[Frontiers in Bioscience, Scholar, 9, 88-110, January 1, 2017]

Identification of natural peptides as a new class of antimalarial drugs by *in silico* approaches

Ramar Perumal Samy[1], Sok Lin Foo[1], Octavio L. Franco[2,3], Bradley G. Stiles[4,5], Alan P. Kumar[6,7,8], Gautam Sethi[7,9], Lina HK. Lim[1,10]

[1]Department of Physiology, NUS Immunology Programme, Centre for Life Sciences, National University Health System (NUHS), Yong Loo Lin School of Medicine, National University of Singapore, Singapore -117456, [2]Centro de Analises Proteomicas e Bioquimicas/Programa de Pos-Graduacao em Ciencias Genomicas e Biotecnologia, UCB, Brasilia, DF, Brazil, [3]S-inova, Programa de Pos-Graduacao em Biotecnologia, Universidade Catolica Dom Bosco, Campo Grande-MS, Brazil, [4]Integrated Toxicology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702-5011, USA, [5]Department of Biology, Wilson College, 1015 Philadelphia Avenue, Chambersburg, Pennsylvania 17201, USA, [6]Cancer Science Institute, National University of Singapore, Singapore-117597, [7]School of Biomedical Sciences, Curtin University, Western Australia, [8]Department of Biological Sciences, University of North Texas, Denton, Texas, USA, [9]Department of Pharmacology, Yong Loo Lin School of Medicine, National University Singapore, Singapore-117597, [10]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore

TABLE OF CONTENTS

1. ABSTRACT

Malaria is one of the most widespread and serious parasitic diseases worldwide. Currently available antimalarial drugs have side effects, and many strains of *Plasmodia* have developed resistance to such drugs. The present review examines the use of annexins and of natural peptides from snake venom as a new class of anti-malarial agents, with the key property of reducing inflammation. Severe cases of malaria manifest elevated serum levels of liver enzymes, inflammation, fibrin deposition, apoptosis, and reduction in peripheral $CD8^+$ T cells. The annexin-A1/5 proteins trigger inflammation via increased expression of diverse cytokines (tumor necrosis factor alpha, interleukin-1beta, interleukin-10), however, by shielding microbial phospholipids they prevent injury via damage-associated molecular patterns (DAMPs). Here, we also review an *in silico*-based bioengineering approach that may allow for a better design, synthesis and characterization of novel peptides from snake venom as a more effective approach to treatment due to their improved antimalarial activity.

2. INTRODUCTION

Malaria, a mosquito-borne disease caused by *plasmodium* species, is a major public health problem, annually affecting over 300-500 million people worldwide (1-3). In 2012 in sub-Saharan Africa, 207 million new cases of malaria caused 627,000 deaths, mostly of young children (4-7). Malaria reduces personal productivity and contributes to the development of poverty; the cost of treatment also causes an economic burden of 12 billion dollars per year. In Singapore, the annual incidence of malaria ranges from 2.9 - 11.1 per 100,000 population, causing 38 deaths per year. Among these, 92.1 percent of the cases are caused by *P. falciparum,* while the remaining cases are caused by *P. vivax* (8, 9). The *Anopheles* vector surveillance data (1983-2007) collected by the National Environmental Agency revealed that the majority of the reported cases (91.4 - 98.3 percent) were from Southeast Asia and the Indian subcontinent (9-11). Central and Southeast Asia contribute to 39 percent of the global malaria burden by *P. falciparum* (3, 4, 12), with high endemicity in Orissa state (Eastern India), Western Myanmar and the lowlands of New Guinea (4). The global population at risk of *P. vivax* transmission not only spans India, but also includes other parts of Asia, particularly China, Indonesia, Pakistan, Vietnam, the Philippines, Myanmar, Thailand and Bangladesh (13, 14). South Korea, on the other hand, has been declared malaria-free after having 937,634 indigenous cases reported in the years 1993 to 2005 (15).

Currently available serological diagnoses provide indirect evidence of human exposure to the parasite. Nevertheless, advances in the field have led to the development of a rapid diagnostic test for malaria that has completed phase IV clinical trials (16). Preventive treatments for malaria have so far been challenging, especially for non-immune travelers visiting endemic areas, due to the lack of data for the at-risk population (17, 18). In fact, the systemic use of insecticide-impregnated bed nets showed that the incidence of malarial infection was only reduced by 50 percent (19). The lack of effective vaccines and the emergence of drug resistance against *P. falciparum* have been a concern for the last three decades, due to the possibility that malaria-attributable child mortality will soon double in Eastern and Southern Africa, as well as globally in the near future (4, 20-29). Although the use of antimalarial drugs such as artemisinin is generally considered safe, long-term treatment may pose severe toxic side-effects, including death (30).

To date, newly developed antimalarial drugs and vaccines are only at experimental stages or in phase I-IV clinical trials. In fact, most of the agents derived from natural compounds are still at experimental stages (16), and most of the leading malarial vaccine candidates, such as Mosquirix^{TM} (RTS,S trade name), have shown only limited efficacy (31). In light of this, snake venom might offer a promising therapeutic potential for malaria (32). The cocktail of enzymes and non-enzymatic proteins in snake venom has myriad functions including anesthetic (33-35), bactericidal (36, 37), anti-inflammatory and anti-parasitic (39), and it inhibits human immunodeficiency virus (HIV) (38).

This review focuses on the role of annexin A-1/5 (ANXA1/5) peptides and their pathophysiological interactions in the plasma of malaria patients. *In silico*-based approaches aimed at producing new antimalarials from natural snake venom molecules, targeting these mechanisms, are poised to enhance significantly the opportunities for antimalarial agents.

3. PATHOLOGICAL CHANGES INDUCED BY MALARIAL PARASITES

The clinical symptoms of malaria include pulmonary edema, cerebral malaria, acute kidney injury, hypoglycemia, lactic acidosis, and anemia. Severe infection of the liver by *P. falciparum* can lead to high mortality and morbidity due to liver failure. Liver is the main organ involved in the pathogenesis of malaria as it is implicated in the development of plasmodial sporozoites into merozoites (hepatic stage), before being released into the circulation to penetrate erythrocytes (erythrocytic stage). The parasitized red blood cells (PRBCs) or erythrocytes are then sequestered into small blood vessels, resulting in thrombosis. Degraded hemozoin pigments are also engulfed by local tissue macrophages, such as Kupffer cells and alveolar macrophages (Figure 1).

The clinical-pathologic features of liver infected by *P. falciparum* include reactive Kupffer cells, retention of hemozoin pigment, and minimal levels of PRBC sequestration (40, 41). A high load of hepatomegaly, PRBC and elevated levels of liver enzymes are also observed in malaria patients (42). Earlier studies confirmed that liver pathology, such as the induction of inflammation, leads to important changes in intervillous fibrin deposition (43), infarction regulation and cellular apoptosis in liver tissues, which affects the organ's function. Another study reported that infections by *P. falciparum*, but not by *P. vivax*, induced erythrocytic apoptosis due to strong inflammatory imbalance after the infection. PRBCs are not only involved in the development of anemia, but are also the main factor contributing to the development of thrombosis. The pathogenesis of anemia can be exacerbated by the increase in apoptosis in non-PRBCs (44).

The transcription factor NF-kappaB is known to regulate diverse cellular processes such as inflammation, immunity, cell proliferation and apoptosis (46). Apoptosis and NF-kappaB activation in malaria (*P. falciparum*

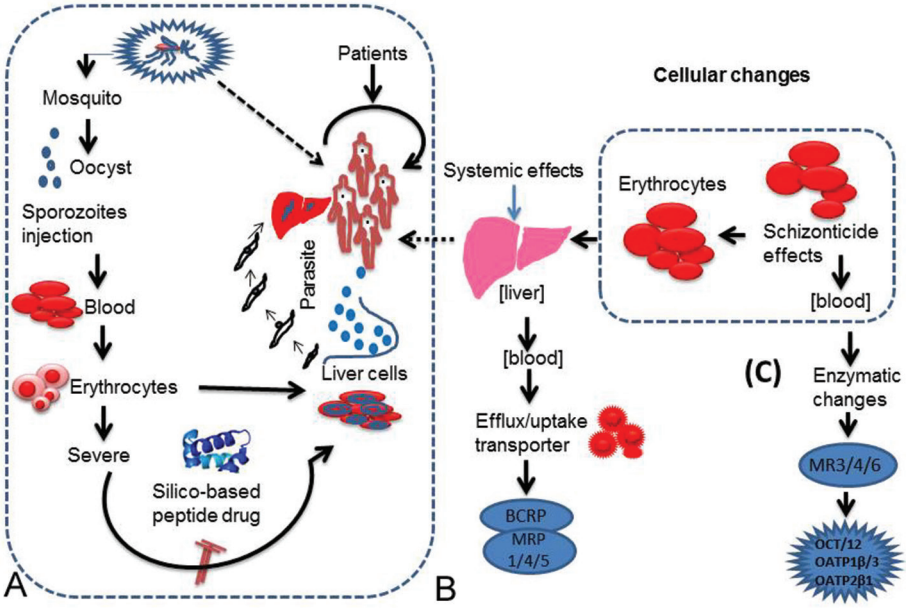

Figure 1. A-C. Flow chart showing disease pathology and involvement of liver in the malaria parasite's life cycle during the hepatic stage, which includes development of malaria sporozoites into merozoites. Merozoites are released into the circulation, penetrate erythrocytes, and cause diverse cellular and enzymatic changes that involve systemic effects in the liver, neurotoxic effects in the brain and schizoniticide effects in blood uptake of efflux or transporter molecules.

infection) are often associated with elevated levels of total bilirubin (TB). In addition, one study has shown that NF-kappaB and pro-inflammatory responses are activated in human brains exposed to PRBCs (47). The pathological changes, on the contrary, are correlated with the presence of Kupffer cells and lymphocytes in the portal tracts (45). However, ANXA5, which is often associated with apoptosis, is found, surprisingly, in the non-apoptotic cells that undergo plasma membrane damage and shrinkage, as demonstrated by flow cytometry (48).

4. ROLE OF ANNEXIN (ANX) IN PLASMA OF MALARIA PARASITE-INFECTED PATIENTS

Endogenously, ANXA1 is constantly expressed in leukocytes, epithelial cells and sub-populations of lymphocytic cells, and its expression is usually enhanced to regulate inflammatory processes (50-53). Studies exploring the association between malarial parasitic infection and ANXA1 in the plasma of malaria patients have revealed that ANXA1 expression differs among lymphocytes, being decreased in CD4+/CD8+ T cells, but increased in regulatory T cells (Treg) (49). This difference in ANXA1 expression may influence cell proliferation and IL-10 release into the plasma of patients infected with *P. vivax.*

In combination with the T-cell receptor (TCR), cytokines modulate the differentiation of T-helper lymphocytes (59, 60). Low-affinity binding of TCR to the antigen presented in a major histocompatibility complex (MHC) leads to the induction of Th2 responses, whereas high-affinity binding leads to Th1 responses (59). Involvement of the regulatory T cells during *P. vivax* infection is balanced by pro- and anti-inflammatory cytokines (58). Host immunity against intracellular pathogens is orchestrated by the production of inflammatory cytokines, such as interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha, to mediate the elimination of plasmodium-infected hepatocytes (pre-erythrocytic) by activated CD8+ T cells (56). CD4+ T cell responses, on the other hand, have been associated with the restriction of parasitic growth within PRBCs via secretion of cytokines (IL-1beta, IL-10, TNF-alpha), activation of macrophages and mediation of humoral immunity (57). Nevertheless, severe infection with *P. vivax* can lead to dysfunctional T cells and lymphopenia, which is implicated in sustaining the disease (54, 55)

ANX is an important protein that targets several phospholipids and DAMPs in reducing inflammatory events during infection (64, 65). ANXA5 has been shown to be involved in repairing damaged membranes by acting as a shield to phospholipid DAMPs that trigger inflammatory response, besides resealing mechanically-damaged membrane to decrease inflammation. This protein is not only a universal repair tool with multiple functions, it also plays a critical role in malaria and ageing (61). Therefore, ANXA5 presents a unique therapeutic strategy to prevent injury at the DAMP recognition level, thus limiting or controlling the most proximal end of the inflammatory response (62, 63).

In patients infected with *P. falciparum,* the pro-inflammatory S100 calcium binding protein A8 (S100A8) was reported to be increased, resulting in the inhibition of cytotoxic T cells that kill the parasites by Treg cells ($CD4^+CD25^+$Foxp3). This, in turn, causes the diminished immune response that is observed in malarial patients (66). In addition, hospital-based study findings showed that high-density lipoprotein (HDL) was also elevated and implicated in the pathogenesis of malaria (67). In the absence of a positive blood film, low levels of cholesterol and platelets in malaria patients can provide a useful clinical diagnosis (68). In children infected with *P. falciparum*, higher levels of IL-10 in the plasma and specific antibody production against merozoite surface protein 3 have been documented (69). The induction of severe host immune responses by the parasite involves pathogen-associated molecular patterns (PAMPs) being released into the circulation during schizogony (70). Particularly, glycosylphosphatidylinositol (GPI) toxin from *P. falciparum* activates leukocytes and stimulates the release of inflammatory cytokines to induce the expression of adhesion molecules via toll-like receptors-2 and -4 (TLR-2 and -4) (71).

5. ANXS

ANXs have been divided into five extensively studied groups, namely human (ANXA1- ANXA13), animal (ANX B9, ANX B10, ANX B11, ANX B12), fungal (ANX C1-C5), plant (ANX D1- D25) and protist (ANX E1-E3) (72, 73). Of all these, annexin-A1 (ANXA1) is well-known for its anti-inflammatory effects induced by glucocorticoids, and it is associated with a diverse range of inflammatory mediators to control inflammatory responses. This protein belongs to the multi-gene family of calcium-mediated phospholipid binding proteins and regulates several pathophysiological processes, such as inflammation, phagocytosis, cell proliferation, cell migration, differentiation and apoptosis (53, 74, 75).

5.1. Molecular structure of ANX

ANXs are membrane-interacting, calcium (Ca^{2+}) and phospholipid binding proteins expressed in eukaryotes throughout the plant and animal kingdoms (76). The vertebrate ANXs are characterized by a highly conserved alpha-helical region and four repeat regions that are tightly packed in the protein core domain, and two principal domains consisting of an NH_2-terminal head and a conserved COOH-terminal protein core (75, 79) (Figure 2). Having approximately 70 amino-acid residues that represent a Ca^{2+} regulated membrane binding module (72, 76-78), the protein also contains a unique central core for a hydrophilic pore, proposed to function as a Ca^{2+} channel.

5.2. Structural activity relationship of ANX binding to bilayers

ANXs are cytosolic proteins that bind to phospholipids, including the intercellular leaflet of phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) or phosphatidylinositol (PI) (82, 83). ANXA2 and 8, for example, are more specifically associated with membrane lipids of phosphatidylinositol-4,5-bisphophate (PIP2) (84). Structural studies reveal that ANXA5/ANX B12 bind to phospholipid bilayers in the liquid crystal phase, whereas ANXA1/2 bind to the phospholipid head groups in the bilayer, along with Ca^{2+} binding sites (80). A molecular dynamics simulation confirms that two water molecules are replaced by an oxygen from the polar head groups of the protein in the presence of phospholipids (81). The conserved hydrophobic amino acids (AA) in the AB loops allow communication of the protein with the lipid bilayers via the hydrophobic acyl chains of phospholipids. The net charge of the polar head groups of the phospholipids is important for recruiting ANX to the membrane. As such, the molecular interaction of the lipid head groups and the specific residues strongly influences the binding affinity with the lipid binding sites. Experimental studies have also validated the hypothesis that the antimalarial drug hydroxychloroquine (HCQ) reverses the anti-phospholipid-mediated disruptions of ANXA5 on phospholipid bilayers (PLBs) in cultured cells as well as in the plasma of anti-phospholipid (aPL) syndrome (APS) patients (85, 86). In addition, HCQ reduces the binding of aPL immunoglobulin G (IgG)-beta 2GPI complexes to phospholipid bilayers, and this disintegration of aPL-beta2GPI complexes is evident using image analysis of atomic force microscopy (AFM) (87, 88). This drug not only reduces thrombosis in animal models of APS, but also reverses aPL-mediated platelet activation (89, 90).

5.3. ANXA1 and NF-κB mediated signaling pathway

ANXA1 is an endogenous protein that is constantly expressed in leukocytes and epithelial cells ((51, 52, 93, 94). Previous studies have shown that ANXA1 exhibits anti-inflammatory properties and potent anti-migratory activity on neutrophils by regulating the adhesion and transmigration of leukocytes to inflammation sites (51, 52, 74, 91). In addition, the protein also serves as an important homeostatic regulator in mature T cells by modulating TCR signaling, provides an important molecular target in the differentiation and proliferation of lymphocytes, and inhibits various enzymes implicated in inflammatory response, including phospholipase A_2 (PLA_2), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). The cleaved N-terminal domain binds to the formyl peptide receptor (FPR), accelerating cell disconnection and inhibiting leukocyte movement to resolve inflammatory response (92). A recent study confirmed that ANXA1, besides regulating IFN-gamma, IL-17, TNF-alpha, and IL-6 production by T cells (95), also suppresses the effects of apoptotic cells on the immune reaction (96). Mechanistically, ANXA1 has been reported to interact with NEMO and RIP1 to constitutively activate IKK

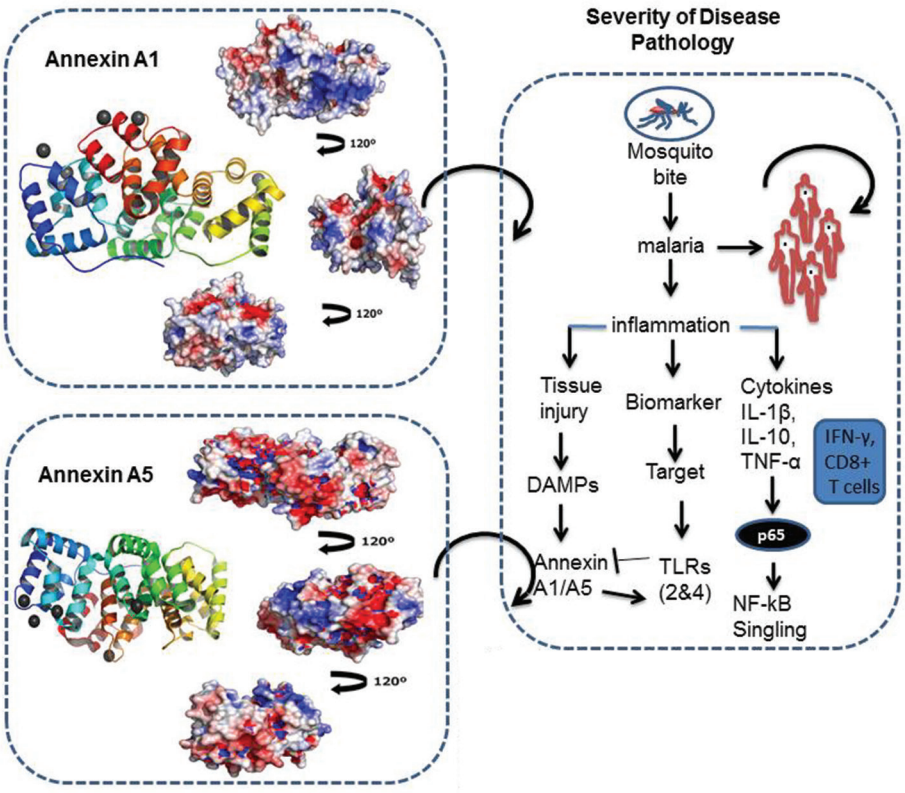

Figure 2. Severe infection caused by the parasite induces inflammatory cytokines (IL-1beta, IL-10, TNF-alpha), NF-kappaB, and activation of macrophages. Annexin A1 (ANXA1) levels increase in plasma of patients infected with malaria, causing elevated lymphocyte levels but reduced numbers of T cells. ANXA1 regulates the inflammatory processes, leading to diverse cellular and molecular changes. Annexin A2 (ANXA2) structure (DOI: 4FTG from PDB), consisting of amino-terminal head and carboxyl domains.

complex and NF-kappaB, which are thought to play a central role for disease progression. The nuclear transcription factor NF-kappaB pathway involves hemozoin (malarial pigment) and 15 (S,R)-hydroxy-6,8,11,13-eicosatetraenoic acid (15-HETE)-mediated activation of matrix metalloproteinase-9 (MMP-9) in human monocytes (95). Therefore, depending on cell stimulus, ANXA1 expression may be upregulated to control the inflammatory response (53).

5.4. Interaction of ANXA1 with PLA_2

ANXA1 was initially reported as a PLA_2 inhibitor. There are two isoforms of PLA_2 − secretory PLA_2 ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$). The latter is an enzyme activated by an elevation in the cytosolic calcium to catalyze the release of arachidonic acid from the sn-2 arachidonyl chain of a 1-O-ether-linked choline-containing glycerophospholipid, providing the precursor for eicosanoids (97, 98). Eicosanoids are the important secondary messenger molecules that play a key role in inflammation. Immunoprecipitation studies have suggested that ANXA1 inhibits $cPLA_2$ directly, rather than by substrate depletion (98). This is further supported by enzyme kinetic studies that measure the inhibitory potential of substrate and calcium concentrations, and the results showed that inhibition of the enzyme was largely due to substrate concentration (100). However, $cPLA_2$ activity can only be inhibited by full-length and C-terminal region of ANXA1, not by the N-terminal region (99), and this interaction is cell type-specific, being more prominent in hepatocytic cell lines (101).

6. ANTIMALARIAL POTENTIAL OF SNAKE VENOM PLA_2

Snake venoms (SVs) contain a mixture of pharmacologically active molecules with varied enzymatic and non-enzymatic properties. Most SVs contain PLA_2 as the main component along with phosphodiesterase, phosphomonoesterase, L-amino acid oxidase, and specific and non-specific endopeptidases (102, 103). The plethora of molecules in SVs contribute to the variety of biological properties found in them, including anti-inflammatory (104), anti-diabetic (105), anti-viral (also against HIV) (106), anti-cancer (107), and anti-arthritic. For instance, the antibacterial potential of a basic PLA_2 obtained from Russell's viper (*Daboia russellii pulchella*) can be effective against *Staphylococcus aureus* and *Bacillus subtilis* at 13-24 micrograms per milliliter (108). Antibacterial and antiparasitic activities attributable to other

Table 1. Antimalarial potency of venoms/proteins/peptides derived from animal sources

Proteins/peptides	Species derived	Testing	Properties	References
Secreted phospholipase ($sPLA_2$) such as IA, IB, IIA and III	Different intra-erythrocytic developmental stages of *P. falciparum*	Antiparasitic	Antiparasitic activities even at an IC_{50} of 0.1 μM	(113)
PLA_2s	Bee Scorpion Snake venoms	Antiparasitic	Effectively prevents the intra-erythrocyte development of *P. falciparum*	(114)
Crotoxin complex PLA_2 (Crotoxin B)	*Crotalus durissus cumanensis*	Antiparasitic fraction II possesses activity against the parasite	0.76 microgram per milliliter) (0.6 microgram per milliliter)	(115)
Natural toxin	*Bothrops marajoensis* venom	Antiparasitic	Effect of at 0.13 microgram per milliliter, PLA_2 (0.01 microgram per milliliter)	(39)
Phospholipase A_2 (PLA_2)	*Bothrops asper*	Antiplasmodial	At 0.13-22.9 microgram per milliliter concentrations	(116)

SV proteins and non-venomous snake serum-derived peptide analogues are shown in Table 1 (38, 39, 109-116).

Of particular interest, the PLA_2 from *Crotalus adamanteus* inhibits different malarial parasites (*P. falciparum* and *P. gallinaceum*) during their development in the mosquito midgut epithelium by preventing ookinete association/oocyst formation (38). Besides these natural proteins, synthetic propeptides have the capabilities to disrupt ookinete interactions with the midgut as well (117). In fact, transgenic *Anopheles stephensi* mosquitoes expressing a PLA_2 venom gene have been shown to block the development of *P. berghei* oocysts and their transmission to mice (118-121).

6.1. Antimalarial potency of SV-derived peptides

Antimicrobial peptides (AMPs) that are diverse in sizes (~20 amino acids), cationic at physiological pH, hydrophobic, having unique secondary structures (36) and linear α-helices free of cysteine residues can be easily synthesized. Previous studies showed that these AMPs (PIP-8, PNT-II, AMP-1 and AMP-2) exert potent anti-inflammatory, anti-bacterial (also against MRSA), wound-healing and anti-viral actions (110). Compared to original proteins used as models, these novel peptides can be more effective anti-malarial candidates, being devoid of cellular toxicities and lytic effects on human erythrocytes.

6.2. Antimalarial activity of other peptides

Recently, several anti-plasmodial peptides were synthesized from N-and C-terminal regions of *P. falciparum* serine repeat antigen 5 (PfSERA5), which is critical for the intra-erythrocytic development of malarial parasites. These peptides co-localize with PfSERA5 within the parasite and reduce its activity, thus conclusively demonstrating the potential of inhibiting the PfSERA5 enzyme as a target for new peptide-based anti-malarials (122). A synthetic derivative of NK-2 has been reported to be effective on *P. falciparum*-infected erythrocytes (PfRBC) at an IC_{50} of 1-10 micromolar (123) and for synthetic peptide D-HALO-rev, the IC_{50} was reported at 0.1 micromolar (125). Dermaseptin NC7-P peptide from *Phyllomedusa sauvagii* (K4-S4) was shown to affect the PfRBC ring stage at a 5.3 micromolar dose, but inhibited the trophozoite stage at 6.2 micromolar (124), whereas K4K20-S4 (dermaseptin) and K4-s4 (1-13) affected PfRBC at 0.2. micromolar and 6 micromolar, respectively (126). Cationic host-defense peptides (defensins/cecropins), membrane-active peptides (gramicidins), hydrophobic peptides (cyclosporins) and thiopeptides (thiostrepton), in contrast, cause severe damage to membrane integrity and affect specific intracellular targets of the mosquito parasites.

Cationic, lysine-branched peptides (Ac-GXRKXHKXWA-NH) display anti-plasmodial effects at IC_{50} greater than 100 micromolar (127), while tetrapeptides Phe-Orn-Phe-Orn (4) and Lys-Phe-Phe-Orn (5) exert activity at IC_{50} of 2.5 and 3.3 micromolar, respectively, against *Plasmodium berghei* (128). Synthetic hybrid peptides (1-3) from cecropin B and melittin caused at least 60 percent mortality of *P. berghei* ookinetes at 50 micromolar concentration (129). Scorpion venom from *Pandinus imperator* effectively inhibits *P. berghei* ookinetes and gametes at approximately 1 micromolar and 10 micromolar, respectively (130, 131), while drosomycin isolated from *Drosophila melanogaster* inhibits 70 percent of *P. berghei* gametocytes at 20 micromolar dose (134). Peptides from mosquitoes, such as Gambicin isolated from *Anopheles gambiae,* inhibit 54.6 percent of *P. berghei* ookinetes (133), while the defensin A peptide from *Aedes aegypti* cleared approximately 85 percent of *P. gallinaceum* oocysts in transgenic mosquitoes (135). Even the peptide derivative (IDR-1018) of bactenicin,

which can be isolated from bovine neutrophils, has been demonstrated to be protective against cerebral malaria (132). A more recent report has also confirmed the promising antimalarial activities of peptides based on those that are already in phase I (NVB302) and phase II (omiganan and OP-145) clinical trials (136). Few peptides have been studied in preclinical animal models for severe malaria (137), and none of them has progressed towards pre-clinical or clinical development as a therapeutic agent due to the concern that most of them cause systemic toxicity, and the fact that their mechanisms of action are relatively unknown.

Secondary metabolites derived from fungi are also rich sources of chemotherapeutic agents. Peptides isolated from fungal species consist of unusual amino acids, (128) such as alpha-aminoisobutyric acid, isovaline, beta-alanine, and hydroxyproline. For example, efrapeptins produced by the fungus *Tolypocladium niveum*, which belongs to the subspecies *inflatum*, inhibit mitochondrial F0F1 ATPase and some bacterial ATP synthases (138). This biological effect of efrapeptins leads to altered plasma membrane potential in the parasite (139). Another study has also revealed that three antibiotic peptides (efrapeptins, zervamicins and antiamoebin) can kill *P. falciparum* in culture with 50 percent inhibitory effects at micromolar concentrations (140). Diverse antimalarial peptides have been synthesized or isolated from natural sources (128, 141). Linear synthetic peptides such as leukinostatin A, efrapeptin, zervamicin, and antiamoebin have been shown to elicit potent activity (140). Dimer, trimer, tetramer, and pentameric peptides including cyclic analogs (142) exert inhibitory activity against *P. falciparum* at an IC_{50} of 7.5. micromolar concentration. A series of several short synthetic peptides, like pentapeptides derived from several *Actinomyces* species (IC_{50}=31 nanograms per milliliter) (140), cyclotetrapeptides such as apicidin and apicidin A derived from HC-toxin I (IC_{50}=125 nanograms per milliliter) (143), and a linear tetrapeptide hirsutellic A acid isolated from *Hirsutella* species BCC 1528 fermentation broth that contains an anthranilic acid residue at the C-terminus (IC_{50}=8.0. micromolar) (144), have all been demonstrated to display promising antimalarial effects. Dipeptides such as 2-(2-tert-butoxycarbonylamino-4-methylpentanoyl-amino)-cyclooctanecarboxylic acid methyl ester and 2-(2-tert-butoxycarbonylamino-3-phenyl-propionylamino)-cyclooctanecarboxylic acid methyl ester are very effective at inhibiting late schizonts at 3.8 and 3.6 micrograms per milliliter concentrations, respectively (142).

7. THERAPEUTIC POTENTIAL OF PEPTIDES BY SILICO APPROACHES AND DRUG DESIGN

Silico drug design involves the identification of possible target proteins of a drug via computational tools. *In silico* methods can be used to analyze target structures for active binding sites, and with this information, they can develop candidate molecules. In addition, they can check the molecules for their drug likeness and attempt to dock these molecules with the target, after which they can proceed to rank these molecules according to their binding affinities and attempt to improve other binding characteristics. The quantitative structure activity-relationship (QSAR) regression model can then be used to summarize and interpret the quantitative relationship between the activity of the candidate molecule and its structure. It does so via statistical and modern data mining techniques, which include ligand-based virtual screening and virtual profile affinity (145). Identifying any drug-induced alterations in either electrophysiology or neurochemical networks can be accomplished using neurochemical and biophysical models (146-148).

7.1. Diverse silico methods for drug design and discovery

In silico, or silico, approaches are used to describe experiments that involve the use of computers. In most cases, *in silico* methods are used to generate data for the accurate modeling and validation of applications. Although it is a comparatively novel tool, there have been many diverse *in silico* drug-designing efforts described (149, 150). These include homology modelling (112) and molecular docking, which are used to determine, score and rank the affinity of particular ligands towards an active site of specific receptors. Currently, there are different methods available to assess the docking of ligands to different binding sites, which include ArgusDock, DOCK, FRED, eHITS, AutoDock and FTDock (151), hologram quantitative structure activity relationship (HQSAR), comparative molecular field analysis (152), and molecular similarity indices analysis (CoMSIA).

Other *in silico* approaches involve three-dimensional (3D) pharmacophore mapping and microarray techniques. Both significantly contribute towards the elucidation and optimization of molecules discovered by high-throughput screening from combinatorial libraries (153, 154). This enables a large number of classical drug candidates to be screened more rapidly within a short period of time, compared to conventional virtual screening (154, 158-160). Microarray techniques are also widely used in different fields for the development of efficient new drugs (155), conformational analysis, Monte Carlo simulation (156) and molecular dynamic simulation (157). These virtual ligand screening and profiling methods play an important role in drug discovery throughout pharmacology. Ligand design and optimization is useful for characterizing basic pharmacological actions of molecules, including absorption, distribution, metabolism, excretion and toxicity (154). Thus, these approaches may lead to improved drug design and represent a “discovery gate” into the future (161).

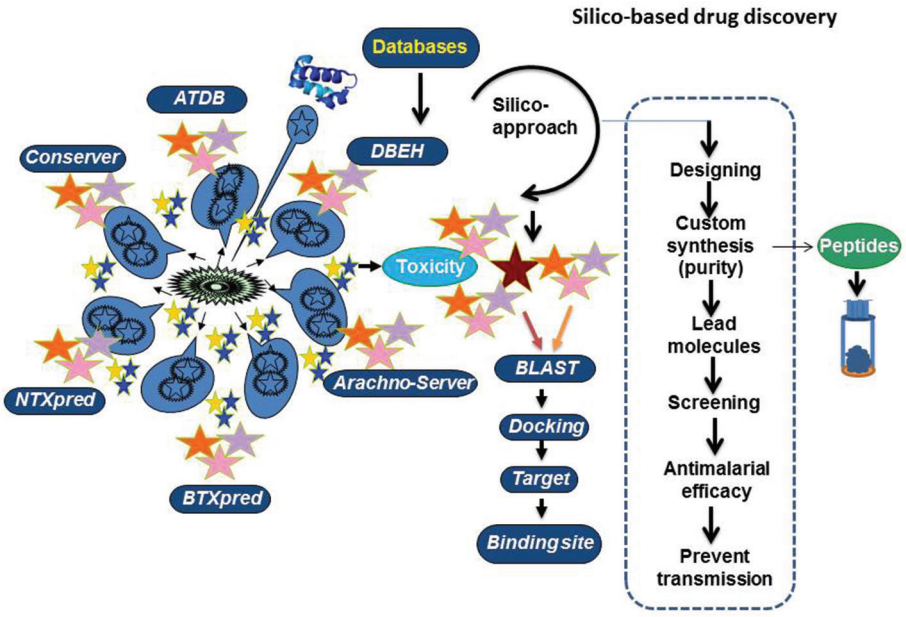

Figure 3. Schematic representation shows the designing of small molecules by bioinformatics tools for the *in silico*-based drug discovery of novel antimalarial drugs.

7.2. Significance and impact of silico methods

Synthetic chemical drugs currently used for the treatment of *P. falciparum* infection in humans are losing therapeutic efficacy because of drug resistance. Therefore, viable silico-based strategies might serve as a novel anti-malarial drug discovery pipeline in producing new drugs to fight these parasites (162). Many efforts yield some anti-malarial agents following high-throughput screening of chemicals from the chemical libraries on parasite cultures (163-165). An estimated two million small molecules were screened against *P. falciparum,* of which 13,533 agents inhibited parasitic growth (80 percent or greater) at 2 micromolar concentration, while 800 agents were reported to have promising activity against a drug-resistant strain (Dd2) (163). Several recent studies used a bioluminescent parasite (*P. falciparum)* to detect shifts in the viability of microcultures (165). For instance, a dermaseptin-4 (DS4) peptide was designed, synthesized and evaluated by screening platforms described above. The results proved that a reduction in parasite viability was consistent with bioluminescence emitted from the parasite micro-cultures after exposure to bacterial extracts containing the DS4 peptide (165-167). The DS4 peptide is lipophilic, highly electrostatic, and possesses an affinity to cell membranes (168). Chemically diverse types of CoA pathway inhibitors can also effectively control malarial parasites at 120 nanomolar to 6 millimolar dose range (169). There are many beneficial features of the candidate molecules from the novel class of peptides in terms of high specificity, biological properties, penetration and low production cost (170, 171). However, major concerns for developing peptide-based drugs are still present, due to the lack of stability, severe toxicity and immunogenicity. Nevertheless, instability can be overcome by incorporation of alpha-aminoxy amino acid and D-amino acids (172, 173).

7.3. Development of new silico approaches for predicting toxicity

Recently, there was an attempt to develop new silico approaches for predicting toxicity of peptides by using diverse databases such as BTXpred (174), NTXpred (175), Conoserver (176), ATDB (177), DBETH (178) and Arachno-Server (179). These silico based models will be useful not only in predicting toxicity of protein/peptides, but also in designing less toxic peptides and discovering toxic regions in proteins (180). Overall, these *in silico* approaches may be useful for peptide/protein-based drug discovery (Figure 3).

8. DELIVERY METHOD OF PEPTIDE DRUGS

There are many problems associated with therapeutic peptides. These often include low bioavailability via the oral route (181), poor permeability/ metabolic inactivation (182), proteolysis or enzymatic degradation, binding to plasma proteins, toxicity and resistance (183). Additionally, costs for peptide production can be very high if there is a need to scale-up production, and as a result, drugs will become less affordable for people in poorly developed countries. This can be overcome by using alternative approaches, such as AMPs that coat contact lenses to prevent lens-related infections, in the case of eye infections (184). For malaria, bed nets, cream-based drugs for topical application and/ or a combination of drugs using a suitable delivery method

may make peptide use more cost effective. Currently, biotechnological approaches that graft bioactive peptides into suitable biocompatible carriers can enhance peptide bioavailability at the site of action (39, 185, 186). Peptide analogues that are encapsulated in biodegradable polymers can be injected at an appropriate site (187). PEGylated poly-l-lysine-based peptide dendrimers, which are prepared and coated with chondroitin sulphate A (CSA), can be used to encapsulate drug molecules by a dialysis method for a controlled and sustained delivery of a blood schizonticide, chloroquine phosphate (CQ). This method promisingly reduced the trophozoite stage of *P. falciparum* in culture (188). These types of drug delivery approaches will improve some of the prevailing negative effects attributed to current anti-malarial management.

9. MARKET POTENTIAL OF THERAPEUTIC PEPTIDE-BASED DRUGS

Current market potential in the US for synthetic therapeutic peptides exceeds 13 billion dollars per year, representing 1.5 percent of total drug sales globally. In Europe, it is Germany and the UK that account for 63 percent of the peptide therapeutic market, with France, Italy, Scandinavia and Spain making up the rest of the major markets. Furthermore, the market for synthetic peptides rose from 5.3 billion dollars in 2003 to 8 billion dollars in 2005. It was estimated that one peptide share would reach 14.1 billion dollars in 2013 and that it will reach 25.4 billion by 2018 (189, 190). New synthetic strategies to improve productivity and reduce metabolism of peptides, along with alternative routes of administration, have been developed in recent years, with a large number of peptide-based drugs now being marketed (191, 192). With increased approval of peptide-based drugs, and the advances in peptide-associated technologies, peptide-based drug therapeutics will become more significant, thus opening up more commercial opportunities for treating malarial infections (186).

10. DEVELOPMENT OF DRUG TARGETS BASED ON MALARIAL PARASITE PROTEINS

Protein phosphorylation, a form of post-translational modification, significantly regulates protein function. Recently, the systematic analyses of *Plasmodium* protein (PP) phosphatases, such as phosphoprotein phosphatases (PPPs), metallo-dependent protein phosphatases (PPMs), protein tyrosine phosphatases (PTPs) and gene product NLI-interacting factor-like phosphatases (NIFs) (193-197), revealed that they play a vital role during ookinete to oocyst transition and subsequent transmission in a rodent malaria model (197, 198). PPM2, PPM5 and PPM10 in particular are regulated by phosphorylation (199) and are involved in important functions during asexual development/ differentiation of *P. falciparum* (200). During every stage of development, the parasite uses a number of signal transduction mechanisms, including reversible protein phosphorylation catalysed by protein kinases (PKs) and phosphatases (PPs). While the PKs are known to be important therapeutic targets (201), the PPs are only now emerging as a target for clinical intervention (202).

11. CLINICAL SIGNIFICANCE AND CONCLUSIONS

Malaria is still one of the most important and urgent global medical challenges. This is predominantly due to the number of people affected by the disease, and the severe complications associated with inappropriate treatment. The majority of fatal malaria cases are caused by *P. falciparum,* and rarely by *P. vivax* or *P. malariae*. Infection by *P. falciparum* causes serious complications, and often results in relapses after treatments and subsequently death (203). These mortality rates peak during monsoon season in Southeast Asia and other parts of the world (204). Patients with severe cases of malaria are given intravenous quinine/quinidine, while artemisinin derivatives are recommended for quinine-resistant cases (205). The main symptoms of malaria include cerebral and pulmonary edema, acute renal failure, severe anemia, and bleeding, which make malaria a disease in need of a panacea. With the lack of effective current treatments, alternatives are clearly necessary (206). Gaining a better understanding of the underlying molecular mechanisms will aid in identifying the appropriate targets that are linked to clinical manifestations, and one important area to focus on would be the role of DAMPs, which are known to trigger inflammation. Furthermore, a better understanding of the mechanisms will also allow for proper diagnosis and better management of malarial parasitic infections.

Understanding the mechanisms associated with ANXA1/5 will be useful to better understand the disease pathology and may be useful as a target for developing diagnostic biomarkers in clinical settings. Cytokines like TNF-alpha and IL-1beta most likely contribute to the pathogenesis of severe malaria parasitic infections, which can progress rapidly and lead to mortality in a few hours or days. The lack of advance diagnostic approaches and non-microscopic methods that are only now being introduced aggravate the outcomes of the disease (207). In addition, as drug resistance develops rapidly in parasites, novel and more efficacious antimalarial drugs that halt malarial parasite development are required. Despite all these obstacles, therapy with a monoclonal antibody against TNF-alpha for controlling fever, when used in combination with drugs such as artemether-lumefantrin or atovaquone-proguanil, lowers the risk of resistance and yields better results than treatment with mefloquine or quinine alone (55). Thus, peptides from snake venom proteins may potentially serve as more

potent anti-infective and antimalarial agents using *in silico* approaches, which ultimately will contribute to improved malaria management.

12. ACKNOWLEDGEMENTS

This study was partially supported by a NUHS Bench to Bedside to Product grant to GS. This work was supported by grants from the National Medical Research Council of Singapore (R-713-000-177-511), and by the NCIS Yong Siew Yoon Research Grant through donations from the Yong Loo Lin Trust to APK. APK was also supported by the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centers of Excellence initiative for the Cancer Science Institute of Singapore, National University of Singapore. OLF is supported by CAPES, CNPq, FAPDF and FUNDECT. The authors confirm that this article content has no conflicts of interest.

13. REFERENCES

1. Pan American Health Organization: Malaria in the Americas: Time Series Epidemiological Data from 2000 to 2007. In: Pan American Health Organization, Regional Office for the Americas 2008., 525 Twenty-third Street, N.W., Washington, D.C. 20037, United States of America (2008)
2. W. H. Organization: World malaria report: 2012. In: *World malaria report* World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (2012)
3. R. W. Snow, C. A. Guerra, A. M. Noor, H. Y. Myint, S. I. Hay: The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 434(7030), 214-217 (2005) DOI: 10.1038/nature03342 PMid:15759000 PMCid:PMC3128492
4. S. I. Hay, E. A. Okiro, P. W. Gething, A. P. Patil, A. J. Tatem, C. A. Guerra, R. W. Snow: Estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007. *PLoS Med* 7(6), e1000290 (2010)
5. World Health Organization: World malaria report: 2013. In: *World malaria report*. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (2013)
6. Centers for Disease Control and Prevention: Impact of Malaria. In: Centers for Disease Control and Prevention, Atlanta, GA 30329-34027 (2012)
7. J. T. Griffin, N. M. Ferguson, A. C. Ghani: Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. *Nat Commun* 5, 3136 (2014)
8. O. T. Ng, E. E. Ooi, C. C. Lee, P. J. Lee, L. C. Ng, S. W. Pei, T. M. Tu, J. P. Loh, Y. S. Leo: Naturally acquired human *Plasmodium knowlesi* infection, Singapore. *Emerg Infect Dis* 14(5), 814-816 (2008) DOI: 10.3201/eid1405.070863 PMid:18439370 PMCid:PMC2600232
9. Y. C. Lee, C. S. Tang, L. W. Ang, H. K. Han, L. James, K. T. Goh: Epidemiological characteristics of imported and locally-acquired malaria in Singapore. *Ann Acad Med Singapore* 38(10), 840-849 (2009)
10. M. E. Sinka, M. J. Bangs, S. Manguin, T. Chareonviriyaphap, A. P. Patil, W. H. Temperley, P. W. Gething, I. R. Elyazar, C. W. Kabaria, R. E. Harbach, S. I. Hay: The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic precis. *Parasit Vectors* 4, 89 (2011)
11. M. E. Sinka, M. J. Bangs, S. Manguin, Y. Rubio-Palis, T. Chareonviriyaphap, M. Coetzee, C. M. Mbogo, J. Hemingway, A. P. Patil, W. H. Temperley, P. W. Gething, C. W. Kabaria, T. R. Burkot, R. E. Harbach, S. I. Hay: A global map of dominant malaria vectors. *Parasit Vectors* 5, 69 (2012)
12. A. P. Patil, E. A. Okiro, P. W. Gething, C. A. Guerra, S. K. Sharma, R. W. Snow, S. I. Hay: Defining the relationship between *Plasmodium falciparum* parasite rate and clinical disease: statistical models for disease burden estimation. *Malaria J* 8, 186 (2009)
13. W. Khan, H. A. Zakai, E. A. Umm: Clinico-pathological studies of *Plasmodium falciparum* and *Plasmodium vivax* - malaria in India and Saudi Arabia. *Acta Parasitol* 59(2), 206-212 (2014) DOI: 10.2478/s11686-014-0227-1 PMid:24827088
14. C. A. Guerra, R. E. Howes, A. P. Patil, P. W. Gething, T. P. Van Boeckel, W. H. Temperley, C. W. Kabaria, A. J. Tatem, B. H. Manh, I. R. F. Elyazar, J. K. Baird, R. W. Snow, S. I. Hay: The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *Plos Negl Trop Dis* 4(8), e774 (2010)

15. E. T. Han, D. H. Lee, K. D. Park, W. S. Seok, Y. S. Kim, T. Tsuboi, E. H. Shin, J. Y. Chai: Reemerging vivax malaria: changing patterns of annual incidence and control programs in the Republic of Korea. *Korean J Parasitol* 44(4), 285-294 (2006)
DOI: 10.3347/kjp.2006.44.4.285
PMid:17170570 PMCid:PMC2559126

16. S. Sinha, B. Medhi, R. Sehgal: Challenges of drug-resistant malaria. *Parasite* 21, 61 (2014)

17. R. McGready, E. A. Ashley, F. Nosten: Malaria and the pregnant traveller. *Travel Med Infect Dis* 2(3-4), 127-142 (2004)

18. P. Schlagenhauf, E. Petersen: Malaria chemoprophylaxis: strategies for risk groups. *Clin Microbiol Rev* 21(3), 466-472 (2008)
DOI: 10.1128/CMR.00059-07
PMid:18625682 PMCid:PMC2493087

19. N. T. Marbiah, E. Petersen, K. David, E. Magbity, J. Lines, D. J. Bradley: A controlled trial of lambda-cyhalothrin-impregnated bed nets and/or dapsone/pyrimethamine for malaria control in Sierra Leone. *Am J Trop Med Hyg* 58(1), 1-6(1998)

20. P. E. Crewther, J. G. Culvenor, A. Silva, J. A. Cooper, R. F. Anders: *Plasmodium falciparum*: two antigens of similar size are located in different compartments of the rhoptry. *Exp Parasitol* 70(2), 193-206 (1990)
DOI: 10.1016/0014-4894(90)90100-Q

21. R. Carter, K. N. Mendis: Evolutionary and historical aspects of the burden of malaria. *Clin Microbiol Rev* 15(4), 564-594 (2002)
DOI: 10.1128/CMR.15.4.564-594.2002
PMid:12364370 PMCid:PMC126857

22. S. I. Hay, C. A. Guerra, A. J. Tatem, P. M. Atkinson, R. W. Snow: Urbanization, malaria transmission and disease burden in Africa. *Nat Rev Microbiol* 3(1), 81-90 (2005)
DOI: 10.1038/nrmicro1069
PMid:15608702 PMCid:PMC3130901

23. A. K. Rowe, S. Y. Rowe, R. W. Snow, E. L. Korenromp, J. R. A. Schellenberg, C. Stein, B. L. Nahlen, J. Bryce, R. E. Black, R. W. Steketee: The burden of malaria mortality among African children in the year 2000. *Int J Epidemiol* 35(3), 691-704 (2006)
DOI: 10.1093/ije/dyl027
PMCid:PMC3672849

24. A. Roca-Feltrer, I. Carneiro, J. R. Armstrong Schellenberg: Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years. *Trop Med Int Health* 13(6), 771-783 (2008)
DOI: 10.1111/j.1365-3156.2008.02076.x
PMid:18363586

25. C. A. MacRaild, R. F. Anders, M. Foley, R. S. Norton: Apical membrane antigen 1 as an anti-malarial drug target. *Curr Top Med Chem* 11(16), 2039-2047 (2011)
DOI: 10.2174/156802611796575885
PMid:21619512

26. R. W. Snow, E. Eckert, A. Teklehaimanot: Estimating the needs for artesunate-based combination therapy for malaria case-management in Africa. *Trends Parasitol* 19(8), 363-369 (2003)
DOI: 10.1016/S1471-4922(03)00168-5

27. K. Marsh: Malaria disaster in Africa. *Lancet* 352(9132), 924 (1998)

28. E. L. Korenromp, B. G. Williams, S. J. de Vlas, E. Gouws, C. F. Gilks, P. D. Ghys, B. L. Nahlen: Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa. *Emerg Infect Dis* 11(9), 1410-1419 (2005)
DOI: 10.3201/eid1109.050337
PMid:16229771 PMCid:PMC3310631

29. Y. A. Afrane, G. Zhou, A. K. Githeko, G. Yan: Clinical malaria case definition and malaria attributable fraction in the highlands of western Kenya. *Malar J* 13, 405 (2014)

30. S. Campos, P. de la Cerda, A. Rivera: Fatal artesunate toxicity in a child. *J Pediatric Infect Dis* 3(1), 69-76 (2008)

31. D. I. Stanisic, A. E. Barry, M. F. Good: Escaping the immune system: How the malaria parasite makes vaccine development a challenge. *Trends Parasitol* 29(12), 612-622 (2013)
DOI: 10.1016/j.pt.2013.10.001
PMid:24176554

32. H. Zieler, D. B. Keister, J. A. Dvorak, J. M. Ribeiro: A snake venom phospholipase A(2) blocks malaria parasite development in the mosquito midgut by inhibiting ookinete association with the midgut surface. *J Exp Biol* 204(Pt 23), 4157-4167 (2001)

33. D. D. Denson, R. T. Worrell, D. C. Eaton: A possible role for phospholipase A2 in the action of general anesthetics. *Am J Physiol* 270(2 Pt 1), C636-644 (1996)

34. M. M. Thwin, S. D. Satyanarayanajois, L. M. Nagarajarao, K. Sato, P. Arjunan, S. L. Ramapatna, P. V. Kumar, P. Gopalakrishnakone: Novel peptide inhibitors of human secretory phospholipase A2 with antiinflammatory activity: solution structure and molecular modeling. *J Med Chem* 50(24), 5938-5950 (2007)
DOI: 10.1021/jm070385x
PMid:17973469

35. M. M. Thwin, E. Douni, P. Arjunan, G. Kollias, P. V. Kumar, P. Gopalakrishnakone: Suppressive effect of secretory phospholipase A2 inhibitory peptide on interleukin-1beta-induced matrix metalloproteinase production in rheumatoid synovial fibroblasts, and its antiarthritic activity in hTNFtg mice. *Arthritis Res Ther* 11(5), R138 (2009)

36. C. Santamaria, S. Larios, Y. Angulo, J. Pizarro-Cerda, J. P. Gorvel, E. Moreno, B. Lomonte: Antimicrobial activity of myotoxic phospholipases A2 from crotalid snake venoms and synthetic peptide variants derived from their C-terminal region. *Toxicon* 45(7), 807-815 (2005)
DOI: 10.1016/j.toxicon.2004.09.012
PMid:15904676

37. R. P. Samy, M. M. Thwin, B. G. Stiles, S. Satyanarayana-Jois, A. Chinnathambi, M. E. Zayed, S. A. Alharbi, K. S. Siveen, S. Sikka, A. P. Kumar, G. Sethi, L. H. Lim: Novel phospholipase A2 inhibitors from python serum are potent peptide antibiotics. *Biochimie* 111, 30-44 (2015)
DOI: 10.1016/j.biochi.2015.01.003

38. D. Fenard, G. Lambeau, E. Valentin, J. C. Lefebvre, M. Lazdunski and A. Doglio: Secreted phospholipases A(2), a new class of HIV inhibitors that block virus entry into host cells. *Journal of Clinical Investigation* 104(5), 611-618 (1999)
DOI: 10.1172/JCI6915
PMid:10487775 PMCid:PMC408539

39. A. F. Costa Torres, R. T. Dantas, M. H. Toyama, E. Diz Filho, F. J. Zara, M. G. Rodrigues de Queiroz, N. A. Pinto Nogueira, M. Rosa de Oliveira, D. de Oliveira Toyama, H. S. Monteiro, A. M. Martins: Antibacterial and antiparasitic effects of *Bothrops marajoensis* venom and its fractions: Phospholipase A2 and L-amino acid oxidase. *Toxicon* 55(4), 795-804 (2010)
DOI: 10.1016/j.toxicon.2009.11.013
PMid:19944711

40. A. B. Rupani, A. D. Amarapurkar: Hepatic changes in fatal malaria: an emerging problem. *Ann Trop Med Parasitol* 103(2), 119-127 (2009)
DOI: 10.1179/136485909X385054
PMid:19208296

41. R. Whitten, D. A. Milner, Jr., M. M. Yeh, S. Kamiza, M. E. Molyneux, T. E. Taylor: Liver pathology in Malawian children with fatal encephalopathy. *Hum Pathol* 42(9), 1230-1239 (2011)
DOI: 10.1016/j.humpath.2010.11.019
PMid:21396681 PMCid:PMC3137725

42. O. Prommano, U. Chaisri, G. D. Turner, P. Wilairatana, D. J. Ferguson, P. Viriyavejakul, N. J. White, E. Pongponratn: A quantitative ultrastructural study of the liver and the spleen in fatal falciparum malaria. *Southeast Asian J Trop Med Public Health* 36(6), 1359-1370 (2005)

43. S. Chaikitgosiyakul, M. J. Rijken, A. Muehlenbachs, S. J. Lee, U. Chaisri, P. Viriyavejakul, G. D. Turner, E. Pongponratn, F. Nosten, R. McGready: A morphometric and histological study of placental malaria shows significant changes to villous architecture in both *Plasmodium falciparum* and *Plasmodium vivax* infection. *Malar J* 13, 4 (2014)

44. P. R. Totino, A. D. Magalhaes, L. A. Silva, D. M. Banic, C. T. Daniel-Ribeiro, F. Ferreira-da-Cruz Mde: Apoptosis of non-parasitized red blood cells in malaria: a putative mechanism involved in the pathogenesis of anaemia. *Malar J* 9, 350 (2010)

45. P. Viriyavejakul, V. Khachonsaksumet, C. Punsawad: Liver changes in severe *Plasmodium falciparum* malaria: histopathology, apoptosis and nuclear factor kappa B expression. *Malar J* 13, 106 (2014)

46. Q. Li, I. M. Verma: NF-kappaB regulation in the immune system. *Nat Rev Immunol* 2(10), 725-734 (2002)
DOI: 10.1038/nri910
PMid:12360211

47. A. K. Tripathi, W. Sha, V. Shulaev, M. F. Stins, D. J. Sullivan, Jr.: *Plasmodium falciparum*-infected erythrocytes induce NF-kappaB regulated inflammatory pathways in human cerebral endothelium. *Blood* 114(19), 4243-4252 (2009)

DOI: 10.1182/blood-2009-06-226415
PMid:19713460 PMCid:PMC2925626

48. P. R. Totino, A. Magalhaes, E. B. Alves, M. R. Costa, M. V. de Lacerda, C. T. Daniel-Ribeiro, F. Ferreira-da-Cruz Mde: *Plasmodium falciparum*, but not *P. vivax*, can induce erythrocytic apoptosis. *Parasit Vectors* 7, 484 (2014)

49. Q. I. Borges, C. J. Fontes, A. S. Damazo: Analysis of lymphocytes in patients with *Plasmodium vivax* malaria and its relation to the annexin-A1 and IL-10. *Malar J* 12, 455 (2013)

50. D. Nantakomol, A. M. Dondorp, S. Krudsood, R. Udomsangpetch, K. Pattanapanyasat, V. Combes, G. E. Grau, N. J. White, P. Viriyavejakul, N. P. Day, K. Chotivanich: Circulating red cell-derived microparticles in human malaria. *J Infect Dis* 203(5), 700-706 (2011)
DOI: 10.1093/infdis/jiq104
PMid:21282195 PMCid:PMC3072726

51. A. M. Kamal, R. J. Flower, M. Perretti: An overview of the effects of annexin 1 on cells involved in the inflammatory process. *Mem Inst Oswaldo Cruz* 100 Suppl 1, 39-48 (2005)
DOI: 10.1590/S0074-02762005000900008

52. L. Spurr, S. Nadkarni, M. Pederzoli-Ribeil, N. J. Goulding, M. Perretti, F. D'Acquisto: Comparative analysis of Annexin A1-formyl peptide receptor 2/ALX expression in human leukocyte subsets. *Int Immunopharmacol* 11(1), 55-66 (2011)
DOI: 10.1016/j.intimp.2010.10.006
PMid:20974309

53. F. D'Acquisto, M. Perretti, R. J. Flower: Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. *Br J Pharmacol* 155(2), 152-169 (2008)
DOI: 10.1038/bjp.2008.252
PMid:18641677 PMCid:PMC2538690

54. K. Kemp, B. D. Akanmori, V. Adabayeri, B. Q. Goka, J. A. L. Kurtzhals, C. Behr, L. Hviid: Cytokine production and apoptosis among T cells from patients under treatment for *Plasmodium falciparum* malaria. *Clin Exp Immunol* 127(1), 151-157 (2002)
DOI: 10.1046/j.1365-2249.2002.01714.x
PMid:11882046 PMCid:PMC1906283

55. A. Trampuz, M. Jereb, I. Muzlovic, R. M. Prabhu: Clinical review: Severe malaria. *Crit Care* 7(4), 315-323 (2003)
DOI: 10.1186/cc2183
PMid:12930555 PMCid:PMC270697

56. N. W. Schmidt, N. S. Butler, J. T. Harty: *Plasmodium*-host interactions directly influence the threshold of memory CD8 T cells required for protective immunity. *J Immunol* 186(10), 5873-5884 (2011)
DOI: 10.4049/jimmunol.1100194
PMid:21460205 PMCid:PMC3087867

57. T. Imai, J. Shen, B. Chou, X. Duan, L. Tu, K. Tetsutani, C. Moriya, H. Ishida, S. Hamano, C. Shimokawa, H. Hisaeda, K. Himeno: Involvement of CD8+ T cells in protective immunity against murine blood-stage infection with *Plasmodium yoelii* 17XL strain. *Eur J Immunol* 40(4), 1053-1061 (2010)
DOI: 10.1002/eji.200939525
PMid:20101613

58. L. L. Bueno, C. G. Morais, F. F. Araujo, J. A. Gomes, R. Correa-Oliveira, I. S. Soares, M. V. Lacerda, R. T. Fujiwara, E. M. Braga: *Plasmodium vivax*: induction of CD4+CD25+FoxP3+ regulatory T cells during infection are directly associated with level of circulating parasites. *PLoS One* 5(3), e9623 (2010)

59. J. M. Blander, D. B. Sant'Angelo, K. Bottomly, C. A. Janeway: Alteration at a single amino acid residue in the T cell receptor alpha chain complementarity determining region 2 changes the differentiation of naive CD4 T cells in response to antigen from T helper cell type 1 (Th1) to th2. *J Exp Med* 191(12), 2065-2073 (2000)
DOI: 10.1084/jem.191.12.2065
PMCid:PMC2193209

60. B. B. Andrade, A. Reis-Filho, S. M. Souza-Neto, J. Clarencio, L. M. Camargo, A. Barral, M. Barral-Netto: Severe *Plasmodium vivax* malaria exhibits marked inflammatory imbalance. *Malar J* 9 (13), (2010)

61. A. Villegas-Mendez, E. Gwyer Findlay, J. B. de Souza, L. M. Grady, C. J. Saris, T. E. Lane, E. M. Riley, K. N. Couper: WSX-1 signalling inhibits CD4(+) T cell migration to the liver during malaria infection by repressing chemokine-independent pathways. *PLoS One* 8(11), e78486 (2013)

62. J. Q. Shi, C. C. Zhang, X. L. Sun, X. X. Cheng, J. B. Wang, Y. D. Zhang, J. Xu, H. Q. Zou:

Antimalarial drug artemisinin extenuates amyloidogenesis and neuroinflammation in APPswe/PS1dE9 transgenic mice via inhibition of nuclear factor-kappaB and NLRP3 inflammasome activation. *CNS Neurosci Ther* 19(4), 262-268 (2013)
DOI: 10.1111/cns.12066
PMid:23406388

63. W. G. Land: The Role of Damage-Associated Molecular Patterns (DAMPs) in Human Diseases: Part II: DAMPs as diagnostics, prognostics and therapeutics in clinical medicine. *Sultan Qaboos Univ Med J* 15(2), e157-e170 (2015)
64. F. N. Gavins, M. J. Hickey: Annexin A1 and the regulation of innate and adaptive immunity. *Front Immunol* 3, 354 (2012)
65. R. T. Gazzinelli, P. Kalantari, K. A. Fitzgerald, D. T. Golenbock: Innate sensing of malaria parasites. *Nat Rev Immunol* 14(11), 744-757 (2014)
DOI: 10.1038/nri3742
PMid:25324127
66. H. W. Lee, T. S. Kim, Y. J. Kang, J. Y. Kim, S. Lee, W. J. Lee, Y. Sohn: Up-regulated S100 calcium binding protein A8 in *Plasmodium*-infected patients correlates with CD4(+) CD25(+)Foxp3 regulatory T cell generation. *Malar J* 14 (1), 385 (2015)
67. N. Nwobodo, P. O. Okonkwo, E. Nwobodo, S. A. Igwe: Evaluation of effects of malaria infection on serum lipid profile of patients attending two district hospitals in Enugu, Nigeria. *Orient J Chem* 24 (2), 415-418 (2008)
68. I. A. Al-Omar, A. M. Eligail, R. M. Al-Ashban, A. H. Shah: Effect of falciparum malaria infection on blood cholesterol and platelets. *J Saudi Chem Soc* 14(1), 83-89 (2010)
DOI: 10.1016/j.jscs.2009.12.013
69. V. Guiyedi, C. Becavin, F. Herbert, J. Gray, P. A. Cazenave, M. Kombila, A. Crisanti, C. Fesel, S. Pied: Asymptomatic *Plasmodium falciparum* infection in children is associated with increased auto-antibody production, high IL-10 plasma levels and antibodies to merozoite surface protein 3. *Malaria J* 14, 162 (2015)
70. C. T. Daniel-Ribeiro, G. Zanini: Autoimmunity and malaria: what are they doing together? *Acta Trop* 76(3), 205-221 (2000)
71. L. R. Gomes, Y. C. Martins, M. F. Ferreira-da-Cruz, C. T. Daniel-Ribeiro: Autoimmunity, phospholipid-reacting antibodies and malaria immunity. *Lupus* 23(12), 1295-1298 (2014)
DOI: 10.1177/0961203314546021
PMid:25228731
72. V. Gerke, S. E. Moss: Annexins: from structure to function. *Physiol Rev* 82(2), 331-371 (2002)
DOI: 10.1152/physrev.00030.2001
PMid:11917092
73. A. Chander, T. Gerelsaikhan, P. K. Vasa, K. Holbrook: Annexin A7 trafficking to alveolar type II cell surface: Possible roles for protein insertion into membranes and lamellar body secretion. *BBA-Mol Cell Res* 1833(5), 1244-1255 (2013)
DOI: 10.1016/j.bbamcr.2013.02.006
74. F. N. Gavins, S. Yona, A. M. Kamal, R. J. Flower, M. Perretti: Leukocyte antiadhesive actions of annexin 1: ALXR- and FPR-related anti-inflammatory mechanisms. *Blood* 101(10), 4140-4147 (2003)
DOI: 10.1182/blood-2002-11-3411
PMid:12560218
75. V. Gerke, C. E. Creutz, S. E. Moss: Annexins: linking Ca2+ signalling to membrane dynamics. *Nat Rev Mol Cell Biol* 6(6), 449-461 (2005)
DOI: 10.1038/nrm1661
PMid:15928709
76. P. K. Kodavali, M. Dudkiewicz, S. Pikula, K. Pawlowski: Bioinformatics analysis of bacterial annexins - putative ancestral relatives of eukaryotic annexins. *Plos One* 9(1) (2014)
77. R. Huber, J. Romisch, E. P. Paques: The crystal and molecular-structure of human annexin-V, an anticoagulant protein that binds to calcium and membranes. *Embo J* 9(12), 3867-3874 (1990)
78. M. A. Swairjo, B. A. Seaton: Annexin structure and membrane interactions: a molecular perspective. *Annu Rev Biophys Biomol Struct* 23, 193-213 (1994)
DOI: 10.1146/annurev.bb.23.060194.001205
PMid:7522665
79. J. I. Kourie, H. B. Wood: Biophysical and molecular properties of annexin-formed channels. *Prog Biophys Mol Biol* 73(2-4), 91-134 (2000)
80. D. R. Patel, J. M. Isas, A. S. Ladokhin, C. C. Jao, Y. E. Kim, T. Kirsch, R. Langen, H.

T. Haigler: The conserved core domains of annexins A1, A2, A5, and B12 can be divided into two groups with different Ca2+-dependent membrane-binding properties. *Biochemistry* 44(8), 2833-2844 (2005)
DOI: 10.1021/bi047642+
PMid:15723527

81. M. A. Lizarbe, J. I. Barrasa, N. Olmo, F. Gavilanes, J. Turnay: Annexin-phospholipid interactions. Functional implications. *Int J Mol Sci* 14(2), 2652-2683 (2013)
DOI: 10.3390/ijms14022652
PMid:23358253 PMCid:PMC3588008

82. V. Goebeler, D. Ruhe, V. Gerke, U. Rescher: Annexin A8 displays unique phospholipid and F-actin binding properties. *FEBS Lett* 580(10), 2430-2434 (2006)
DOI: 10.1016/j.febslet.2006.03.076

83. K. Morozova, S. Sridhar, V. Zolla, C. C. Clement, B. Scharf, Z. Verzani, A. Diaz, J. N. Larocca, K. A. Hajjar, A. M. Cuervo, L. Santambrogio: Annexin A2 promotes phagophore assembly by enhancing Atg16L(+) vesicle biogenesis and homotypic fusion. *Nat Commun* 6, 5856 (2015)

84. M. J. Hayes, D. M. Shao, A. Grieve, T. Levine, M. Bailly, S. E. Moss: Annexin A2 at the interface between F-actin and membranes enriched in phosphatidylinositol 4,5,-bisphosphate. *BBA-Mol Cell Res* 1793(6), 1086-1095 (2009)
DOI: 10.1016/j.bbamcr.2008.10.007

85. J. H. Rand, X. X. Wu, A. S. Quinn, A. W. Ashton, P. P. Chen, J. J. Hathcock, H. A. Andree, D. J. Taatjes: Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: evidence for a novel effect for an old antimalarial drug. *Blood* 115(11), 2292-2299 (2010)
DOI: 10.1182/blood-2009-04-213520
PMid:19965621 PMCid:PMC2844013

86. C. R. Albert, W. J. Schlesinger, C. A. Viall, M. J. Mulla, J. J. Brosens, L. W. Chamley, V. M. Abrahams: Effect of hydroxychloroquine on antiphospholipid antibody-induced changes in first trimester trophoblast function. *Am J Reprod Immunol* 71(2), 154-164 (2014)
DOI: 10.1111/aji.12184
PMid:24325143

87. J. H. Rand, X. X. Wu, A. S. Quinn, P. P. Chen, J. J. Hathcock, D. J. Taatjes: Hydroxychloroquine directly reduces the binding of antiphospholipid antibody-beta2-glycoprotein I complexes to phospholipid bilayers. *Blood* 112(5), 1687-1695 (2008)
DOI: 10.1182/blood-2008-03-144204
PMid:18577708 PMCid:PMC2518879

88. A. Kolyada, I. Karageorgos, P. Mahlawat, N. Beglova: An A1-A1 mutant with improved binding and inhibition of beta2GPI/antibody complexes in antiphospholipid syndrome. *FEBS J* 282(5), 864-873 (2015)
DOI: 10.1111/febs.13185
PMid:25546421

89. R. G. Espinola, S. S. Pierangeli, A. E. Gharavi, E. N. Harris: Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies. *Thromb Haemost* 87(3), 518-522 (2002)

90. C. B. Chighizola, T. Ubiali, P. L. Meroni: Treatment of thrombotic antiphospholipid syndrome: the rationale of current management-an insight into future approaches. *J Immunol Res* 2015, 951424 (2015)

91. M. Perretti: Endogenous mediators that inhibit the leukocyte-endothelium interaction. *Trends Pharmacol Sci* 18(11), 418-425 (1997)
DOI: 10.1016/S0165-6147(97)01116-4

92. E. Solito, A. Kamal, F. Russo-Marie, J. C. Buckingham, S. Marullo, M. Perretti: A novel calcium-dependent proapoptotic effect of annexin 1 on human neutrophils. *FASEB J* 17(11), 1544-1546 (2003)
DOI: 10.1096/fj.02-0941fje

93. E. F. Morand, C. M. Jefferiss, J. Dixey, D. Mitra, N. J. Goulding: Impaired glucocorticoid induction of mononuclear leukocyte lipocortin-1 in rheumatoid arthritis. *Arthritis Rheum* 37(2), 207-211 (1994)
DOI: 10.1002/art.1780370209
PMid:8129776

94. N. J. Goulding, J. Dixey, E. F. Morand, R. A. Dodds, L. S. Wilkinson, A. A. Pitsillides, J. C. Edwards: Differential distribution of annexins-I, -II, -IV, and -VI in synovium. *Ann Rheum Dis* 54(10), 841-845 (1995)
DOI: 10.1136/ard.54.10.841
PMid:7492225 PMCid:PMC1010021

95. Y. H. Yang, W. Song, J. A. Deane, W. Kao, J. D. Ooi, D. Ngo, A. R. Kitching, E. F. Morand, M. J. Hickey: Deficiency of annexin

A1 in CD4+ T cells exacerbates T cell-dependent inflammation. *J Immunol* 190(3), 997-1007 (2013)
DOI: 10.4049/jimmunol.1202236
PMid:23267026

96. H. Weyd, L. Abeler-Dorner, B. Linke, A. Mahr, V. Jahndel, S. Pfrang, M. Schnolzer, C. S. Falk, P. H. Krammer: Annexin A1 on the surface of early apoptotic cells suppresses CD8+ T cell immunity. *PLoS One*, 8(4), e62449 (2013)
DOI: 10.1371/journal.pone.0062449
PMid:23638088 PMCid:PMC3640057

97. Y. Hefner, A. G. Borsch-Haubold, M. Murakami, J. I. Wilde, S. Pasquet, D. Schieltz, F. Ghomashchi, J. R. Yates, 3rd, C. G. Armstrong, A. Paterson, P. Cohen, R. Fukunaga, T. Hunter, I. Kudo, S. P. Watson, M. H. Gelb: Serine 727 phosphorylation and activation of cytosolic phospholipase A2 by MNK1-related protein kinases. *J Biol Chem* 275(48), 37542-37551 (2000)
DOI: 10.1074/jbc.M003395200
PMid:10978317

98. R. Mukherjee, L. Jow, D. Noonan, D. P. McDonnell: Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J Steroid Biochem Mol Biol* 51(3-4), 157-166 (1994)

99. S. W. Kim, H. J. Rhee, J. Ko, Y. J. Kim, H. G. Kim, J. M. Yang, E. C. Choi, D. S. Na: Inhibition of cytosolic phospholipase A2 by annexin I. Specific interaction model and mapping of the interaction site. *J Biol Chem* 276(19), 15712-15719 (2001)
DOI: 10.1074/jbc.M009905200
PMid:11278580

100. K. M. Kim, D. K. Kim, Y. M. Park, C. K. Kim, D. S. Na: Annexin-I inhibits phospholipase A2 by specific interaction, not by substrate depletion. *FEBS Lett* 343(3), 251-255 (1994)
DOI: 10.1016/0014-5793(94)80566-0

101. E. Solito, C. de Coupade, S. Canaider, N. J. Goulding, M. Perretti: Transfection of annexin 1 in monocytic cells produces a high degree of spontaneous and stimulated apoptosis associated with caspase-3 activation. *Br J Pharmacol* 133(2), 217-228 (2001)
DOI: 10.1038/sj.bjp.0704054
PMid:11350857 PMCid:PMC1572776

102. J. E. Burke, E. A. Dennis: Phospholipase A(2) structure/function, mechanism, and signaling. *J Lipid Res* 50(Suppl), S237–S242 (2009)

103. H. M. Creed-Kanashiro, R. M. Bartolini, M. N. Fukumoto, T. G. Uribe, R. C. Robert, M. E. Bentley: Formative research to develop a nutrition education intervention to improve dietary iron intake among women and adolescent girls through community kitchens in Lima, Peru. *J Nutr* 133(11 Suppl 2), 3987S-3991S (2003)

104. A. Wadood, S. A. Ali, R. Sattar, M. A. Lodhi, Z. Ul-Haq: A novel pharmacophore model to identify leads for simultaneous inhibition of anti-coagulation and anti-inflammatory activities of snake venom phospholipase A(2). *Chem Biol Drug Des* 79(4), 431-441 (2012)
DOI: 10.1111/j.1747-0285.2011.01281.x
PMid:22136624

105. D. Fenard, G. Lambeau, T. Maurin, J. C. Lefebvre, A. Doglio: A peptide derived from bee venom-secreted phospholipase A2 inhibits replication of T-cell tropic HIV-1 strains via interaction with the CXCR4 chemokine receptor. *Mol Pharmacol* 60(2), 341-347 (2001)

106. V. D. Muller, R. O. Soares, N. N. dos Santos, Jr., A. C. Trabuco, A. C. Cintra, L. T. Figueiredo, A. Caliri, S. V. Sampaio, V. H. Aquino: Phospholipase A2 isolated from the venom of *Crotalus durissus terrificus* inactivates dengue virus and other enveloped viruses by disrupting the viral envelope. *PLoS One* 9(11), e112351 (2014)

107. R. Zouari-Kessentini, N. Srairi-Abid, A. Bazaa, M. El Ayeb, J. Luis, N. Marrakchi: Antitumoral potential of Tunisian snake venoms secreted phospholipases A2. *Biomed Res Int* 2013, 391389 (2013)

108. S. Sudarshan, B. L. Dhananjaya: Antibacterial potential of a basic phospholipase A2 (VRV-PL-V) of *Daboia russellii pulchella* (Russell's Viper) venom. *Biochemistry (Mosc)* 79(11), 1237-1244 (2014)
DOI: 10.1134/S000629791411011X
PMid:25540009

109. R. Perumal Samy, P. Gopalakrishnakone, M. M. Thwin, T. K. Chow, H. Bow, E. H. Yap and T. W. Thong: Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes.

J Appl Microbiol 102(3), 650-659 (2007)
DOI: 10.1111/j.1365-2672.2006.03161.x
PMid:17309613

110. R. Perumal Samy, P. Gopalakrishnakone, V. T. Chow: Therapeutic application of natural inhibitors against snake venom phospholipase A(2). *Bioinformation* 8(1), 48-57. (2012)

111. R. P. Samy, M. Kandasamy, P. Gopalakrishnakone, B. G. Stiles, E. G. Rowan, D. Becker, M. K. Shanmugam, G. Sethi, V. T. Chow: Wound healing activity and mechanisms of action of an antibacterial protein from the venom of the eastern diamondback rattlesnake (*Crotalus adamanteus*). *PLoS One* 9(2), e80199 (2014)

112. A. Wadood, Z. U. Ulhaq: In silico identification of novel inhibitors against *Plasmodium falciparum* dihydroorate dehydrogenase. *J Mol Graph Model* 40, 40-47 (2013)
DOI: 10.1016/j.jmgm.2012.11.010
PMid:23353582

113. C. Guillaume, C. Deregnaucourt, V. Clavey, J. Schrevel: Anti-*Plasmodium* properties of group IA, IB, IIA and III secreted phospholipases A2 are serum-dependent. *Toxicon* 43(3), 311-318 (2004)
DOI: 10.1016/j.toxicon.2004.01.006
PMid:15033330

114. C. Guillaume, C. Calzada, M. Lagarde, J. Schrevel, C. Deregnaucourt: Interplay between lipoproteins and bee venom phospholipase A2 in relation to their anti-*Plasmodium* toxicity. J Lipid Res 47(7),1493-1506 (2006)
DOI: 10.1194/jlr.M600111-JLR200
PMid:16607035

115. J. C. Quintana, A. M. Chacon, L. Vargas, C. Segura, J. M. Gutierrez, J. C. Alarcon: Antiplasmodial effect of the venom of *Crotalus durissus cumanensis*, crotoxin complex and Crotoxin B. *Acta Trop* 124(2), 126-132 (2012)
DOI: 10.1016/j.actatropica.2012.07.003
PMid:22884508

116. J. C. Castillo, L. J. Vargas, C. Segura, J. M. Gutierrez, J. C. Perez: *In vitro* antiplasmodial activity of phospholipases A2 and a phospholipase homologue isolated from the venom of the snake *Bothrops asper*. *Toxins (Basel)* 4(12), 1500-1516 (2012)
DOI: 10.3390/toxins4121500
PMid:23242318 PMCid:PMC3528259

117. R. K. Bhatnagar, N. Arora, S. Sachidanand, M. Shahabuddin, D. Keister, V. S. Chauhan: Synthetic propeptide inhibits mosquito midgut chitinase and blocks sporogonic development of malaria parasite. *Biochem Biophys Res Commun* 304(4), 783-787 (2003)
DOI: 10.1016/S0006-291X(03)00682-X

118. L. A. Moreira, J. Ito, A. Ghosh, M. Devenport, H. Zieler, E. G. Abraham, A. Crisanti, T. Nolan, F. Catteruccia, M. Jacobs-Lorena: Bee venom phospholipase inhibits malaria parasite development in transgenic mosquitoes. *J Biol Chem* 277(43), 40839-40843 (2002)
DOI: 10.1074/jbc.M206647200
PMid:12167627

119. F. G. Rodrigues, M. N. Santos, T. X. de Carvalho, B. C. Rocha, M. A. Riehle, P. F. Pimenta, E. G. Abraham, M. Jacobs-Lorena, C. F. Alves de Brito, L. A. Moreira: Expression of a mutated phospholipase A2 in transgenic *Aedes fluviatilis* mosquitoes impacts Plasmodium gallinaceum development. *Insect Mol Biol* 17(2), 175-183 (2008)
DOI: 10.1111/j.1365-2583.2008.00791.x
PMid:18353106 PMCid:PMC4137777

120. R. C. Smith, C. Kizito, J. L. Rasgon, M. Jacobs-Lorena: Transgenic mosquitoes expressing a phospholipase A(2) gene have a fitness advantage when fed *Plasmodium falciparum*-infected blood. *Plos One* 8(10), e76097 (2013)

121. D. Paton, A. Underhill, J. Meredith, P. Eggleston, F. Tripet: Contrasted fitness costs of docking and antibacterial constructs in the EE and EVida3 strains validates two-phase genetic transformation system. *PLoS One* 8(6), e67364 (2013)

122. S. Kanodia, G. Kumar, L. Rizzi, A. Pedretti, A. N. Hodder, S. Romeo, P. Malhotra: Synthetic peptides derived from the C-terminal 6kDa region of *Plasmodium falciparum* SERA5 inhibit the enzyme activity and malaria parasite development. *Biochim Biophys Acta* 1840(9), 2765-2775 (2014)
DOI: 10.1016/j.bbagen.2014.04.013
PMid:24769454

123. C. Gelhaus, T. Jacobs, J. Andra, M. Leippe: The antimicrobial peptide NK-2, the core region of mammalian NK-lysin, kills intraerythrocytic *Plasmodium falciparum*. *Antimicrob Agents Chemother* 52(5), 1713-1720 (2008)
DOI: 10.1128/AAC.01342-07

PMid:18332165 PMCid:PMC2346629

124. L. Efron, A. Dagan, L. Gaidukov, H. Ginsburg, A. Mor: Direct interaction of dermaseptin S4 aminoheptanoyl derivative with intraerythrocytic malaria parasite leading to increased specific antiparasitic activity in culture. *J Biol Chem* 277(27), 24067-24072 (2002)
DOI: 10.1074/jbc.M202089200
PMid:11937508

125. A. J. Mason, W. Moussaoui, T. Abdelrahman, A. Boukhari, P. Bertani, A. Marquette, P. Shooshtarizaheh, G. Moulay, N. Boehm, B. Guerold, R. J. Sawers, A. Kichler, M. H. Metz-Boutigue, E. Candolfi, G. Prevost, B. Bechinger: Structural determinants of antimicrobial and antiplasmodial activity and selectivity in histidine-rich amphipathic cationic peptides. *J Biol Chem* 284(1), 119-133 (2009)
DOI: 10.1074/jbc.M806201200
PMid:18984589

126. M. Krugliak, R. Feder, V. Y. Zolotarev, L. Gaidukov, A. Dagan, H. Ginsburg, A. Mor: Antimalarial activities of dermaseptin S4 derivatives. *Antimicrob Agents Chemother* 44(9), 2442-2451 (2000)
DOI: 10.1128/AAC.44.9.2442-2451.2000
PMid:10952593 PMCid:PMC90083

127. N. K. Kaushik, J. Sharma, D. Sahal: Anti-plasmodial action of de novo-designed, cationic, lysine-branched, amphipathic, helical peptides. *Malar J* 11, 256 (2012)

128. L. Perez-Picaso, B. Velasco-Bejarano, A. B. Aguilar-Guadarrama, R. Argotte-Ramos, M. Y. Rios: Antimalarial activity of ultra-short peptides. *Molecules* 14(12), 5103-5114 (2009)
DOI: 10.3390/molecules14125103
PMid:20032878

129. R. B. G. Arrighi, C. Ebikeme, Y. Jiang, L. Ranford-Cartwright, M. P. Barrett, U. Langel, I. Faye: Cell-penetrating peptide TP10 shows broad-spectrum activity against both *Plasmodium falciparum and Trypanosoma brucei brucei. Antimicrob Agents Chemother* 52(9), 3414-3417 (2008)
DOI: 10.1128/AAC.01450-07
PMid:18519720 PMCid:PMC2533502

130. R. Conde, F. Z. Zamudio, M. H. Rodriguez, L. D. Possani: Scorpine, an anti-malaria and anti-bacterial agent purified from scorpion venom. *FEBS Lett* 471(2-3), 165-168 (2000)

131. R. Carballar-Lejarazu, M. H. Rodriguez, F. de la Cruz Hernandez-Hernandez, J. Ramos-Castaneda, L. D. Possani, M. Zurita-Ortega, E. Reynaud-Garza, R. Hernandez-Rivas, T. Loukeris, G. Lycett, H. Lanz-Mendoza: Recombinant scorpine: a multifunctional antimicrobial peptide with activity against different pathogens. *Cell Mol Life Sci* 65(19), 3081-3092 (2008)
DOI: 10.1007/s00018-008-8250-8
PMid:18726072

132. M. Wieczorek, H. Jenssen, J. Kindrachuk, W. R. Scott, M. Elliott, K. Hilpert, J. T. Cheng, R. E. Hancock, S. K. Straus: Structural studies of a peptide with immune modulating and direct antimicrobial activity. *Chem Biol* 17(9), 970-980 (2010)
DOI: 10.1016/j.chembiol.2010.07.007
PMid:20851346

133. J. Vizioli, P. Bulet, M. Charlet, C. Lowenberger, C. Blass, H. M. Muller, G. Dimopoulos, J. Hoffmann, F. C. Kafatos, A. Richman: Cloning and analysis of a cecropin gene from the malaria vector mosquito, *Anopheles gambiae*. *Insect Mol Biol* 9(1), 75-84 (2000)
DOI: 10.1046/j.1365-2583.2000.00164.x
PMid:10672074

134. C. Tian, B. Gao, C. Rodriguez Mdel, H. Lanz-Mendoza, B. Ma, S. Zhu: Gene expression, antiparasitic activity, and functional evolution of the drosomycin family. *Mol Immunol* 45(15), 3909-3916 (2008)
DOI: 10.1016/j.molimm.2008.06.025
PMid:18657321

135. V. Kokoza, A. Ahmed, S. Woon Shin, N. Okafor, Z. Zou, A. S. Raikhel: Blocking of *Plasmodium* transmission by cooperative action of cecropin A and defensin A in transgenic *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci USA* 107(18), 8111-8116 (2010)
DOI: 10.1073/pnas.1003056107
PMid:20385844 PMCid:PMC2889521

136. J. L. Fox: Antimicrobial peptides stage a comeback. *Nat Biotechnol* 31(5), 379-382 (2013)
DOI: 10.1038/nbt.2572
PMid:23657384

137. A. H. Achtman, S. Pilat, C. W. Law, D. J. Lynn, L. Janot, M. L. Mayer, S. Ma, J. Kindrachuk, B. B. Finlay, F. S. Brinkman, G. K. Smyth,

R. E. Hancock, L. Schofield: Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria. *Sci Transl Med* 4(135), 135ra64 (2012)

138. C. G. Jackson, P. E. Linnett, R. B. Beechey, P. J. Henderson: Purification and preliminary structural analysis of the efrapeptins, a group of antibiotics that inhibit the mitochondrial adenosine triphosphatase. *Biochem Soc Trans* 7(1), 224-226 (1979)
DOI: 10.1042/bst0070224
PMid:155542

139. J. P. Abrahams, S. K. Buchanan, M. J. Van Raaij, I. M. Fearnley, A. G. Leslie, J. E. Walker: The structure of bovine F1-ATPase complexed with the peptide antibiotic efrapeptin. *Proc Natl Acad Sci USA* 93(18), 9420-9424 (1996)
DOI: 10.1073/pnas.93.18.9420
PMid:8790345 PMCid:PMC38443

140. G. Nagaraj, M. V. Uma, M. S. Shivayogi, H. Balaram: Antimalarial activities of peptide antibiotics isolated from fungi. *Antimicrob Agents Chemother* 45 (1), 145-149 (2001)
DOI: 10.1128/AAC.45.1.145-149.2001
PMid:11120957 PMCid:PMC90252

141. K. Kaur, M. Jain, T. Kaur, R. Jain: Antimalarials from nature. *Bioorg Med Chem* 17(9), 3229-3256 (2009)
DOI: 10.1016/j.bmc.2009.02.050
PMid:19299148

142. M. Sathe, D. Thavaselvam, A. K. Srivastava, M. P. Kaushik: Synthesis and antimalarial evaluation of cyclic beta-amino acid-containing dipeptides. *Molecules* 13(2), 432-443 (2008)
DOI: 10.3390/molecules13020432
PMid:18305429

143. P. T. Meinke, P. Liberator: Histone deacetylase: a target for antiproliferative and antiprotozoal agents. *Curr Med Chem* 8(2), 211-235 (2001)
DOI: 10.2174/0929867013373787
PMid:11172676

144. J. Thongtan, J. Saenboonrueng, P. Rachtawee, M. Isaka: An antimalarial tetrapeptide from the entomopathogenic fungus *Hirsutella sp*. BCC 1528. *J Nat Prod* 69(4), 713-714 (2006)
DOI: 10.1021/np050549h
PMid:16643062

145. T. I. Oprea, H. Matter: Integrating virtual screening in lead discovery. *Curr Opin Chem Biol* 8(4), 349-358 (2004)
DOI: 10.1016/j.cbpa.2004.06.008
PMid:15288243

146. M. Graupner, B. Gutkin: Modeling nicotinic neuromodulation from global functional and network levels to nAChR based mechanisms. *Acta Pharmacol Sin* 30(6), 681-693 (2009)
DOI: 10.1038/aps.2009.87
PMid:19498415 PMCid:PMC4002372

147. H. R. Noori, W. Jager: Neurochemical oscillations in the basal ganglia. *Bulletin of Mathematical Biology* 72(1), 133-147 (2010)
DOI: 10.1007/s11538-009-9441-7
PMid:19588207

148. H. R. Noori: The effects of the acute administration of low-dosage ethanol on the phasic neurochemical oscillations of the basal ganglia. *Math Med Biol* 29(3), 231-244 (2012)
DOI: 10.1093/imammb/dqr006
PMid:21543550

149. T. A. de Beer, G. A. Wells, P. B. Burger, F. Joubert, E. Marechal, L. Birkholtz, A. I. Louw: Antimalarial drug discovery: in silico structural biology and rational drug design. *Infect Disord Drug Targets* 9(3), 304-318 (2009)
DOI: 10.2174/1871526510909030304
PMid:19519484

150. S. Kaczanowski, P. Zielenkiewicz: Why similar protein sequences encode similar three-dimensional structures? *Theor Chem Acc* 125(3-6), 643-650 (2010)

151. X. Y. Meng, H. X. Zhang, M. Mezei, M. Cui: Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des* 7(2), 146-157 (2011)
DOI: 10.2174/157340911795677602
PMid:21534921 PMCid:PMC3151162

152. P. Labrie, S. P. Maddaford, S. Fortin, S. Rakhit, L. P. Kotra, R. C. Gaudreault: A comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) of anthranilamide derivatives that are multidrug resistance modulators. *J Med Chem* 49(26), 7646-7660 (2006)
DOI: 10.1021/jm060239b
PMid:17181148

153. E. F. Meyer, S. M. Swanson, J. A. Williams: Molecular modelling and drug design. *Pharmacol Ther* 85(3), 113-121 (2000)
DOI: 10.1016/S0163-7258(99)00069-8

154. S. Ekins, J. Mestres, B. Testa: In silico pharmacology for drug discovery: methods

for virtual ligand screening and profiling. *Br J Pharmacol* 152(1), 9-20 (2007)
DOI: 10.1038/sj.bjp.0707305
PMid:17549047 PMCid:PMC1978274

155. H. Gmuender: Perspectives and challenges for DNA microarrays in drug discovery and development. *Biotechniques* 32(1), 152-154, 156??, 158?? (2002)
156. M. J. Yu: Simulating the drug discovery pipeline: a Monte Carlo approach. *J Cheminform* 4(1), 32 (2012)
157. M. E. Tuckerman, G. J. Martyna: Understanding modern molecular dynamics: Techniques and applications. *J Phys Chem B* 104(2), 159-178 (2000)
DOI: 10.1021/jp992433y
158. Y. Marrero-Ponce, M. Iyarreta-Veitia, A. Montero-Torres, C. Romero-Zaldivar, C. A. Brandt, P. E. Avila, K. Kirchgatter, Y. Machado: Ligand-based virtual screening and in silico design of new antimalarial compounds using nonstochastic and stochastic total and atom-type quadratic maps. *J Chem Inf Mode* 45(4), 1082-1100 (2005)
DOI: 10.1021/ci050085t
PMid:16045304
159. G. D. Geromichalos: Importance of molecular computer modeling in anticancer drug development. *J BUON* 12(Suppl 1), S101-118 (2007)
160. C. Suh, K. Rajan: Virtual screening and QSAR formulations for crystal chemistry. *QSAR Comb Sci* 24(1), 114-119 (2005)
DOI: 10.1002/qsar.200420057
161. H. R. Noori, R. Spanagel: In silico pharmacology: drug design and discovery's gate to the future. *In Silico Pharmacol* 1, 1 (2013)
162. D. A. Fidock: Drug discovery: Priming the antimalarial pipeline. *Nature* 465(7296), 297-298 (2010)
DOI: 10.1038/465297a
PMid:20485420
163. F. J. Gamo, L. M. Sanz, J. Vidal, C. de Cozar, E. Alvarez, J. L. Lavandera, D. E. Vanderwall, D. V. Green, V. Kumar, S. Hasan, J. R. Brown, C. E. Peishoff, L. R. Cardon, J. F. Garcia-Bustos: Thousands of chemical starting points for antimalarial lead identification. *Nature* 465(7296), 305-310 (2010)
DOI: 10.1038/nature09107
PMid:20485427
164. W. A. Guiguemde, A. A. Shelat, D. Bouck, S. Duffy, G. J. Crowther, P. H. Davis, D. C. Smithson, M. Connelly, J. Clark, F. Zhu, M. B. Jimenez-Diaz, M. S. Martinez, E. B. Wilson, A. K. Tripathi, J. Gut, E. R. Sharlow, I. Bathurst, F. El Mazouni, J. W. Fowble, I. Forquer, P. L. McGinley, S. Castro, I. Angulo-Barturen, S. Ferrer, P. J. Rosenthal, J. L. Derisi, D. J. Sullivan, J. S. Lazo, D. S. Roos, M. K. Riscoe, M. A. Phillips, P. K. Rathod, W. C. Van Voorhis, V. M. Avery, R. K. Guy: Chemical genetics of *Plasmodium falciparum*. *Nature* 465(7296), 311-315 (2010)
DOI: 10.1038/nature09099
PMid:20485428 PMCid:PMC2874979
165. E. Lucumi, C. Darling, H. Jo, A. D. Napper, R. Chandramohanadas, N. Fisher, A. E. Shone, H. Y. Jing, S. A. Ward, G. A. Biagini, W. F. DeGrado, S. L. Diamond, D. C. Greenbaum: Discovery of potent small-molecule inhibitors of multidrug-resistant *Plasmodium falciparum* using a novel miniaturized high-throughput luciferase-based assay. *Antimicrob Agents Chemother* 54(9), 3597-3604 (2010)
DOI: 10.1128/AAC.00431-10
PMid:20547797 PMCid:PMC2934977
166. A. Mongui, F. J. Perez-Llanos, M. M. Yamamoto, M. Lozano, M. M. Zambrano, P. Del Portillo, C. Fernandez-Becerra, S. Restrepo, H. A. Del Portillo, H. Junca: Development of a genetic tool for functional screening of anti-malarial bioactive extracts in metagenomic libraries. *Malar J* 14, 233 (2015)
167. G. Siciliano, P. Alano: Enlightening the malaria parasite life cycle: bioluminescent *Plasmodium* in fundamental and applied research. *Front Microbiol* 6, 391 (2015)
168. M. Amiche, C. Galanth: Dermaseptins as models for the elucidation of membrane-acting helical amphipathic antimicrobial peptides. *Curr Pharm Biotechno* 12(8), 1184-1193 (2011)
DOI: 10.2174/138920111796117319
169. S. Fletcher, V. M. Avery: A novel approach for the discovery of chemically diverse anti-malarial compounds targeting the *Plasmodium falciparum* coenzyme A synthesis pathway. *Malaria J* 13, 343 (2014)
170. P. Vlieghe, V. Lisowski, J. Martinez, M. Khrestchatisky: Synthetic therapeutic

peptides: science and market. *Drug Discov Today* 15(1-2), 40-56 (2010)

171. J. Thundimadathil: Cancer treatment using peptides: current therapies and future prospects. *J Amino Acids* 2012, 967347 (2012)
172. L. Gentilucci, R. De Marco, L. Cerisoli: Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. *Curr Pharm Des* 16(28), 3185-3203 (2010)
DOI: 10.2174/138161210793292555
PMid:20687878
173. F. Chen, B. Ma, Z. C. Yang, G. Lin, D. Yang: Extraordinary metabolic stability of peptides containing alpha-aminoxy acids. *Amino Acids* 43(1), 499-503 (2012)
DOI: 10.1007/s00726-011-1095-8
PMid:21964968
174. S. Saha, G. P. Raghava: BTXpred: prediction of bacterial toxins. *In Silico Biol* 7(4-5), 405-412 (2007)
175. S. Saha, G. P. Raghava: Prediction of neurotoxins based on their function and source. *In Silico Biol* 7(4-5), 369-387 (2007)
176. Q. Kaas, J. C. Westermann, R. Halai, C. K. Wang, D. J. Craik: ConoServer, a database for conopeptide sequences and structures. *Bioinformatics* 24(3), 445-446 (2008)
DOI: 10.1093/bioinformatics/btm596
PMid:18065428
177. Q. Y. He, Q. Z. He, X. C. Deng, L. Yao, E. Meng, Z. H. Liu and S. P. Liang: ATDB: a uni-database platform for animal toxins. *Nucleic Acids Res* 36(Database issue), D293-297 (2008)
178. A. Chakraborty, S. Ghosh, G. Chowdhary, U. Maulik, S. Chakrabarti: DBETH: a database of bacterial exotoxins for human. *Nucleic Acids Res* 40(Database issue), D615-620 (2012)
179. D. L. Wood, T. Miljenovic, S. Cai, R. J. Raven, Q. Kaas, P. Escoubas, V. Herzig, D. Wilson, G. F. King: ArachnoServer: a database of protein toxins from spiders. *BMC Genomics* 10, 375 (2009)
180. S. Gupta, P. Kapoor, K. Chaudhary, A. Gautam, R. Kumar, C. Open Source Drug Discovery and G. P. Raghava: In silico approach for predicting toxicity of peptides and proteins. *PLoS One* 8(9), e73957 (2013)
181. C. Teixeira, N. Vale, B. Perez, A. Gomes, J. R. Gomes, P. Gomes: "Recycling" classical drugs for malaria. *Chem Rev* 114(22), 11164-11220 (2014)
DOI: 10.1021/cr500123g
PMid:25329927
182. D. J. Craik, D. P. Fairlie, S. Liras, D. Price: The future of peptide-based drugs. *Chem Biol Drug Des* 81(1), 136-147 (2013)
DOI: 10.1111/cbdd.12055
PMid:23253135
183. T. N. Wells, E. M. Poll: When is enough enough? The need for a robust pipeline of high-quality antimalarials. *Discov Med* 9(48), 389-398 (2012)
184. D. Dutta, M. D. Willcox: Antimicrobial contact lenses and lens cases: a review. *Eye Contact Lens* 40(5), 312-324 (2014)
DOI: 10.1097/ICL.0000000000000056
PMid:25083781
185. F. R. Maia, M. Barbosa, D. B. Gomes, N. Vale, P. Gomes, P. L. Granja, C. C. Barrias: Hydrogel depots for local co-delivery of osteoinductive peptides and mesenchymal stem cells. *J Control Release* 189, 158-168 (2014)
DOI: 10.1016/j.jconrel.2014.06.030
PMid:24979208
186. R. Lax, C. Meenan: Challenges for therapeutic peptides part 1: on the inside, looking out. *Innov Pharm Tech* 42, 54-56 (2012)
187. L. Anthony, P. U. Freda: From somatostatin to octreotide LAR: evolution of a somatostatin analogue. *Curr Med Res Opin* 25(12), 2989-2999 (2009)
DOI: 10.1185/03007990903328959
PMid:19842996 PMCid:PMC3678951
188. D. Bhadra, S. Bhadra, N. K. Jain: PEGylated peptide dendrimeric carriers for the delivery of antimalarial drug chloroquine phosphate. *Pharm Res* 23(3), 623-633 (2006)
DOI: 10.1007/s11095-005-9396-9
PMid:16374532
189. A.A. Kaspar, J.M. Reichert: Future directions for peptide therapeutics. *Drug Discov Today* 18, 807-817 (2013)
DOI: 10.1016/j.drudis.2013.05.011
PMid:23726889
190. K. Fosgerau, T. Hoffmann: Peptide therapeutics: current status and future directions. *Drug Discov Today* 20,

122-128 (2015)
DOI: 10.1016/j.drudis.2014.10.003
PMid:25450771

191. P. W. Latham: Therapeutic peptides revisited. *Nat biotechnol* 17, 755-758 (1999)
DOI: 10.1038/11686
PMid:10429238

192. A. K. Sato, M. Viswanathan, R. B. Kent, C. R. Wood: Therapeutic peptides: technological advances driving peptides into development. *Curr Opin Biotechnol* 17(6), 638-642 (2006)
DOI: 10.1016/j.copbio.2006.10.002
PMid:17049837

193. D. S. Guttery, B. Poulin, A. Ramaprasad, R. J. Wall, D. J. Ferguson, D. Brady, E. M. Patzewitz, S. Whipple, U. Straschil, M. H. Wright, A. M. Mohamed, A. Radhakrishnan, S. T. Arold, E. W. Tate, A. A. Holder, B. Wickstead, A. Pain, R. Tewari: Genome-wide functional analysis of *Plasmodium* protein phosphatases reveals key regulators of parasite development and differentiation. *Cell Host Microbe* 16(1), 128-140 (2014)
DOI: 10.1016/j.chom.2014.05.020
PMid:25011111 PMCid:PMC4094981

194. M. A. Kutuzov, A. V. Andreeva: Protein Ser/Thr phosphatases of parasitic protozoa. *Mol Biochem Parasitol* 161(2), 81-90 (2008)
DOI: 10.1016/j.molbiopara.2008.06.008
PMid:18619495

195. J. M. Wilkes, C. Doerig: The protein-phosphatome of the human malaria parasite *Plasmodium falciparum*. *BMC Genomics* 9, 412 (2008)

196. G. B. Moorhead, V. De Wever, G. Templeton, D. Kerk: Evolution of protein phosphatases in plants and animals. *Biochem J* 417(2), 401-409 (2009)
DOI: 10.1042/BJ20081986
PMid:19099538

197. D. S. Guttery, B. Poulin, D. J. Ferguson, B. Szoor, B. Wickstead, P. L. Carroll, C. Ramakrishnan, D. Brady, E. M. Patzewitz, U. Straschil, L. Solyakov, J. L. Green, R. E. Sinden, A. B. Tobin, A. A. Holder, R. Tewari: A unique protein phosphatase with kelch-like domains (PPKL) in *Plasmodium* modulates ookinete differentiation, motility and invasion. *PLoS Pathog*, 8(9), e1002948 (2012)
DOI: 10.1371/journal.ppat.1002948
PMid:23028336 PMCid:PMC3447748

198. E. M. Patzewitz, D. S. Guttery, B. Poulin, C. Ramakrishnan, D. J. P. Ferguson, R. J. Wall, D. Brady, A. A. Holder, B. Szoor, R. Tewari: An ancient protein phosphatase, SHLP1, is critical to microneme development in *Plasmodium* ookinetes and parasite transmission. *Cell Reports* 3(3), 622-629 (2013)
DOI: 10.1016/j.celrep.2013.01.032
PMid:23434509 PMCid:PMC3617505

199. M. Treeck, J. L. Sanders, J. E. Elias, J. C. Boothroyd: The phosphoproteomes of *Plasmodium falciparum* and *Toxoplasma gondii* reveal unusual adaptations within and beyond the parasites' boundaries. *Cell Host Microbe* 10(4), 410-419 (2011)
DOI: 10.1016/j.chom.2011.09.004
PMid:22018241 PMCid:PMC3254672

200. M. H. Wright, B. Clough, M. D. Rackham, K. Rangachari, J. A. Brannigan, M. Grainger, D. K. Moss, A. R. Bottrill, W. P. Heal, M. Broncel, R. A. Serwa, D. Brady, D. J. Mann, R. J. Leatherbarrow, R. Tewari, A. J. Wilkinson, A. A. Holder, E. W. Tate: Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach. *Nat Chem* 6(2), 112-121 (2014)
DOI: 10.1038/nchem.1830
PMid:24451586 PMCid:PMC4739506

201. C. Doerig, A. Abdi, N. Bland, S. Eschenlauer, D. Dorin-Semblat, C. Fennell, J. Halbert, Z. Holland, M. P. Nivez, J. P. Semblat, A. Sicard, L. Reininger: Malaria: targeting parasite and host cell kinomes. *Biochim Biophys Acta* 1804(3), 604-612 (2010)
DOI: 10.1016/j.bbapap.2009.10.009

202. G. B. Moorhead, L. Trinkle-Mulcahy and A. Ulke-Lemee: Emerging roles of nuclear protein phosphatases. *Nat Rev Mol Cell Biol* 8(3), 234-244 (2007)
DOI: 10.1038/nrm2126
PMid:17318227

203. H. Habeeb, J. R. Ripper, A. Cohen, P. B. Hinfey: A case of imported severe *Plasmodium falciparum* malaria in the emergency department and the current role of exchange transfusion treatment. *J Emerg Med* 44(2), e211-215 (2013)
DOI: 10.1016/j.jemermed.2012.02.051
PMid:22609412

204. M. S. Memon, S. Solangi, S. Lakho, Z. I. Arain, F. Naz, M. Zaki: Morbidity and mortality of

malaria during monsoon flood of 2011: South East Asia experience. *Iran J Public Health* 43(1), 28-34 (2014)

205. G. Pasvol: The treatment of complicated and severe malaria. *Br Med Bull* 75-76, 29-47 (2005)

206. Q. Li, S. Remich, S. R. Miller, B. Ogutu, W. Otieno, V. Melendez, P. Teja-Isavadharm, P. J. Weina, M. R. Hickman, B. Smith, M. Polhemus: Pharmacokinetic evaluation of intravenous artesunate in adults with uncomplicated falciparum malaria in Kenya: a phase II study. *Malar J* 13, 281 (2014)

207. P. C. Mohapatra, A. Sarangi, A. K. Sarangi, R. K. Dalai, D. Sahoo: Sequential serum cytokine levels of TNF-alpha, IL-4 and IL-12 are associated with prognosis in *Plasmodium falciparum* malaria. *Indian J Clin Biochem* 29(3), 321-326 (2014)
DOI: 10.1007/s12291-013-0359-3
PMid: 24966480 PMCid: PMC4062674

Abbreviations: AMPs: antimicrobial peptides; ANX: annexin; CD8: T cluster of differentiation-8; CSA: chondroitin sulphate A; CQ: chloroquine phosphate; HIV: human immunodeficiency virus; IFN-gamma: interferon gamma; IL-17: interleukin-17; IL-6: interleukin-6; TNF-alpha: tumor necrosis factor-alpha; NEA: National Environmental Agency; NIFs: NLI-interacting factor-like phosphatases; DAMPs; damage-associated molecular pattern molecules; NF-kappaB: nuclear factor kappa-light-chain enhancer of activated B cells; PfSERA5: *Plasmodium falciparum* serine repeats antigen-5; PEGL: Polyethylene pegylated poly-l-lysine based peptide dendrimers; PPPs: phosphoprotein phosphatases; PPMs: metallo-dependent protein phosphatases; PTPs: protein tyrosine phosphatases; PKs: protein kinases; PPs: phosphatases; RBC: Red blood cells; RTS,S: trade name of Mosquirix; $sPLA_2$: secretory phospholipase A_2; TCR: T cell receptor; WHO: World Health Organization; PRBC: parasitized red blood cells; Treg: regulatory T cells; PAMP: pathogen-associated molecular patterns.

Key Words: *Plasmodium* strains, Annexin, Secretory phospholipase A_2 ($sPLA_2$), Antimicrobial peptide (AMPs), Review

Send correspondence to: Ramar Perumal Samy, Department of Physiology, NUS Immunology Programme, Centre for Life Sciences (CeLS), Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, 28 Medical Drive, Singapore 117597, Tel: 65-65165903, Fax: 65-6778 2684, E-mail: phsrp@nus.edu.sg