.*, 2007; Shonhai *et al.*, 2007).

A recent study by Chiang *et al.* (2009) assessed the antimalarial effect of synthetic pyrimidinone molecules (Fig. 2b) on the growth of chloroquine-resistant *P. falciparum* cells. It is interesting to note that the compounds were able to inhibit PfHsp70-1 and human Hsp70 selectively (Chiang *et al.*, 2009). However, the *in vitro* inhibition of PfHsp70-1 was reportedly noticeable at a much higher inhibitor concentration (300 μM), compared with IC_{50} values around 30 nM to 1.6 μM, required for the same compounds to inhibit parasite growth. The authors suggested that this may have been because the inhibitors may have acted on other pathways in the parasite besides the PfHsp70-1 functional pathway. However, additional insights into this discrepancy could be drawn. Naturally, data derived from studies involving inhibition of PfHsp70-1 *in vitro* would not compare with findings from the parasite growth assay used in the study by Chiang *et al.* (2009). For this purpose, incorporation of a model substrate into the PfHsp70-1 inhibition assay along with the Hsp40 protein would have improved the reliability of the biochemical assay. Nonetheless, the fact that some of the pyrimidinones had variable effects on the activity of PfHsp70-1 compared with human Hsp70 and its yeast counterpart, Ssa1 (Chiang *et al.*, 2009), is encouraging towards drug design efforts. Another important aspect is that some of the compounds stimulated the basal ATPase activity of PfHsp70-1 several fold, while only a mild modulation was observed in the presence of a J domain (Chiang *et al.*, 2009). The enhanced stimulation of ATP hydrolysis by PfHsp70-1 could compromise its role (Chiang *et al.*, 2009), particularly under stressful conditions, because ATP is depleted faster during such periods. However, the binding site of these compounds on PfHsp70-1 is yet to be mapped out and it is not clear whether this site is unique to PfHsp70-1 or whether it occurs in other Hsp70s from the parasite (Chiang *et al.*, 2009). All the active compounds had an ester pyrimidinone backbone; thus, this substructure is thought to constitute the basic feature essential for the activity of these compounds (Chiang *et al.*, 2009). On the basis of this, it is now possible to modify these compounds to improve their efficacy and binding kinetics.

Malarial small heat shock proteins as drug targets

sHsp occur in all organisms and have molecular weight that ranges between 16 and 30 kDa. sHsp are known to be important for disaggregation and refolding of denatured proteins (Jakob *et al*., 1993). *Plasmodium falciparum* polyubiquitin (PfUB), a sHsp, has been described (Horrocks & Newbold, 2000). The protein is constitutively expressed, but is induced by stress and during development, with its peak expression at the late trophozoite stages (Horrocks & Newbold, 2000). *Plasmodium vivax* also expresses a sHsp with metalloprotease activity at its erythrocytic stage of development (Fakruddin *et al*., 2000). This protein was inhibited by 1,10-phenanthroline, while zinc successfully reversed the inhibition (Fakruddin *et al*., 2000). However, typical inhibitors of cysteine, serine and aspartate families of proteases were not able to inhibit the function of sHsp from *P. vivax*. The fact that proteases also operate as molecular chaperones suggests a crucial role for these proteins in the development of the parasite. Proteases are known to be essential for the survival of the malaria parasite as they are involved in host cell invasion, nutrition and growth, among other functions. Incidentally, proteases and chaperones were found to dominate the proteomic content of the parasite PV, suggesting an important role for these molecules in the protein quality control of parasite proteins (Nyalwidhe & Lingelbach, 2006). Given the important role of proteases in the development of the parasite, they have been proposed as drug targets, and some inhibitor compounds have been shown to block parasite growth *in vitro* and parasites in infected mice (Rosenthal, 2002). One of the major divergences between the parasite and the human genome is the fact that the parasite contains a few genes for sHsp compared with the human genome, which has several genes encoding for these proteins (Pavithra *et al*., 2007). It is not clear why there is such a huge difference between the number of genes for sHsp in the human host and the malaria parasite. However, it is possible that the role of sHsp is less essential in the parasite than it is in humans. In contrast, assuming that the few genes encoding for sHsp in the parasite are essential, it is possible that disruption of at least one of these genes would compromise the parasite's survival.

The implications of functional partnerships among heat shock proteins in drug design

The fact that heat shock proteins from *P. falciparum* exhibit functional partnerships (LaCount *et al*., 2005; Pavithra *et al*., 2007) is important for drug design as it means that the inhibition of one heat shock protein could impact on the role of other heat shock proteins down the functional cascade. For example, simultaneous reduction of the expression of both human Hsc70 and Hsp70 in human cancer cell lines has been found to compromise Hsp90 function (Powers *et al*., 2008). In addition, greater success could be achieved by targeting more than one heat shock protein in a functional pathway. In contrast, the existence of heat shock proteins in functional pathways with some degree of functional overlap, means that cells can upregulate the expression of a gene encoding a particular heat shock protein in order to compensate for loss of function of the other.

Hsp70 plays a central chaperone role through its functional links with other chaperones and co-chaperones (Borges & Ramos, 2005). In this way, Hsp70 serves as the intersectional chaperone by receiving unfolded proteins before passing them on to other chaperones. DnaK is involved in reciprocal exchange of peptide substrates with GroEL, the prokaryotic Hsp60 (Buchberger *et al*., 1996). In fact, in *E. coli*, the chaperone machinery exists in functional partnerships, some of which involve overlapping responsibilities; consequently, the overproduction of one group of chaperone may compensate for the other (El Hage *et al*., 2001). A second, yet to be characterized counterpart of PfHsp70-1, previously denoted as PfHsp70-x (Shonhai *et al*., 2007), possesses an EEVN motif to which DSG probably binds. It is possible that DSG inhibits both of these proteins. Therefore, the characterization of PfHsp70-x to investigate its possible inhibition by DSG would be important as it is likely that it has overlapping functions with PfHsp70-1 (Shonhai *et al*., 2007).

The regulation of Hsp70s by their respective Hsp40 co-chaperone partners is known to confer Hsp70s with a specialized function. This could facilitate drug design efforts as chemotherapeutic agents that specifically influence various Hsp70/Hsp40 partnerships could be designed. To this end, it has been suggested that Hsp70/Hsp40 partnerships in *P. falciparum* be established as a priority (Botha *et al*., 2007; Chiang *et al*., 2009). This would facilitate the screening of compounds that inhibit the various Hsp70/Hsp40 partnerships using high-throughput Hsp70 inhibitor screening methods such as the one that was previously described for screening inhibitors of *E. coli* DnaK (Chang *et al*., 2008).

Concluding remarks

The identification and development of small molecule inhibitors with antimalarial activity has opened a new horizon in the area of malaria drug discovery. It is encouraging that some of the molecules identified so far inhibit the parasite at IC_{50} levels that are nontoxic and comparable to traditional antimalarial drugs such as chloroquine. However, there is a need to focus more attention on understanding the modes of action of some of these compounds such as the pyrimidinone and DSG families of inhibitors.

It is also important to further identify specific functional partnerships and metabolic pathways that the target heat shock proteins are involved in to facilitate modification of the compounds to improve their inhibitory potential. For example, the identification of key Hsp70/Hsp40 partnerships in the parasite could guide drug designers to develop compounds that have high selectivity for parasite metabolic pathways, with minimum interference on the host system. It is also important to establish whether PfHsp90 inhibitors would reverse parasite drug resistance as this may give a second chance to some of the traditional antimalaria drugs such a chloroquine, which have become largely ineffective due to drug resistance mounted by the parasite.

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