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CHARACTERIZATION AND OPTIMIZATION OF THE ANTIMALARIAL AND DRUG METABOLISM AND PHARMACOKINETIC ACTIVITIES OF BETA-HYDROXY ETHYLAMINE BASED PLASMEPSIN INHIBITORS

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Abstract

First, in malaria-endemic areas, antimalarial drugs were often given to high-risk individuals including pregnant women and children at improperly low doses. The drug's shelf life will be

reduced if resistance spreads more rapidly. As a result of the current method for determining dose, this is a logical fallout. Another approach to dose discovery is to use phase 2 research to first characterize the PK-PD and calibrate in vitro susceptibility data in vivo. It is feasible to accurately quantify the early therapeutic responses by mPlasmepsins, a kind of aspartic protease found in Plasmodium, are critical to the parasite's ability to survive in the host. Plasmeptins in the digestive vacuole break down hemoglobin, providing food for the parasite. Several parasite proteins are processed for export into the erythrocyte by the protease known as Plasmepsin V. For the time being, we don't know how the human body uses the other plasmepsins that have not been identified. There has been a lot of interest in the digestive vacuole-targeting plasmepsin inhibitors throughout the past decade. Due to the need for new antimalarial drugs that may quickly lower parasitemia, precise parasitemia modulation data from in vivo models is required. A new family of antimalarial drugs has been proposed based on the pharmacological properties of hydroxyethyl amines, a group of alkanolamine molecules.

Keywords: Antimalarial Drug Metabolism, Hydroxy-ethylamine, Plasmepsin, Pharmacokinetic, Plasmodium.

Introduction

For thousands of years, malaria has been a major cause of human suffering. The world's poorest countries are at risk from malaria (around 40 percent of the population). Malaria kills an estimated 1.5-2.7 million people each year, infecting between 300 million and 500 million people each year, according to the World Health Organization. P. falciparum is the most common cause of death in instances of malaria [1,2]. This parasite is transmitted via the bite of an infected female Anopheles mosquito, which is responsible for transmitting malaria. P malariae, P falciparum, and P vivax, P ovale are the four types of parasites that infect humans. P falciparum is the most widespread cause of mortality and illness in Africa, accounting for the majority of infections. Because reactivated hypnozoites may cause a clinical relapse month or even years after the initial infection, it is crucial to keep a watch out for these parasites. Reactivated hypnozoites include Malaria is spread by female anopheles' mosquitoes. However, in 2013, there were still more than 177,767 cases in Brazil [3]. WHO recommends artemisinin-based combination therapy for antimalarial treatment because of its efficacy and ability to slow the rate at which resistance develops. Many cases have emerged over the last few years, first along the Cambodia-Thailand border [4] and now throughout Southeast Asia, showing artemisinin derivatives resistance [5]. Antimalarial drug development is essential in this scenario. Antimalarial drug discovery depends on a wide range of methods. This includes finding ways to stop the spread of infection, cut down on recurrences, or treat both mild and severe forms of malaria with one medication. However, the major goal of new treatments will be the rapid decrease of parasitemia. The biological characteristics of the hydroxyethyl amine core have been extensively studied. It's been known for some time now that hydroxyethyl amines serve as HIV protease inhibitors and that research into their antimalarial effects is ongoing. In a study Plasmodium parasite cannot produce their own proteins if they are treated with enzyme inhibitors like hydroxyethyl amines, which are employed in the treatment of malaria. An antimalarial drug

class based on hydroxyethyl amine derivatives might be a promising new alternative for treating malaria, according to preliminary research. Alkylamine-based antimalarial hydroxyethyl amine molecules have recently been found to be improved by the addition of a cyclohexyl group. Antimalarial activity of nine newly synthesized hydroxyethyl amines generated from the ring-opening of (2S,3S)-Boc phenylalanine epoxide in isopropanol was investigated in a mouse in vivo model of infection with green fluorescent protein-expressing P. berghei [6-7].

Malaria

Classification of Malaria [8]

As per the severity of illness, malaria classified into two types:

a. Uncomplicated (Benign) malaria

b. Complicated (Malignant) malaria

P. falciparum is responsible for the vast majority of malaria infections and almost all of the disease's fatalities. Confusion, sleepiness, and acute weakness are all symptoms of falciparum malaria in its most severe forms. Adult respiratory distress syndrome (ARDS) and acute renal failure (ARF) are all possible side effects of falciparum malaria. There is also a chance that falciparum malaria may cause brain malaria and generalized convulsions (ARDS).

Life Cycle of Malaria Parasites

In order for human malarial plasmodia to thrive, they must pass through an extremely complicated life cycle. The mosquito is the location of the parasite's sexual reproduction, while humans are the site of its asexual reproduction. Female Anopheles mosquitoes that feed on human blood secrete the infectious phase of the malaria parasite in their salivary glands. An estimated 15 to 20 sporozoites are transferred from a mosquito's saliva into the blood stream during blood extraction. There are between 10,000 and 30.000 merozoites in each of these tissue schizonts, which penetrate the liver cells and mature into sporozoites. To begin the erythrocytic life cycle of the parasite, one to two weeks after it has ruptured, schizonts release the merozoites. Plasmodium vivax and Plasmodium ovale both produce hypnozoites, which may lay latent in liver cells for months or years after the original infection. This can lead to relapses months or years later.

When treated ineffectively, Plasmodium falciparum and Plasmodium malariae do not survive in the liver for long periods of time. Invading erythrocytes allows merozoites discharged into the bloodstream to re-escape detection by the host's immune system. The parasites progress from the ring stage through the trophozoite stage to the blood schizont stage in the erythrocytes. The erythrocyte bursts, releasing 16-32 new merozoites into the bloodstream, which in turn re-infects the erythrocytes and begins the erythrocytic cycle all over again. In the course of a second blood meal, gametocytes, the sexual form of the merozoites, are transmitted to the mosquito. When the erythrocytes burst, parasite waste and cell debris are released into the bloodstream, generating some of the clinical signs of malaria [7]. These sporozoites travel to the salivary glands, where they are ready for future infection. Frequent fever is the most common symptom but not in the typical tertian (every 48 hr) or quartan patterns. Chills, headache, back and stomach discomfort,

and nausea and vomiting are all possible side effects. Predators of vivax, ovale, and malaria have differential preferences for infected erythrocytes of different ages. As a result, the level of total parasitemia is restricted. All age groups are infected by P. falciparum, resulting to a high parasitemia [9-10].

Development of Drug Resistance

Drug-resistant parasites have made it more difficult to give effective treatment. Chloroquine and Quinine, for example, have become mostly ineffective as a result of this. Malaria cases are expected to increase unless innovative antimalarial treatment approaches are utilized, according to the WHO in response to parasite resistance. Falciparum malaria therapy has become significantly more challenging as a result of the growing problem of medicine resistance. Although chloroquine and sulfadoxine/pyrimethamine were once assumed to be effective against most infections, this is no longer the case, prompting the exploration of other regimens. As well as looking at medication resistance, other variables like expired antimalarials or patient compliance should also be taken into consideration. Continuing a failing treatment strategy after the emergence of drug resistance leads to an increase in malaria transmission, an increase in epidemics, and a decrease in public confidence in malaria control efforts, rather than a cure [11].

Over the preceding generation, the falciparum parasite has evolved resistance to a broad range of routinely used antimalarial medications. As a consequence of the selection of drug-resistant mutants that emerge spontaneously during evolution, drug resistance is formed. Parasites which survive after a drug-sensitive parasite population has been eradicated will be considered drug-insensitive. Point mutations that affect drug accumulation or efflux in the erythrocyte or decrease the affinity of the medication to the target molecule are the most common causes of antimalarial drug resistance. One patient's treatment failure is one manifestation of antimalarial drug resistance; nevertheless, the majority of instances see an increase in the apparent number of cases of malaria. A single infection that is inhibited by insufficient medication, observed by a parasite comeback in the sick individual's body, is the source of the many infections. Malaria cases are on the rise, even in previously well-controlled areas, and outbreaks are popping up in formerly low-risk areas of transmission, all of which point to this being the case. All that is needed to disseminate medication resistance geographically is the movement of ill people to new areas with Anopheles vectors that are capable. Multidrug-resistant Plasmodium falciparum geographic distribution maps must be generated at a slower pace than the parasite's movement and should not be considered as an indication of lack of drug resistance, according to researchers. Multiple drug resistance has definitely reached in India, and it is especially widespread in the northeastern parts of the nation. As soon as a previously treated patient begins to relapse with their illness, the first indicators of medication resistance are visible. Trials with follow-up periods less than four weeks will almost certainly provide erroneous and perhaps misleading results when used to determine drug resistance for public health purposes [12-15].

- **Chloroquine-Resistance:** Because it was the sole malaria medication that worked against both falciparum and vivax parasites for about a decade after its introduction, chloroquine was the medicine of choice for many malaria patients. For vivax malaria in most regions, chloroquine is still a great medication. Chloroquine, on the other hand, is ineffective in the treatment of falciparum malaria. The growth of chloroquine resistance has led to a false feeling of security among physicians, which must now be removed. When used alone, chloroquine is not thought to be an effective therapy for falciparum malaria because of its anti-inflammatory effects [16-17].

- **Antifolate-Resistance:** The parasite's capacity to synthesize tetrahydrofolate, which hinders the creation of nucleic acids, is inhibited by antifolate drugs. In the therapy of lupus, sequential blockades of the manufacturing pathway using both pyrimethamine and sulfadoxine have been shown to be efficacious. Due to their ability to quickly accumulate many genetic alterations, falciparum parasites have become resistant to antimalarial medications. Sulfadoxine/pyrimethamine (SP) was widely accessible in Thailand in the 1970s, but it quickly failed as the primary therapy for tuberculosis. Sulfadoxine/pyrimethamine therapy for uncomplicated falciparum infections is typically ineffective because of the fast expansion of these resistance genes in Asia and Africa. As a consequence, a medication's capacity to be used in conjunction with a more effective treatment is substantially limited if it fails to treat a significant number of diseases [18].

- **Mefloquine-Resistance:** Mefloquine-resistant parasites may have existed previous to the drug's introduction, based on historical data. Many drug-resistant falciparum parasites were selected due to the widespread use of mefloquine, which had a direct impact on the spread of the illness. Long-acting drugs like mefloquine or sulfadoxine/pyrimethamine, notwithstanding the case of single-dose administration, carry the seeds of their own demise inside their own structure. It is possible for parasites to come into touch with sub-inhibitory levels of mefloquine in persons who were treated weeks or months ago because of its prolonged elimination half-life. To put it another way: The selection and development of mefloquine resistance will only take place if mefloquine is administered extensively without the use of another effective treatment [19].

- **Atovaquone-Resistance:** Unlike other antimalarial drugs, atovaquone disrupts the parasite's cytochrome electron transport mechanism, making it less effective. Even within a single patient, a single nucleotide mutation in the cytochrome b gene may lead to very high levels of medication resistance. Atovaquone can only be used in conjunction with Proguanil; it is not accessible on its own. In uncomplicated falciparum patients, the oral combination of Atovaquone and Proguanil resulted in a very high cure rate. Because of its lengthy half-life, atovaquone is more likely to select for drug-resistant strains. Due to Atovaquone's high cost, its broad usage is improbable, postponing the inevitable development of drug resistance [20].

- **Multiple-Drug Resistance:** Malaria parasites that have previously developed resistance to one type of antimalaria medication might acquire resistance to yet another. This is called multiple

drug resistance (MDR). In order to eradicate all remaining parasites, a combination of fast-acting drugs (such as quinine artemisinin) and slower-acting drugs (such as mefloquine or tetracycline) is used. Combination chemotherapy is the medical term for this course of treatment, which is administered for a minimum of four parasite generations, or around eight days. Commercially available combinations that have been found to be successful in field trials include quinine-tetracycline, chlorproguanil-dapsone, artemisinin-lumefantrine and atovaquone-proguanil [21-23].

Optimization of Antimalarial Drug

Target populations must have their pharmacokinetic and pharmacodynamic characteristics studied to determine the best dose. Antimalarial pharmacokinetics, parasite susceptibility, host susceptibility and parasite susceptibility are the four main factors that determine the therapeutic response. There may also be an issue with a combination of infections. Antimalarial drug dosing is still best determined by in-vivo investigations, even if ex vivo approaches may predict resistance [24] and provide considerable pharmacodynamic information [25]. For the treatment of uncomplicated falciparum malaria, combinations, especially fixed-dose combos (FDC), are indicated. However, in the event of vivax malaria, chloroquine and primaquine may be considered a combination. When a new drug is first discovered, there is only a little window of time to characterize the dose-response link [26]. Individual component dosages cannot be optimized once an FDC is given solely. FDCs, by definition, have a predetermined dosage ratio. Recording the blood concentrations associated with submaximal antimalarial effects is essential to a suitable dose modification in drug development. The study of P falciparum in animals may be beneficial, but human studies are also required. Recognizing that some volunteers may have short-term treatment failures is very critical here. In spite of our reluctance, sensitive parasite identification methods now provide us with safe ways that should avoid the patient from suffering any injury or pain [27]. An alternative way to determining dosage is addressed here, which if proven might help reduce under-prescription and hence under-dosing by speeding up and enhancing the process of determining dosage. In addition to being more efficient and less expensive, it may also be faster. Study objective is to identify the in vivo MIC so that the medication may be dosed correctly in a clinical setting. Following study of the antimalarial pharmacokinetics, antimalarial pharmacodynamics and PK-PD linkages must be evaluated.

Plasmepsin Biology

Plasmodium falciparum, P. malariae, and *P. vivax, P. ovale* are the four primary species of Plasmodium that cause malaria in humans. When a person is infected with P. falciparum malaria they are at a greater risk of complications and death. When Plasmodium infects a human host, it does so by infecting erythrocytes with merozoites, which then multiply and spread to other red blood cells, causing the parasite to explode in numbers. Malaria is caused by intraerythrocytic parasites, which is why drugs are being developed to combat it. In addition, the mosquito has a sexual development cycle and a replicative stage in the liver. Because fewer parasites are available from which to choose mutants, a claim may be made that hitting the sexual stages is desirable

because it inhibits transmission and delays resistance [28]. Plasmepsin IV seems to have a crucial role in the mosquito's ookinete stage, as far as we know [29]. Medicines active in the liver stage of certain Plasmodium species may be able to prevent recurrence of infection, although little is known about latent liver form plasmepsin synthesis (hypnozoites). A total of 10 plasmepsins are found in Plasmodium falciparum. Even though this Plasmodium species does not have as many as other species, it possesses four digestive vacuoles instead of only one [30]. Plasmepsins I, II, IV, and HAP are found in the digesting vacuole [31], whereas Plasmepsin V is found in the endoplasmic reticulum [32]. Plasmepsins VI, VII, and VIII are not expressed by intraerythrocytic parasites. There is indirect evidence that the parasite exports IX and X into cells, but we don't know what they're employed for [33]. At least 55% to 75% similarity is seen between the digestive vacuole plasmepses [34].

Plasmepsins As Antimalarial Drug Targets

It's difficult, but not impossible, to intervene therapeutically using aspartic proteases. [35]; Proteases found in our digestive system, as well as fungi like Candida and parasite plasmepsins. have been demonstrated to be excellent targets for all of these medications. FDA-approved aspartic protease inhibitors include a renin inhibitor. Several of these successful drug discovery programmes relied heavily on structure-based drug design [36]. Potent inhibitors' physical and peptidic physico-chemical characteristics stifled early attempts. Subsequent updates will need to contain even more drug-like characteristics. It is possible to use the discoveries of the last twenty years in aspartic protein drug development to identify additional Asp inhibitors, such as plasmepsins. It's now easier than ever to identify potential new Asp protease targets because to the vast library of existing inhibitors. Plasmepsin I-IV x-ray crystal structures containing inhibitors are available to aid in the development of new inhibitors (Table 1). Recently, a comprehensive assessment of the present status of plasmepsin computational inhibitor design was published [37]. As antimalarial drugs, the discovery of plasmepsin inhibitors is a challenge. (1) In order to construct inhibitors with drug-like features, such as a MW of 500 and a nonpeptidic nature, it is necessary to have a wide and diversified peptide binding site for proteases. Despite this, a slew of aspartic protease inhibitors, such as the HIV-1 protease inhibitor, have been developed. In order to achieve the requisite degree of efficiency against P. falciparum, most researchers believe that all four DV plasmepsins must be inhibited [38]. There has been several research looking for inhibitors of plasmepsin that may block a variety of different enzymes. It may be difficult to develop inhibitors that target many targets. On the other hand, the plasmepsin binding sites are known to be forgiving, thus this method may be effective. This versatility may be useful in dealing with drug-resistant mutations, since it allows plasmepsin inhibitor to engage a wide range of targets. Plasepsin inhibitors for erythrocyte testing have been identified, as will be shown in the next sections. Non-digestive vacuole plasmepsin inhibitors may also influence parasite-killing plasmepsins, which the plasmepsin inhibitors may affect. Digestional vacuole plasmepsin inhibitors' SAR does not match their parasite killing efficiency, as can be shown in the study mentioned above. This drug's primary target. digestive vacuole plasmepsins, may be acting as a

stand-in for the more crucial one. Non-DV inhibitors of plasmepsin V-X have not yet been discovered. The Medicines for Malaria Venture (MMV) has said that the ideal antimalarial medication for the poor in developing countries should have the following desirable features: Oral effectiveness against P. falciparum, the deadliest plasmodium species, and resistant strains, for example, are crucial. Plasmodium species such as P. vivax and others would be good targets. In animal studies, it must be at least 50 times safer than current medicines for pregnant women and new-borns. An adult's daily dose of new antimalarials should not exceed 100 mg, should be oral, and should cost no more than one dollar (USD) each treatment. In order to keep manufacturing costs low, candidate compounds should be synthesized from low-cost starting ingredients in as little as five steps. An antimalarial drug that targets plasmepsins must have an appropriate candidate profile with these challenging qualities [39].

Plasmepsin Inhibitors

Research on inhibitors of the digestion-related enzyme plasmepsins, which are found in the vacuoles of the digestive tract, has been going on since the late 1990s. as shown by previous studies [40-41]. For the purposes of this article, we will not present a full overview of all plasmepsin inhibitors, but we will concentrate on the most recent findings and their implications for the future development of plasmepsin inhibitors as antimalarial medicines. The hydroxyethyl amine scaffold, which is typical of plasmepsin inhibitors, provides the bas for 74 drugs [42].

Peptidomimetic Plasmepsin Inhibitors

Inhibitors of PM-I, PM-II. HAP, and PMIV with picomolar efficacy include Pepstatin A. In fact. only a little amount of parasite development is prevented by pepstatin A (iRBC IC50 = 4 M) [43]. This might be because of the need to traverse four cellular membranes, as well as the requirement to inhibit falcipain-2. Pepstatin A. being a peptide, is unlikely to be useful as a medication. Peptidomimetic inhibitors, on the other hand, have been the subject of many different techniques. Inspired by aspartic proteases, these transition-state inhibitors resemble the non-hydrolysable residue that mimics the tetrahedral hydrolysis intermediate. The HIV protease and BACE literature [44] has several descriptions of transition state peptidomimetics of this kind. Peptidomimetic inhibitors of plasmepsins have been studied by many groups, and the findings were recently reviewed [45]. Researchers working with Kyoto Pharmaceutical University and John Hopkins University have developed "adaptive inhibitors" that bind plasmepsin II with extraordinary potency while keeping some of the DV plasmepsin's activity. It may be found at [46] Using this strategy, an inhibitor must be designed with the most precise interactions against the conserved binding site portions, but it must also be flexible enough to be used in less conserved areas [47]. Table I shows the many PM-II crystal structures used to optimize the KNI series. The enthalpic and entropic contributions to binding energy were determined in large part by the use of isothermal calorimetry (ITC). Enthalpy contribution is the most difficult component to optimize owing to the very precise interactions, hence compounds with a higher contribution are preferred. For example, the effective use of this strategy relies on H-bonding to residues shared by all four DV plasmepsins.

The HIV protease inhibitor KNI-10006 is a transitional form. HMC-isostere allophenylnorstatine and a new thiazolidine scaffold duplicate the Phe-Leu in this peptidomimetic peptide's hemoglobin chain at P1-P1' locations. There is little to no effect on parasite development even when provided in combination with the PM-II and the rest of the D-Plasmepsins (Ki = 0.01nM). Antimalarial efficacy of these PM-II inhibitors has been considerably enhanced by adding 2-aminoethyl groups to their terminal phenyl ethers, according to the study. With an IC50 of less than 360 nM and a PM-II efficacy of less than 0.01 nM, KNI-10743 surpasses KNI-10006. According to this study's authors, acidic food vacuoles are to blame. However, a rise in potency on another target might also be the culprit [48].

Table 1: peptidomimetic Plasmepsin Inhibitors [60]

Compound	Ki (nM)				iRBCa
	PM-I	PM-II	PM-III(HAP)	PM-IV	iRBCa IC50(nM)
KNI-10006	-	0.5	-	-	6800
Pepstatin A	0.39	0.025	0.081	0.31	4000
KNI-10743	-	<0.1	-	-	360
KNI-10125	-	10	-	-	1100
KNI-10283	-	25	-	-	450

Non-Peptidomimetic Plasmepsin Inhibitors

There have been new attempts to identify and produce nonpeptidic plasmepsin inhibitors because of the difficulty in developing peptidomimetics as medications [49]. Nonpeptidic inhibitors may be found using virtual screens, which are less costly than high-throughput screening (HTS). PM-II and PM-IV are the best candidates for virtual screening due to the availability of x-ray crystal structures. Because of the protease's flexibility, only a few structures are typically evaluated in virtual screening efforts because of computational limits. All of these compounds have been found to be successful in the virtual screening procedure for the development of plasminogen activator protein (PAP) inhibitors [50] Despite the fact that many of the inhibitors they target are weak, these compounds have been useful non-peptidomimetic prerequisites for medicinal chemistry optimization despite their typically poor druggability. It's impossible to assess the worth of potential strikes since there aren't any statistics on parasite deaths.

Friedman and his colleagues employed a molecular docking technique based on fragments to evaluate 40,000 molecules in virtual reality as part of their study [51]. 13 substances were found to have IC50 values of less than 100 M and four were found to have IC50 values of 2 to 5 mg/mL. These substances will now be tested. An antimalarial drug with low nanomolar potency. Halofantrine has no known mechanisms for its activity. It's a molecule from this list as shown in table 2. The major mechanism of action of halofantrine is unlikely to be plasmepsin inhibition

unless it is a function of concentration in the food vacuole since plasmepsin inhibitors like mefloquine and halofantrine have lesser micromolar potencies and structural similarities.

Mendoza and associates recently revealed that antimalarial hydroxyethyl piperazines, such as 1 (RBC IC50 0.5-1.3 M), are structurally similar [52]. It is possible that these compounds might be PM-II inhibitors, even though they have not been tested for plasmepsin inhibition by the authors, who mention docking investigations [53]. Many new chemical categories were discovered during the post-docking study. This enzyme was inhibited by 30 commercially available medications from four distinct classes, with inhibitory levels ranging from 4 to 1800 nanograms per millilitre. These new inhibitor groups cannot be evaluated since there is no proof of parasite growth suppression or toxicity in human cells. HIV protease and renin have also been tested to see whether they can limit the activity of plasmepsin. Pyrrolidine-based PM-II and PM-IV inhibitors, such as the Klebe group's molecule 5, are symmetrical [54]. Initially, these complexes were meant to inhibit HIV protease [55]. In comparison to the 100-fold more active 1-naphthyl derivative 6, 5-phenyl's PM-IV and PM-II activity was found to be low [56]. Despite its lipophilicity (eLogP 5.9), little is known about molecule 6 except that it is an enzyme that has been shown to be capable of converting fatty acids into glucose. Unfortunately, there isn't much evidence available on the usefulness of these medications in tests with parasite development. For further enhancement, CatD selectivity, activity in Pf-infectious bloodstream cells, and pharmacokinetic properties are critical. Similar 3,4-disubstituting piperidine scaffolds were reported to be antimalarial drugs by Roche scientists [57]. In the beginning, it was intended for Asp protease renin [58-59], a chemical family derived from which it was based. We looked for sequence homology with renin inhibitors to see whether they inhibited PM-II inhibition. A large number of potent PM-11 inhibitors were discovered; however, the specific enzyme inhibition potencies were not reported. It was discovered that Plasmodium falciparum strains that were chloroquine sensitive (N54) and chloroquine resistant (KI) were active against these medicines. IC50 values for compound 7 are 60 nM for Kl strain and 50 nM for N54 strain, making it one of the most potent drugs. PM-II inhibitors with low micromolar potencies were discovered by Actelion Pharmaceuticals' researchers using a high-throughput FRET assay and a commercial library of 50,000 chemicals [38]. ACT-056822 (iRBC IC50 = 252 nM, PM-II IC50 = 104 nM) was discovered as a result of parallel medicinal chemistry optimization. Malaria medicine ACT-056822 was tested on mice infected with P. berghei germs by researchers (4 doses over 2 days). ACT-056822, a chemical that improved life expectancy by 161%, was discovered. Due to the compound's poor pharmacokinetic characteristics and restricted solubility as a result of its high viscosity, further medicinal chemistry modifications were required. As a key medicinal chemistry strategy to reduce lipophilicity and increase the pharmacokinetic characteristics and solubility. Actelion has patented heterocyclic moieties [60]. Actelion inhibitors were often effective against PM-1. but less so against PM-II or PM-IV [61] in many cases. Because they must block all four DV plasmepsins, several of the early compounds had little impact on parasites. [62] ACT-056822 and PM-II counterparts x-ray crystal structures have been established. Homology models of PM-I and PM-IV were used to identify drugs that might block all four DV

plasmepsins. Compound 8 inhibited the development of PM-I, PM-II, and PM-IV parasites in experiments that looked at parasite growth. Although closely related, compounds had no effect on parasite creation but did have an influence on parasite development, parasite growth studies were significantly more effective with compounds. Both chemical build-up in the food vacuole and broad-spectrum plasmepsin activity were shown to be responsible for cell movement, as opposed to other plasmepses like as PM-V, IX and X. In contrast to the Actelion series, the catalytic Asp34 of plasmepsin II creates a bidentate contact with amino group 11 [63-64]. This series has nanomolar strength against plasmepsins I, II, and IV: nevertheless, the enzyme strength doesn't really translate into appreciable parasite suppression. Plasmepsin may be overly selective for these chemicals, which limits its ability to suppress parasite development in the digestive vacuole.

Table 2: Inhibitors of Plasmepsin that are not Peptidomimetic [60]

Compound	IC50(nM)				
	PM-I	PM-II	PM-III(HAP)	PM-IV	iRBC
Halofantrine	3000	2000	6000	-	-
ACT-056822	-	104	-	-	252
7	-	-	-	-	50-60
5	-	11000	-	7000	-
1	-	-	-	-	500-1300
4	-	4.4	-	-	-

Table 3: Antimalarials Under Clinical Trials and Their Parasite Stage Targets

Parasite Stage Target	Antimalarial	Phase of Clinical Trial	Reference
	Piperaquine	IV	[65]
	Spiroindolone-NITD609 (Natural Product)	I	[66]
Infected red blood cell	Ozonide-OZ439 (synthetic peroxide)	II	[67]
Infected red blood cell + Gametocytes + Liver stages	Imidazolopiperazine (antifungal + anti-helminthic)	I	[68]
Liver stages	Bulaquine (8-aminoquinoline)	III	[69] [70]

Conclusion

For future antimalarials, study of the pharmacokinetic-pharmacodynamic interactions of antimalarial drugs would likely enhance existing antimalarial dosing regimens and raise the probability of selecting an optimal regimen. Antimalarial plasmepsins inhibitors have been studied over the last decade and many very powerful inhibitors of digestive vacuole plasmepsins have been discovered. Due to severance in their functions for each and other digestive vacuole proteases, data shows that these plasmepsins are not the best targets for drug development. Inhibitor efficacy against malaria may be attributed to binding to various plasmepsins as well as to other targets.

Plasmepsin V's newly discovered position as a protein export gatekeeper provides a once-in-a-lifetime opportunity for researchers. Novel antimalarial drugs might be developed from potent PM-V inhibitors once they are found. Other non-digestive vacuolar plasmepsins must also be studied to establish their roles and functions as possible therapeutic targets.