Malaria Journal

REVIEW

Open Access

Natural products as transmission-blocking agents against malaria: a comprehensive review of bioactive compounds and their therapeutic potential

Patrick Valere Tsouh Fokou[1,2,11*], Mariscal Brice Tchatat Tali[2,11], Cedric Derick Jiatsa Mbouna[2], Lauve Rachel Tchokouaha Yamthe[3], Javad Sharifi-Rad[4,5,6*], Daniela Calina[7*], Radha[8], Manoj Kumar[9], Jean Claude Tchouankeu[10] and Fabrice Fekam Boyom[2,11]

Abstract

Malaria eradication is hindered by the persistence of transmission stages of *Plasmodium falciparum* that enable parasite transfer from humans to mosquitoes. Current therapeutic strategies, such as artemisinin-based combination therapy (ACT) combined with primaquine, are insufficient due to limited efficacy on mature gametocytes and safety concerns in populations with glucose-6-phosphate dehydrogenase deficiency. This highlights the critical need for innovative, safe, and effective transmission-blocking interventions. This review explores the potential of natural sources, including medicinal plants, marine organisms, and microorganisms—as reservoirs of novel bioactive compounds with anti-malarial properties. A comprehensive literature search identified promising natural products with gametocytocidal and sporontocidal activity, validated through advanced bioassays. The review also evaluates various methodologies, such as colorimetric, microscopy, and flow cytometry assays, for assessing transmission-blocking efficacy. The findings emphasize the potent gametocytocidal effects of certain plant extracts, such as *Azadirachta indica* and *Vernonia amygdalina*, and microbial products, including ionophores and proteasome inhibitors. Despite promising in vitro and in vivo data, the transition of these compounds to clinical applications remains limited. Challenges include standardizing assays, addressing resistance to current therapies, and ensuring drug safety for endemic populations. The current review underscores the untapped potential of natural products as transmission-blocking agents and proposes a systematic, stage-specific screening cascade to identify and optimize these compounds. Addressing these gaps could significantly advance global malaria eradication efforts.

Keywords Antimalarial agents, Bioactive compounds, Gametocytocidal activity, Malaria transmission-blocking, *Plasmodium falciparum*

*Correspondence:
Patrick Valere Tsouh Fokou
ptsouh@gmail.com
Javad Sharifi-Rad
javad.sharifirad@gmail.com
Daniela Calina
calinadaniela@gmail.com
Full list of author information is available at the end of the article

Background

Malaria is a parasitic disease caused by apicomplexan parasites of the genus *Plasmodium*, which can infect a diverse range of vertebrate hosts and be transmitted by the female anopheles' mosquito. A number species are involved in human malaria, of which *Plasmodium falciparum* is the most prevalent in sub-Saharan Africa, accounting for 93% of estimated malaria cases in 2018 [1]. In the same year, there were an estimated 228 million cases of malaria occurred worldwide and 405000 deaths from malaria globally compared with 416,000 estimated deaths by the World Health Organization (WHO) in 2017, and 585,000 in 2010 [2]. Children aged under 5 years and pregnant women remains the most vulnerable groups. The life cycle of *P. falciparum* is highly complex, involving several developmental stages in both humans and mosquito. Mature asexual blood stages inside the human host are responsible for all the clinical symptoms of malaria, but only the non-replicating sexual blood stages of the parasite (male and female gametocytes), are able of developing in the mosquito vector and causing onward infection [3]. Though all *P. falciparum* infections are not severe and some are asymptomatic in highly endemic regions, they can contribute to malaria transmission [4].

The major chemotherapeutic agents to fight against malaria remains artemisinin-based combination therapy (ACT) [5]. However, ACT has no sterilizing effect on gametocytes stages V responsible for malaria transmission [6, 7]. To overcome this gap, ACT is currently combined with primaquine in order to block human to mosquito transmission [5]. Unfortunately, primaquine safety remains a major public health concern due to its haemolytic side effect in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency [8]. Therefore, the lack of an effective vaccine and the emergence of drug-resistant parasites suggests that interventions targeting the symptom-causing (asexual) stages of the parasite are insufficient to curb malaria transmission and completely eradicate malaria. Also, research on malaria transmission blocking drug discovery targeting gametocytes stages is still lacking. Hence, efforts should be made to encourage the search for new transmission-blocking drugs, by exploring diverse sources of drugs molecules. To achieve this, one promising way to support this effort toward malaria eradication remains herbal-based medicine. Nature-derived inhibitors are the richest source of novel pharmacophores with diverse biological activity as they are known to occupy biologically important chemical space [9]. Indeed, natural products such as medicinal plants, which have been a source of some anti-plasmodial "hits" such as artemisinin extracted from the Chinese herb *Artemisia annua* and quinine got from the *Cinchona* trees [10], could be an efficient tools for malaria transmission blocking drug discovery. Indeed, most people in endemic regions heavily rely on medicinal plants for the prevention and treatment of malaria and they have served as a source of bioactive drugs against *P. falciparum* asexual-blood stages. Meanwhile, natural products are still poorly investigated for their efficacy against transmissible gametocyte stages of malaria parasites. Studies report the gametocidal activity of some medicinal plants such as *Azadirachta indica, Artemisia afra,* and *Vernonia amygdalina* [11–17] suggesting the need for detailed and depth investigation.

This comprehensive review provides insights into the discovery of malaria transmission-blocking drugs, with a significant emphasis on natural products as a promising and underexplored alternative for future therapies.

Methodology for literature search and selection

The aim of the literature search was to identify studies focused on the discovery of malaria transmission-blocking drugs derived from natural sources, specifically targeting *Plasmodium falciparum* gametocytes and related transmissible stages. A comprehensive search was conducted across six databases: PubMed, Web of Science, Scopus, Embase, Google Scholar, and Cochrane Library. The search utilized both Medical Subject Headings (MeSH) terms and free-text keywords, including "malaria transmission-blocking," "gametocytocidal agents," "natural products," "medicinal plants," "marine-derived compounds," and "microbial bioactive agents." Boolean operators (AND, OR) were applied to combine these terms and optimize the retrieval of relevant studies. Studies were included if they were peer-reviewed articles published in English, investigated natural compounds or extracts for their transmission-blocking potential, and specifically addressed activity against gametocytes or mosquito stages of the *Plasmodium* life cycle. Articles were also included if they described the development or validation of assays used to evaluate the transmission-blocking efficacy of natural products. Systematic reviews and meta-analyses were considered if they provided substantial information on natural product-based drug discovery. Exclusion criteria were studies focusing solely on asexual blood-stage anti-malarials, those with incomplete data or methodological descriptions, and non-peer-reviewed materials such as conference abstracts or opinion pieces. Studies unrelated to natural product drug discovery or without clear relevance to malaria transmission-blocking strategies were also excluded. The most representative data were summarized in Tables and Figures.

Targeting *Plasmodium* gametocytes: biology and transmission-blocking strategies

Plasmodium transmissible stages: gametocytes

The complete *Plasmodium* life cycle includes the sexual developmental stages in the mosquito as well as the hepatic sporozoite and intra-erythrocytic sexual gametocyte stages in humans [7, 18] (Fig. 1). *Plasmodium* gametocytes are the only parasite stage that can be transmitted to the mosquito and derived from the sexual development in a small per cent of parasites and are void of clinical manifestations [3]. Despite their critical role in malaria transmission, understanding gametocyte biology remains a key focus for developing effective transmission-blocking agents.

Once gametocytes are ingested during a blood meal in the mosquito midgut, they become activated and differentiate into male and female gametes. The male gamete then fertilizes the female gamete, resulting in the formation of a zygote, which further develops into a motile ookinete, penetrates the gut epithelium, and subsequently develops into an oocyst. Maturation of the oocyst leads to the production of sporozoites, which migrate to the salivary gland of the mosquito and are ready for the next round of blood meal. Gametocytogenesis progresses through five morphological stages I-V over the course of 8–10 days [19]. Early gametocytes in stages I to III are susceptible to most classical schizontocidal anti-malarial drugs, whereas mature gametocytes in stages IV and V are insensitive to most available anti-malarial drugs. Only mature stage V gametocytes can develop in the *Anopheles* mosquito and remain the main stage to be interrupted in the hope of blocking human to mosquito malaria transmission and vice versa. In addition, gametocyte stages I to IV were reported to sequester in deep organs such as bone marrow and spleen and, therefore, difficult to be targeted by anti-malarial drugs whereas gametocyte stage V is the only circulating stage found in the peripheral blood where they take an additional 3 days to become infectious to mosquito [20, 21]. Gametocytes, specifically stage V provide a plausible link in malaria transmission from the human to mosquito, thereby making them a prime target for transmission-blocking strategies.

Transmission-blocking strategies

For making malaria elimination and eradication a true reality, interruption of parasite transmission is essential. Therefore, the role of transmission-blocking drugs becomes very peremptory [22]. The ability to block transmission relies on identifying (and curing) asymptomatic or semi-immune human hosts carrying transmissible forms of the parasite that represent the major reservoirs of continued infection; eliminating mosquito vectors through multiple and integrated strategies; and eliminating the parasite pool in patients with malaria, which, in the absence of a vaccine, still relies solely on chemotherapy and prophylaxis to prevent new or re-infection [23, 24]. Transmission blocking agent should ideally target and kill gametocyte stages, especially the transmissible mature gametocyte stages V [25], and thereby prevent malaria transmission from human to mosquito by clearing most gametocytes in the human host to render those patients non-infectious to mosquito [26]. In line with

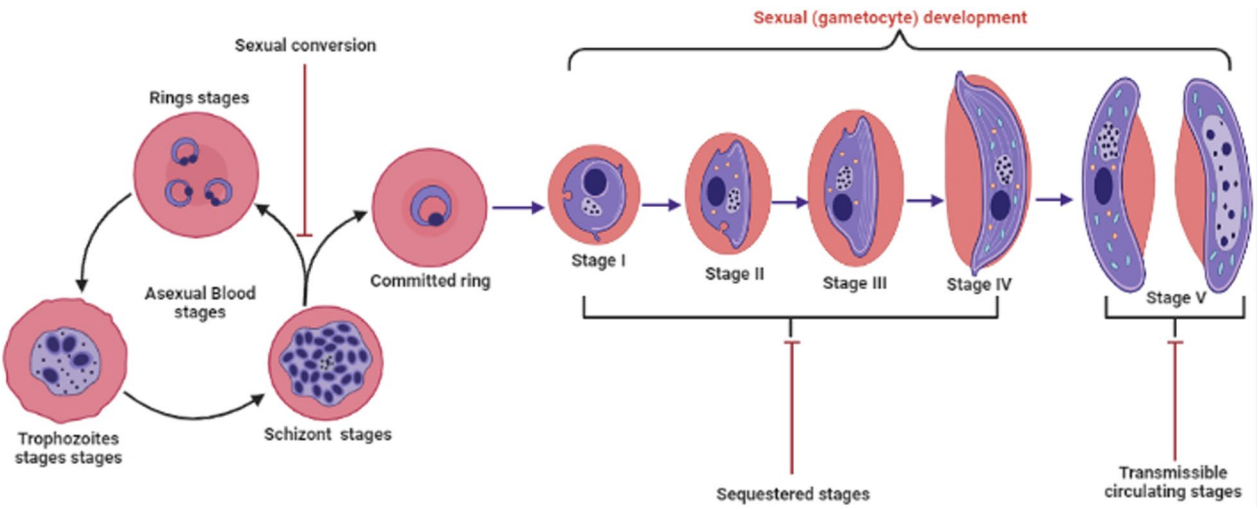


Fig. 1 Developmental stages and transmission potential of *Plasmodium* gametocytes. The diagram depicts the *Plasmodium* gametocyte life cycle, starting with sexual conversion from asexual blood stages to committed rings, which then progress through five gametocyte stages (I–V). Stages I–IV are sequestered in tissues, making them less accessible to drugs. Only mature stage V gametocytes circulate in the blood, becoming transmissible to mosquitoes and critical for malaria spread. This figure was created using BioRender.com elements

this priority, strategies targeting the vector stages would require a drug to be at pharmacologically relevant concentrations for as long as mature gametocytes circulate (up to 30 days). So, the most appropriate point of intervention remains to target the host gametocytes especially stage V.

Current preclinical assays for transmission-blocking drugs discovery: challenges and prospects

Gametocyte production

Gametocytes are currently induced in culture by non-specific stressors, such as changes in hematocrit, addition of mammalian hormones, reticulocytes, nucleic acid synthesis inhibitors (e.g., antifolates), and various drugs like Berenil, chloroquine, and amodiaquine [20, 27–31]. However, standardized, reproducible protocols for gametocyte production are lacking, making it difficult to compare findings across laboratories [32]. Furthermore, newer methods using genetic modification and controlled microenvironments aim to improve consistency in producing high-yield, stage-specific gametocytes for drug screening [33].

Current anti-gametocyte assays

There have been significant investments over the past decade and technological advances in the validation of some standard "methods" for transmission blocking-drug discovery. Several methods for assessing the transmission-blocking potential have been published using a variety of detection methods. These include measurement of metabolic activity such as parasite adenosine triphosphate (ATP) and guanosine triphosphate (GTP) levels [34], expression of a gene reporter [35]; colorimetric assays [36, 37], microscopy and flow cytometry methods.

Colorimetric methods

Colorimetric methods to assess transmission-blocking efficacy of a drug are currently based on metabolic activity such as enzymatic reaction-based spectrophotometry or fluorescence among all *Plasmodium* lactate dehydrogenase (pLDH) based assay [36] relies on the rapid utilization of 3-acetylpyridine adenine dinucleotide (APAD) as a coenzyme by pLDH in the reaction leading to the conversion of lactate to pyruvate [32] and Resazurin/Alamar blue-based assay using resazurin, a cell-permeant blue dye that functions as a cell viability indicator upon reduced to resorufin (red) by the metabolic activity of living cells [37].

These methods are used for both the early and late stages of transmissible gametocytes. pLDH is a reliable marker for gametocyte viability as the enzyme is present at high levels throughout gametocytogenesis [38, 39]. One major advantage of the pLDH assay is that it is performed directly on parasite cultures, therefore minimizing the manipulation of gametocytes. However, late-stage gametocytes exhibit decreased expression of genes responsible for glycolysis, protein biosynthesis, and haemoglobin catabolism [40]. While, redox reactive (oxidoreductive indicators) cell-permeable dyes like Alamar blue have previously been reported as robust assays supporting high-throughput screening, more sensitive than traditionally used tetrazolium dyes [37, 41]. Though these assays enable medium to high-throughput screening against gametocytes, care must be taken especially where compounds target the redox state of the parasite, as it may interfere with the assay readout. Moreover, standard drugs routinely used like methylene blue cannot be used in this assay platform due to colorimetric interference.

Reporter gene methods

This method consists of ATP bioluminescence assay, a luciferase reporter assay, a green fluorescent protein (GFP)-luciferase bioluminescence assay, a luminescence-indirect standard membrane feeding assay (SMFA) and a luminescence-direct SMFA. Luciferase reporter assay consists of both a luciferase reporter enzyme and a detection reagent that provides the enzyme substrate. In this assay, the light emitted is proportional to the reporter gene expression level when the reporter enzyme and detection reagent are combined. Luciferase-expressing *Plasmodium berghei* parasites are currently employed to monitor the parasite's sporogony development in vitro, as well as a tool for the identification of compounds with transmission-blocking activity. This assay has been established to enable accurate, reliable and quantifiable investigations of the stage-specific action of gametocytocidal drugs for each of the early and late gametocyte marker cell lines [42]. However, the major concern with this technique is a higher inter-assay variability between different assays and the problem of ATP interference. However, this method has to be improved in the future by using the recently reported *P. berghei* line expressing the novel luciferase enzyme NanoLuc (PbNLuc) that resulted in delivering a significantly enhanced luminescence signal, enabling single parasite detection in various stages of its life cycle, including in the mosquito vector [43]. Another promising way to improve the assay will be the use of a second reporter gene, placed under the control of a sporozoite-specific promoter, which would enable the specific monitoring of sporozoite formation and maturation [44]. ATP plays a central role in energy exchanges in biological systems (both in eukaryotic and prokaryotic cells), serves as the main donor of free energy, and is produced in all metabolically active cells. The ATP bioluminescent assay is classified as a gold standard for exploring parasite viability. Therefore, this parameter is

currently used as a suitable tool to assess the functional integrity of *P. falciparum* gametocytes. Injury and death lead to a rapid decrease in cytoplasmic ATP, [45] providing a reliable platform to test the effect of small molecules on the gametocyte's ability. In addition, transgenic *P. falciparum* parasites are used to quantify the effects of inhibitors either on asexual blood-stage in vitro using standard assays [46] or on transmission-blocking activity in the mosquito in high-throughput screens using SMFA [47, 48]. For the transmission-blocking assays transgenic *P. falciparum* (*NF54* strain) parasite lines expressing the GFP-luciferase fusion protein under the control of either the strong constitutive *P. falciparum hsp70* [47] or the gametocyte-specific *pfs16* promoters are currently used [48]. The use of transgenic parasite lines expressing GFP-luciferase fusion protein to evaluate the transmission-blocking potential of a given molecule is not without its limitations. First, the promoter is less suitable for high reporter expression in liver stages. Second, when using 'additional copy' chimeric parasites, the gene expression is dependent on the promoter used, which is unlikely to exactly mimic the timing and magnitude of the gene expression [1]. Gametocytes, ookinete, sporozoites, oocytes and liver stages are the main targeted stages in this method. These methods are easy, accurate, reliable, reproducible and quantifiable. However, a higher inter-assay variability has been noticed.

Microscopy methods

Microscopic examination of Giemsa's stained slides remains the 'gold standard' method for several decades for the detection, quantification, speciation and staging of blood-stage malaria parasites [49]. Today, this technique is currently used in the search for malaria transmission-blocking agents. Microscopy is currently used in association with other techniques in the malaria transmission-blocking drug discovery pipeline. Among these, we have, light microscopy-indirect SMFA- *P. falciparum;* light microscopy-direct SMFA-*P. berghei;* automated microscopic analysis and high-content imaging. Light microscopy associated with SMFA is currently used as the confirmatory tool to confirm the transmission-blocking potential of a given molecule. Therefore, *P. falciparum* SMFA remains the current "gold standard" mosquito-based confirmatory transmission-blocking assay for human malaria. However, owing to its complexity, only selected gametocidal molecules are progressed into SMFA. Predictive tools for the evaluation of transmission-blocking behaviour of compounds in SMFA would be extremely beneficial, but the lack of substantially large data sets from many mosquito feeds preempts the ability to perform correlations between outcomes from in vitro assays and in vivo assay through SMFA [47, 50], and have more overlap with the parasite's life cycle than any of the other gametocytocidal or gametogenesis inhibition assays [51]. The major problem encountered with light microscopy is that this technique is tedious, time-consuming and relies on the skills of technicians of microscopy techniques. The technician must be trained to correctly identify the different parasite species and stages [52, 53]. This final point is of greatest concern, as this inter-reader viability, gives rise to the common criticism that microscopy counts are relatively subjective.

Flow cytometry methods

As a laser-based technology, flow cytometry is largely dependent on the light-scattering properties of cells and particles that make it possible to analyse such characteristics as the size of cells, DNA content within a cell, as well as cell granularity among others. *Plasmodium* gametocyte quantification based on flow cytometry has been recently proposed with the goal of increasing precision and objectivity in malaria transmission-blocking drug discovery. Flow cytometry is currently used in association with other methods such as microscopy to assess the effect of promising asexual-blood stages inhibitors to distinguish pan-reactive drugs with multistage activity against the malaria parasite. Previous studies on the flow cytometry method allow highly reproducible quantification of specific drug effects on sexual conversion and early sexual development [54]. Flow cytometry in combination with magnetic enrichment has been a useful tool to estimate the inhibitory concentration of known drugs against *P. falciparum* gametocytes and remains useful to evaluate promising anti-gametocyte drugs. This method is generally based on the detection of *Plasmodium* double-stranded DNA in the infected erythrocytes [55]. However, this technique is subject to many challenges as far as *P. falciparum* gametocytes do not go through DNA replication. In addition, flow cytometry cannot distinguish between live and dead parasites [56].

Overall, these methods are used on the later stage and/or on mature gametocytes, as the immature stages have often been hypothesized to be sensitive to most antimalarial drugs and, hence, of limited concern. To really confirm the transmission-blocking potential of such inhibitors, some of those methods are currently used in association with SMFA known today as a "gold standard" in the field of malaria transmission-blocking drugs discovery. Those available methods already stabilized represent an important starting point for transmission blocking-drug screening. However, each method has its own advantages and limits and thereby required further improvement. Some molecular players such as PfAP2-G could be very useful for inhibition or prevention of sexual

commitment and therefore limiting human to mosquito malaria transmission and vice versa.

Existing therapeutic approaches

Chemical-based transmission-blocking drugs

Currently, the available drugs with therapeutic activity against late-stage gametocytes is artesunate, artemether, methylene blue, tafenoquine, and primaquine (Table 1) [33, 57]. For its activity on gametocytes, primaquine has been recently recommended at a single low dose (0.25 mg/kg) alongside anti-malarial treatment in disease-endemic countries. Again, ACT is currently used in combination with primaquine in order to treat and block transmission from humans to mosquitoes [5]. To protect the patient from the emergence of artemisinin resistance and reduce malaria transmission, researchers have developed a combination of ACT with methylene blue for malaria eradication [58] relying on the effect of methylene blue on parasite asexual-blood stages and, mature male and female gametocytes. However, ACT recommended for malaria transmission-blocking are not active on mature gametocyte stages V involved in transmission and are all threatened by emerging resistance [6, 11]. Besides, the use of primaquine is severely restricted due to its haemolytic issues in individuals with glucose-6-phosphate-dehydrogenase (G6PD) deficiency [33, 59]. Moreover, very few new agents are in development specifically for malaria transmission-blocking, although some have multistage activity [1]. Overall, new tools to specifically target sexual commitment are urgently needed for malaria control and prevention [3]. Indeed, the transmission of malaria can also be interrupted by targeting other parasitic stages, known as the sporogony stages (gametes, zygotes, ookinetes, oocysts and sporozoites), which occur inside the mosquito. These mosquito stages are also attractive drug targets because parasites in the majority of these stages exception of sporozoites, constitute the bottleneck population [22]. Targeting the parasite's sporogony stages remains an indirect transmission-blocking method of drug delivery due to the presence of these stages, not in the bloodstream of the host but inside the body of the mosquito. Drugs targeting the sporogony stages inside the mosquito vector has to be present in the host's blood circulation over and above the concentration required to kill these mosquito-stage parasites, for a sufficient period [51] making gametocytes a more suitable target for a transmission-blocking drug search. In recent years, many potential new antimalarial transmission-blocking drugs have been discovered. Advanced transmission-blocking drugs in

Table 1 Summary on current experimental assays used for transmission-blocking drug discovery

Transmission Blocking assay	Variants	Targeted stages	Turnaround time	Advantages	Limits	References
Colorimetric methods	-pLDH-based assay -Resazurin/Alamar blue based-assay	Gametocytes	48–72 h	-Robust, fast, easy, cheap and reproducible	Colorimetric interference with the drug	[37, 41]
Reporter gene methods	-ATP bioluminescence assay -Luciferase reporter assay -GFP-Luciferase bioluminescence -Luminescence- Indirect SMFA -Luminescence- Direct SMFA	Gametocytes Ookinete Oocyst Sporozites Liver stages	24–72 h	- Minimal interference -Easy, accurate, reproducible, reliable and quantifiable	-Higher inter-assay variability -ATP interference	[47, 48, 99]
Microscopy	-Light microscopy-Indirect SMFA- *P. falciparum* - Light microscopy-Direct SMFA- *P. berghei* - Automated microscopic analysis and High content imaging	Oocyst, Sporozoites, Male gamete, Ookinete	0–72 h	Easy, reliable and reproducible	-Require well-trained microscopist - Inter-reader viability	[44, 100, 101]
Flow cytometry	-Flow cytometry-gametocyte commitment assay	Early and late gametocyte stages	48 h	-Accurate and reproducible -Fast, robust and simple -Precise and objective	-Absence of DNA since these cells do not multiply	[54, 56, 102]

ATP Adenosine Triphosphate, *GFP* Green Fluorescent Protein, *pLDH* Plasmodium Lactate Dehydrogenase, *SMFA* Standard Membrane Feeding Assay

the development pipeline include the synthetic endoperoxides OZ439 that has proven activity against the asexual stages of the parasite [60] and is currently in phase IIa clinical trials, and cipargamin, which has previously demonstrated potent dose-responsive activity against the sexual stages of *P. falciparum* [61]. Cipargamin, the most advanced candidate in blocking human-to-mosquito transmission and OZ439 scientifically called artefenomel (Fig. 2). Their structural design has been inspired by artemisinin [60] and they are two transmission-reducing drugs under development. The effect of cipargamin on gametocytes and the transmission-reducing potential was investigated in vitro and compared to other antimalarials by Van Pelt-Koops et al. [61]. In this investigation, the authors firstly measured the in vitro activity on asexual *P. falciparum* parasites from the drug-sensitive NF54 and K1 strains, which showed favourable results for cipargamin compared to lumefantrine, artemether and primaquine. Secondly, gametocytocidal activity was measured, with cipargamin being the most effective inhibitor of early and late gametocyte development. Thirdly, these four compounds were added to blood meals of blood-feeding Anopheles mosquitoes, where cipargamin and lumefantrine both independent yielded reduced oocyst counts [61]. Additionally, the transmission blocking effect of cipargamin have been reported by Upton and colleagues in 2015 [62]. Results from the studies of Charman and colleagues (2011) demonstrated that the endoperoxide, OZ439, has sterilizing effect against

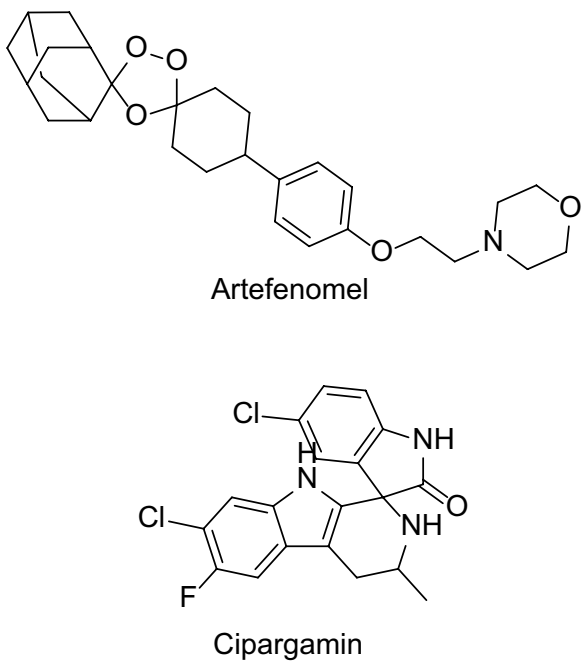


Fig. 2 Chemical structures of Cipargamin and Artefenomel (OZ439)

P. falciparum transmissible gametocytes with several advantages compared to artemisinin derivatives, such as prolonged plasma exposure, higher potency, stability and its synthetic nature [60]. It has shown comparable efficacy in the treatment of *P. falciparum* and *P. vivax* patients [60] are undergoing phase IIa clinical trials and is intended to be used as a single-dose combination therapy for acute malaria. It has previously been demonstrated to completely cure *P. berghei*-infected mice (blood-stage infection) with a single oral dose of 20 mg/kg [60] but does not affect *P. berghei* ookinete development in in vitro assays at 10 μM, suggesting it functions before the ookinete stage [63]. It has additionally demonstrated a potent effect in the SMFA with *P. falciparum* at 10 μM [63], OZ439 significantly reduced oocyst and sporozoite development in the mosquito when administered 24 h pre-feed at the dose of 6.5 mg/kg but did not significantly reduce the number of secondary mouse infections.

In the light of the above limitations encounter by chemical molecules used as transmission-blocking drugs, natural products and their derivative appear as a promising alternative (Fig. 3).

In fact, nature remains an attractive, credible, untapped source of new therapeutic candidate compounds since a tremendous chemical diversity is found in medicinal plants, animals, marine organisms, and microorganisms [12, 64]. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make a significant contribution to drug discovery today and the role of natural products as a source for remedies has been formally recognized [64] (Fig. 4). In addition, natural products have been used as scaffolds for the development of new products with huge therapeutic and industrial potential due to their unusual chemical features [65]. Therefore, the depth investigation of natural products and their derived phytoconstituents toward transmission-blocking drugs discovery needs a well-defined strategy. Hence, The transmission blocking-based natural product agents research strategies might include (i) the exploration of various sources of natural products (such as plants, marine algae and microorganisms) for gametocidal potency, (ii) ethnopharmacology-based plants selection of plants traditionally used in disease endemic country for malaria prevention; (iii) the repurposing of natural products with activity against asexual-blood stages; (iv) develop and validate more suitable, time effective and easier way to run the assay. Overall, natural products remain underexploited and there is no transmission-blocking natural product in clinical development. This underlines the low rate of progress in identifying gametocidal compounds from a natural source and the key importance of designing and implementing a clear, rationale, and sustainable screening test

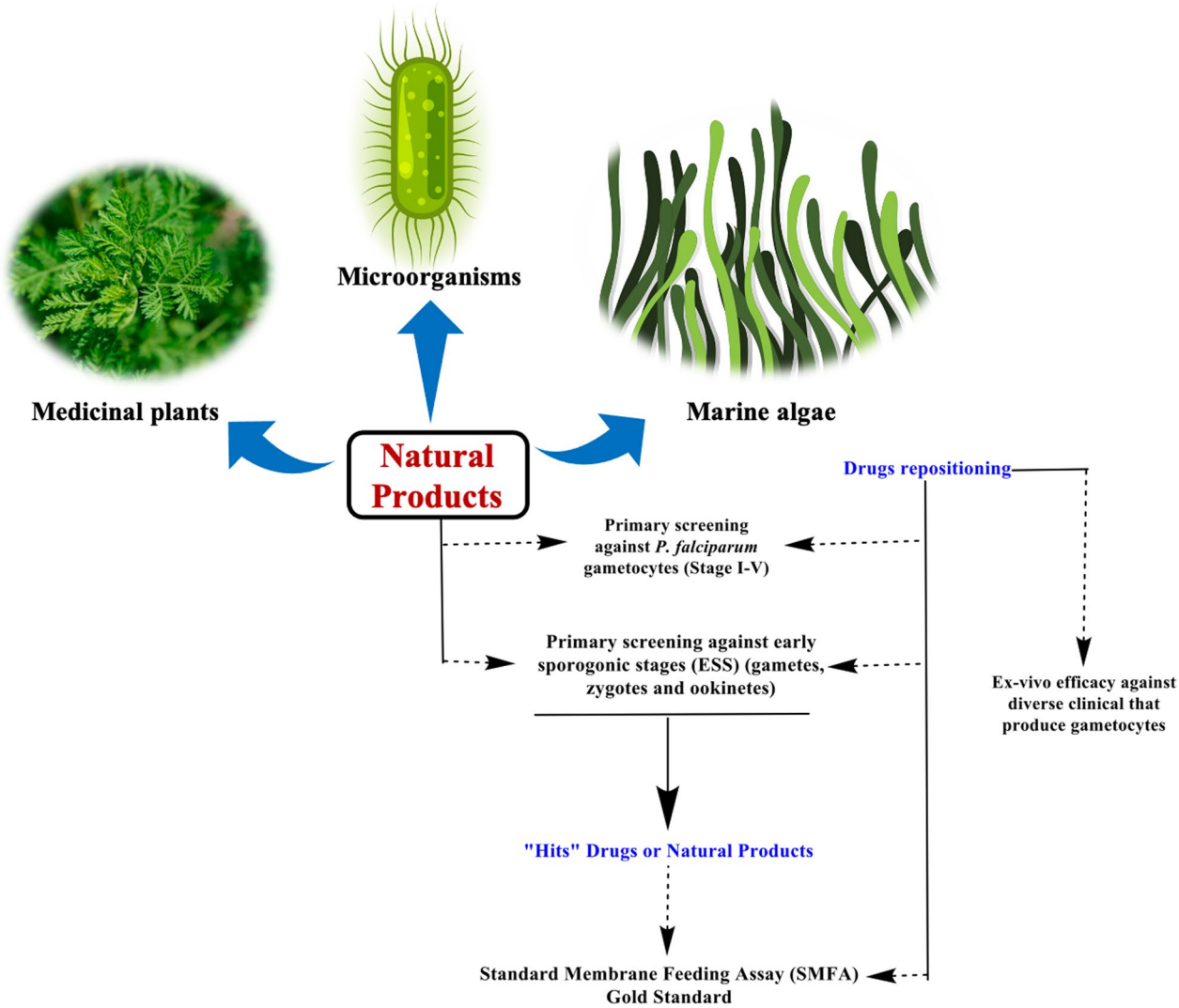


Fig. 3 Diagram with the sources and screening pipeline for identifying natural products with transmission-blocking potential against malaria. Natural products derived from medicinal plants, microorganisms, and marine algae serve as primary sources of bioactive compounds. Initial screening is conducted against Plasmodium falciparum gametocytes (Stages I-V) and early sporogonic stages (ESS), including gametes, zygotes, and ookinetes. Promising compounds, termed "hit" drugs or natural products, undergo further validation in ex vivo efficacy assays and standard membrane feeding assays (SMFA), considered the gold standard for assessing transmission-blocking potential. Additionally, drug repurposing approaches are incorporated to identify established drugs with potential activity against transmissible malaria stages

(See figure on next page.)

Fig. 4 Proposed test cascade approach for screening and isolation of transmission-blocking antimalarial compounds from natural products. Natural products sourced from medicinal plants, microorganisms, and marine algae undergo primary screening against *Plasmodium falciparum* transmissible stages. Promising extracts are identified at 100 µg/mL and subjected to dose–response testing, with active extracts showing $IC_{50} \leq 10$ µg/mL progressing further. Bioassay-guided purification isolates compounds with $IC_{50} \leq 5$ µg/mL, which then undergo studies of mode of action, including time-kill assays and stage-specific activity (early vs. late stages). Additional testing includes target identification and structure–activity relationship (SAR) studies. Active compounds are further assessed in in vitro male/female gamete assays and finally validated in the Standard Membrane Feeding Assay (SMFA) for transmission-blocking efficacy

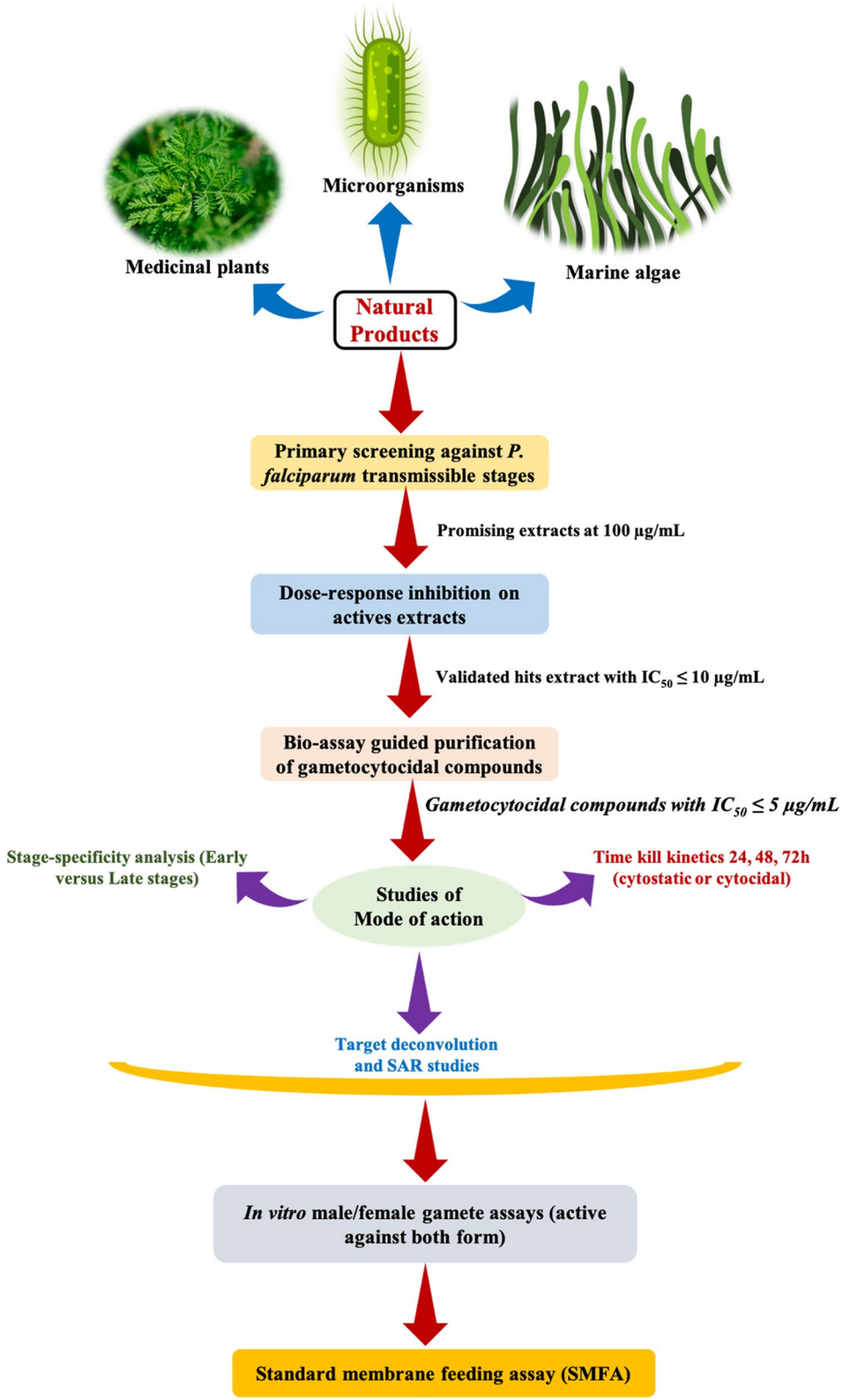


Fig. 4 (See legend on previous page.)

cascade for the isolation of naturally derived transmission-blocking compounds. Hence, the existing approach used for transmission blocking-drug discovery did not take into consideration a comprehensive cascade strategy in order to discover a promising molecule able to inhibit human gametocytes stages and mosquitoes' stages such as ookinete, gametes and zygote. In that regard, the discovery of transmission-blocking drugs from natural resources or from an existing set of drug molecules should first start with the screening of human transmissible gametocytes in order to identify a "hit" molecule that could screen on mosquitoes stages and in vivo using SMFA.

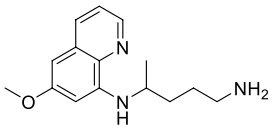
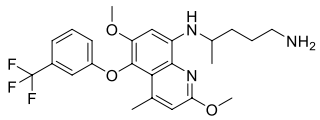
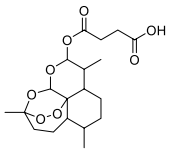
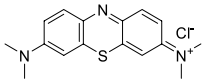
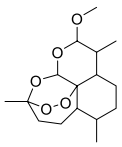
Role of plant extracts and secondary metabolites in malaria transmission-blocking

Medicinal plants used as alternative drugs are indicative of the vital role that plants play in many developing countries, and are also sources of novel plant-derived phytoconstituents that could be lead to the treatment of malaria and other diseases [66, 67]. The WHO estimated that ~65–80% of the world population ensured their primary health care using predominately plant-derived traditional medicines, existing a lower prevalence in developed countries [68]. Drug discovery approaches using medicinal plants as a tool provide diversity and structurally different compounds targeting multiple *P. falciparum* life cycles including liver stages, asexual-blood stages in the individual and transmissible gametocytes stages in the human host and mosquito's vector. Moreover, medicinal plants remain a promising alternative to conventional medicine, considering the rich biodiversity of the continent. Moreover, people living in Africa's endemic countries use medicinal plants even when they are not suffering from malaria. Indeed, they use it as a preventive strategy to stay safe from malaria infection and highlight the fact that, medicinal plants used for malaria prevention could contain preventive bioactive phytoconstituents with the potential to reduce malaria transmission as it has worked in the past for symptom-causing asexual blood stages. Among the few available drugs used in the fight against malaria transmission, only artemisinin used in combination with primaquine, comes from a medicinal plant, the Chinese herb *Artemisia annua* highlighting the fact that medicinal plants have been neglected for transmission-blocking drug discovery. Hence, medicinal plants present in nature remain powerful tools and inexhaustible sources that could accelerate malaria eradication worldwide. However, there is a paucity of studies that demonstrate the transmission-blocking effect of traditionally used medicinal plants involving human malaria parasite *P. falciparum*. The data in Table 2 summarize medicinal plant species investigated for their potential to inhibit or kill transmissible gametocyte stages in vitro or in vivo. In addition, it is evident from the in vitro assays that the pLDH-based assay was the most used to assess the gametocidal effect and therefore, highlighting the fact that, this method could be the easier method for investigating transmission-blocking anti-malarial compounds.

In vitro findings on gametocytocidal activity

Scientific literature only reports very few in vitro studies (Table 2) investigating the potential of medicinal plants or another natural resource to block human-to-mosquito malaria transmission by inhibiting *P. falciparum* gametocytes. Moving in that way, a study conducted by [12] reported the inhibition of *P. falciparum* early and late-stage gametocyte viability by extracts from eight traditional South African medicinal plants (Table 2). As a result, the IC_{50} of tested plant extracts ranged from 5.70 to 35.95 µg/mL in the early stage and from 3.20 to 35.95 µg/mL in the late gametocytes stages. Among the thirteen plant extracts investigated, three of them viz *Artemisia afra*, *Trichilia emetica* and *Turraea floribunda* were more potent in both stages with $IC_{50} < 10$ µg/mL. Besides, the three most active extracts in both early and late stages belong to *Asteraceae* and *Meliaceae* families. Extracts from *Combretaceae* family well-known for their pronounced activity against asexual-blood stages of *P. falciparum* showed moderated activity on both gametocyte stages with IC_{50} ranging from 28.35 to 32.74 µg/mL. In addition, Amoah et al. [14] reported the gametocidal activity of eight Ghanaian herbal medicines on *P. falciparum* gametocytes. From this investigation, authors showed that two of the herbal-antimalarial plant's products CM (in which active ingredients are *Cryptolepis sanguinolenta* and *Azadirachta indica*) and RT (in which active ingredients are *Aloe schweinfurthii*, *Khaya senegalensis*, *Piliostigma thonningii* and *Cassia siamea*) inhibited the growth of late-stage gametocytes by >80% at 100 µg/mL whilst one, KG (in which active principle are *Nauclea latifolia*, *Phyllanthus fraternus*, *Cryptolepis sanguinolenta*) was the most active on early-stage gametocytes at that same concentration. However, at 1 µg/mL, only YF (mixture of *C. sanguinolenta* and *A. indica*) significantly inhibited the survival of late-stage gametocytes although at that same concentration YF barely inhibited the survival of early-stage gametocytes. Soré et al. [15] reported the activity of aqueous, ethanolic, and methanolic extract from leaves and stem bark of five medicinal plants widely used traditionally in western Burkina Faso on *P. falciparum* gametocytes stages using a luciferase assay (Table 2). In the same vein, Udeinya and colleagues showed in vitro gametocidal effects of neem (*A. indica*, Meliaceae) leaf and seed extracts on *P. falciparum* (Table 2). At a dosage of 2.5 g/mL, neem extract kills

Table 2 Current transmission drugs, structure, chemical class, mode of action and preferential gametocytes stages of action

Name of compound	Chemical class	Structures	Mode of action in *P. falciparum*		Stage targeted	Refs.
			Molecular target	Pathway		
Primaquine	8-aminoquinoline		Not yet identified	Precisely unclear but generated metabolites might be interfering with the redox metabolism of the parasite	Early-stage I to III	[103]
Tafenoquine	8-aminoquinoline		Not yet identified	Not yet identified	Early-stage I to III	[104]
Artesunate	Endoperoxide		Proteasome/Existing proteins/Newly synthesized proteins	Inhibition of protein translation	Early (I–III) and Late (V) stage	[105]
Methylene blue	Benzothiazine		Monoamine oxidase Glutathione reductase Hemozoin formation	Glutathione reductase or inhibition of hemozoin formation	Late-stage V	[106, 107]
Artemether	Endoperoxide		Proteasome/Existing proteins/Newly synthesized proteins	Inhibition of protein translation	Early (I–III) and Late (V) stage	[85, 105]

IC_{50} Inhibitory Concentration 50, *ND* Not Determined, *IRBCs* Infected Red Blood Cells

more than 90% of *P. falciparum* immature and mature gametocytes in culture [16, 69].

In vivo findings on transmission-blocking

The results from Table 3 summarize a subset of medicinal plants investigated for their transmission-blocking properties in vitro and in vivo. This investigation shows that the studies were mostly conducted in vitro with very few in vivo. Among all investigated plant extracts, *Azadirachta indica* and *Vernonia amygdalina* [17] are the only ones investigated in vivo and showed a good reduction of oocyst density in mosquitoes, preventing, therefore, malaria transmission back from mosquitoes to humans very few or no study have been carrying out so far toward the investigation of plant-based medicine for their ability to block human to mosquitoes malaria transmission. An in-depth literature review showed that aqueous, ethanolic plant extracts and fractions from *Vernonia amygdalina* display in vivo transmission-blocking potential by reducing the *P. berghei* macrogametocyte density in mice by about 50%. Ver-EtOH reduced *P. berghei* oocyst prevalence and density by 27 and 90%, respectively and inhibited almost completely (> 90%) early sporogony stages (ESS) development in vitro at 50 µg/mL. At the same dose, four fractions obtained from the ethyl acetate phase of the methanol extract displayed inhibitory activity > 90% against ESS [13].

A commercially available standardized extract from neem seeds, '*NeemAzal*' (rich in azadirachtin: azadirachtin A [34%], azadirachtin B to azadirachtin K [16%]) was examined for sporontocidal activity in 'mice–to–mosquito and mosquito–to–mice' transmission model [17]. In this study, ookinete formation and oocyst development were completely blocked inside the *Anopheles stephensi* mosquitoes, when the mosquitoes were fed with blood from the gametocytaemic mice treated with 50 mg/kg "NeemAzal". This complete blockage of transmission was further confirmed when healthy uninfected mice on which these mosquitoes were fed, showed no oocysts. However, upon midgut dissection zygotes and post-zygotic forms were observed but these were less in number and of abnormal morphology. Since no mature ookinetes were observed at 50 mg/kg "NeemAzal", it can be hypothesized that the extract probably interferes

Table 3 Medicinal plants investigated for their transmission-blocking potential

Plants species	Family	Transmission blocking potential							
		In vitro activity			In vivo activity				
		IC_{50} on early stages (µg/ml	IC_{50} on late stages (µg/ml)	Methods used	Micro gametocytesper 2,500 IRBCs (95% CI)	Micro gametocytes per 2,500 IRBCs (95% CI)	Percentage inhibition Early Sporogonic Stages (EES)	Reduction in Oocyst density	References
Argenome mexicana	*Papaveraceae*	35.95 ± 11.1	35.95 ± 11.1	*Luciferase assay*	ND	ND	ND	ND	[15]
Artemisia afra	*Asteraceae*	5.7 ± 0.3	3.2 ± 0.2	*pLDH-based assay*	ND	ND	ND	ND	[108]
Azadirachta indica	*Meliaceae*	ND	ND	Microscopy	ND	ND	ND	86.0–93.5	[109]
Catha edulis	*Celastraceae*	> 10	> 10	*pLDH-based assay*	ND	ND	ND	ND	[13]
Combretum collinum	*Combretaceae*	28.35 ± 3.3	28.35 ± 3.3	*Luciferase assay*	ND	ND	ND	ND	[15]
Guiera senegalensis	*Combretaceae*	ND	ND	Microscopy	ND	ND	ND	No activity	[109]
Khaya anthotheca	*Meliaceae*	> 10	> 10	*pLDH-based assay*	ND	ND	ND	ND	[108]
Leonotis leonurus	*Lamiaceae*	12.8 ± 1.4	13.9 ± 2.6	*pLDH-based assay*	ND	ND	ND	ND	[108]
Leonotis leonurus	*Lamiaceae*	> 10	> 10	*pLDH-based assay*	ND	ND	ND	ND	[108]
Lophira lanceolata	*Ochnaceae*	11.35 ± 3.2	11.35 ± 3.2	Luciferase assay	ND	ND	ND	ND	[15]
Olea europaea sub sp. Africana	*Oleaceae*	> 10	> 10	*pLDH-based assay*	ND	ND	ND	ND	[13]
Terminalia macroptera	*Combretaceae*	32.74 ± 11.9	32.74 ± 11.9	*Luciferase assay*	ND	ND	ND	ND	[15]
Trichilia emetica Vahl subsp. Emetica	*Meliaceae*	7.6 ± 0.9	3.8 ± 0.5	*pLDH-based assay*	ND	ND	ND	ND	[108]
Turraea floribunda	*Meliaceae*	9.2 ± 0.2	4.6 ± 0.7	*pLDH-based assay*	ND	ND	ND	ND	[108]
Vernonia amygdalina	*Asteraceae*	ND	ND	Microscopy	5.4–8.0	32–44.8	93.4–99.1	26–53	[13]
Zanthoxylum zanthoxyloïdes	*Rubiaceae*	20.63 ± 2.3	20.63 ± 2.3	*Luciferase assay*	ND	ND	ND	ND	[15]

with gametogenesis and ookinete development. Also, "NeemAzal" at 50 mg/kg was not found to disrupt oocyst maturation during its incubation with early oocysts, suggesting a lack of any activity against the early oocysts. The observed inhibition of gametogenesis and ookinete formation was attributed to the microtubule-targeted mode of action of neem extracts containing azadirachtin [17]. Moreover, authors equally reported the transmission-blocking potential of an azadirachtin-enriched extract of neem seeds at 50 mg/kg mouse body weight in rodent malaria in vivo model of *Plasmodium berghei*/*Anopheles stephensi*. *Azadirachta indica* is a popular medicinal plant used in various Asian and African countries for the treatment of various illnesses including malaria. Furthermore, two *Az.. indica* fractions codified as PNE and ENM were found to be more active than the third fraction ENS at 0.125% v/v and 0.25% v/v against early and late-stage gametocytes [70]. Two fractions (IRDN-A and IRDN-B) obtained from an extract of neem leaves led to complete inhibition of early-stage and late-stage gametocytes

at 1 and 100 µg/mL respectively (IC_{50} for IRDN-A and IRDN-B against both early and late-stage gametocytes $=10^{-3}$ µg/mL) [17]. *Azadirachta indica* is one of the important medicinal plants which has been investigated via in vivo experimental trial and showed promising results in malaria transmission-blocking experiments.

Plasmodium transmissible gametocytes

Plant-derived compounds

Medicinal plants are investigated as one of the important raw materials which act as excellent malaria transmission-blocking agents. Two compounds 1α,4α-dihydroxybishopsolicepolide and yomogiartemin, isolated from *Artemisia afra* showed good inhibition on *P. falciparum* gametocytes stages and the two others from *Vernonia amygdalina* viz vernolide and vernodalol have the potential to inhibit transmissible gametocytes stage V. It was also reported that azadirachtin from neem tree prevents the formation of motile male gametes in *P. berghei* with an IC_{50} of 3.5 µM and completely inhibited ex-flagellation in *P. falciparum* at 100 µM [71]. In addition to the compounds previously described, several other natural products have shown promising transmission-blocking activity. These include Mallotojaponin C, a clerodane diterpenoid from *Mallotus japonicus*, which exhibits gametocytocidal effects [72]; Parthenin and Parthenolide, sesquiterpene lactones from *Parthenium hysterophorus* and *Tanacetum parthenium*, respectively, reported to impair gametocyte development [73]; and securinine-related alkaloids [74], which have demonstrated activity against sexual stages of *Plasmodium*. These compounds, structurally related to artemisinin, may offer potential scaffolds for developing new transmission-blocking drugs.

Moussavi and co-authors in 2015 identified pellitorine as the main insecticidal component in *Zanthoxylum heitzii* bark against *Anopheles gambiae* mosquitoes [75], while *P. berghei* ookinete conversion has been demonstrated to be inhibited by dihydronitidine (0.59 µg/ml) and heitziquinone (6.2 µg/ml) [76]. Similarly, Cox and co-authors in 2024 identified brusatol, a natural product, as a potential transmission blocker through in vitro screening and in vivo analyses [77]. Furthermore, Pulixin, a chromene compound isolated from *Artemisia afra*, has been identified as a potent inhibitor of gametocyte maturation and parasite transmission [78]. Sterigmatocystin, a fungal metabolite structurally related to aflatoxins, also exhibits notable inhibitory effects on *Plasmodium* sporogonic stages [78]. These additions highlight the chemical diversity of natural sources with potential transmission-blocking activities. The discussion on plant-derived malaria transmission-blocking compounds has been limited here, as this topic has already been comprehensively reviewed by Moyo et al. [79]. Figure 5 shows

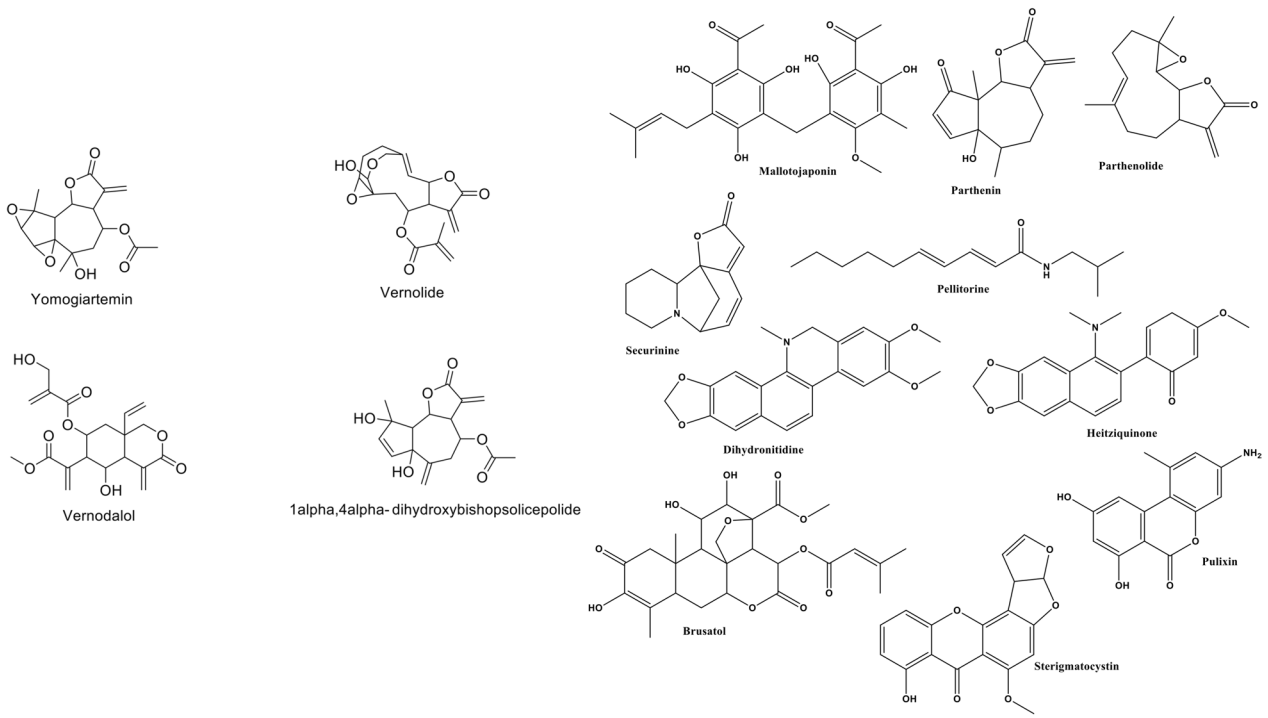


Fig. 5 The structures of medicinal plant-derived compounds with anti-gametocyte properties

the structures of medicinal plant-derived compounds with anti-gametocyte properties.

Microorganisms

Microorganisms such as bacteria and fungi have been invaluable in discovering drugs and lead compounds. In addition, microorganisms produce a wide variety of antimicrobial agents which have evolved to give their hosts an advantage over their competitors in the microbiological world [64]. On the other hand, microorganisms living closely associated with plants present in their neighbourhood and, particularly, their microbiome are also seen as a powerful tool providing a diversity of chemical agents in response to stress and environmental conditions. Therefore, the exploration of new therapeutic approaches based on microorganism-derived phytocompounds or microorganisms living in plants such as bacterial and fungal (endophytes) will be a promising direction toward the discovery of transmission-blocking agents. Amongst the group of some investigated microorganisms-derived natural products, ionophores are one of the very large and important groups of naturally occurring compounds. Ionophores are highly lipophilic substances capable of interacting stoichiometrically with metal ions, thereby serving as a carrier by which these ions can be transported across a bimolecular lipid membrane [80].

Ionophores are toxic to many bacteria, protozoa, fungi, and higher organisms and thus fit the classical definition of antibiotics [81]. Motivated and inspired by the drug repurposing endeavour, D'Alessandro and coworkers (2015) screened in vitro, three ionophores specifically salinomycin, nigericin and monensin isolated from *Streptomyces* sp. against early and late stage of *P. falciparum* gametocytes [82]. Results showed that all three compounds displayed good activity against both early and late stages *P. falciparum* transmissible gametocytes (IC_{50} < 200 nM) with salinomycin displaying preferential inhibition on late-stage gametocytes [82]. In addition, ionophores showed the potential to inhibit the development of *P. berghei* gametocytes into early sporogony stages (ESS) in vitro and the transmission-blocking properties of these compounds were confirmed in vivo using the SMFA. In addition, maduramicin, an ionophore produced by actinomycete *Actinomadura rubra*, has been demonstrated by the works of [83] to have transmission-blocking properties both in vitro and in vivo by killing late-stage *P. falciparum* gametocytes (IC_{50} < 200 nM). Killing kinetics studies showed that, maduramicin is fast-acting by reducing late-stage gametocyte viability by >90% at 12 h post-treatment with morphological change evident within 1 h following drug exposure. Additionally, in vivo, maduramicin displayed good transmission-blocking activity by significantly inhibiting oocyst development following exposure of gametocytes to the drug for only 90 min before mosquito feed [83].

Adriamycin and plicamycin, two glycosides known as a DNA synthesis inhibitors and RNA synthesis inhibitors respectively, display gametocytocidal activity at submicromolar concentrations [84]. These results underline that natural product-derived inhibitors of nucleic acid synthesis are effective against the *P. falciparum* transmissible stage of the malaria parasite [79]. This observation can be justified with transcription inhibitors [35, 48, 85], such as puromycin with equipotent in vitro activity against all five development stages of *P. falciparum* gametocytes [85] and are fast-acting against *P. falciparum* macro-gametes in kill kinetics assay [50] whilst also blocking *P. berghei* ookinetes development [86]. In addition, the macrolide chlorotonil A is highly active against late-stage gametocytes [87]. Some antibiotics such tetracycline, fosmidomycin, and deferoxamine have shown their potential against both *P. falciparum* gametocytes (IC_{50} values >12.5 μM) and macro-gametes and are not able to block *P. berghei* ookinete development in vitro.

Epoxomicin, an inhibitor of *P. falciparum* proteasome is one of the most widely investigated peptides currently used as a reference for in vitro transmission-blocking activity targeting gametocytes stages [50, 83, 88]. Results from some studies showed that epoxomicin displayed a potent in vitro activity against late-stage *P. falciparum* gametocytes with IC_{50}< 10 nM [34, 36, 89] with sex-specific inhibition on *P. falciparum* micro-gametes [50, 90]. In vivo studies showed the potential of epoxomicin to completely block *P. falciparum* oocysts formation in *An. stephensi* [90]. Additionally, another peptide, carmaphycin B targeting the β5 subunit of the yeast 20 s proteasome have been shown to display a potent inhibition against both asexual-blood and transmissible gametocytes stages of *P. falciparum* in vitro (IC_{50}< 1 μM) with 40-fold preferential inhibition on *Plasmodium* asexual-blood stage [91, 92]. The exploration of cyclic oligopeptides including the antibiotic thiostrepton which is known as an inhibitor of *P. falciparum* asexual-blood stage has been shown to be moderately potent against the five development stages of gametocytes with IC_{50} ranging from 1.82 to 3.4 μM [85] with a 14-fold enhanced activity against micro-gametes compared to macro-gametes [63]. Moreover, thiostrepton significantly reduces *P. berghei* oocyst development in *An. stephensi* mosquito midguts as well as reducing the number of sporozoites per mosquito [44]. In addition, two oligopeptides, dactinomycin and romidepsin displayed gametocytocidal activity at a sub-micromolar concentration in vitro and confirmed in vivo transmission-blocking activity for romidepsin [93]. A library of crude fungal extracts for compounds that disrupt the interaction of fibrinogen-related protein

1 (FREP 1) with *Plasmodium* parasites allowed to identified three active extracts with *Aspergillus niger* (92% inhibition of FREP 1-*Plasmodium* association) being the most potent [94]. From this study, *P*-orlandin was identified as the active principle from this extract and showed in vivo transmission-blocking activity against oocysts formation [94]. In addition, aphidicolin, a mycotoxin from *Cephalosporum aphidicola* exhibited good activity toward *P. falciparum* micro-gametes exflagellation [95] without demonstrating any sign of toxicity.

Natural products from marine drugs

The marine environment has been demonstrated to be an interesting reservoir of compounds with unique chemical features serving as a baseline for the molecular modelling and chemical synthesis of new drugs with greater efficacy and specificity for therapeutics purposes [96]. Moreover, marine organisms do not have a distinguished history of use in traditional medicine compared to terrestrial organisms [96]. However, in the last 50 years, advances in new technologies and engineerings such as scuba diving techniques, manned submersibles, and remotely operated vehicles opened up the marine environment to scientific exploration [68] for drugs discovery. The correlation between several species in these habitats of a limited extent increases their competitiveness and complexity. Therefore, marine organisms such as seaweeds have been demonstrated to be an exceptional source of bioactive chemicals agents, some of them with different structural features from those of terrestrial sources and must be exploited for the discovery of malaria transmission-blocking agents. A set of the seaweed extracts were screened on the liver stage (LS) of the rodent malaria parasite *P. berghei* by using a transgenic *P. berghei* parasite expressing the bioluminescent reporter protein luciferase to visualize and quantify parasite development in Huh7 cells, a human hepatoma cell line. Eight seaweed extracts representing all three classes of marine macroalgae [green seaweeds *Cladophora rupestris*, *Codium fragile* ssp. *tomentosoides*, *Ulva lactuca*; brown seaweeds *Cystoseira baccata*, C. *tamariscifolia*; red seaweeds *Osmundea pinnatifida*, *Ceramium virgatum*, and *Halopitys incurvus*], demonstrated moderate activity against hepatic stage parasites with IC_{50} values ranging from 14.9 to 52.9 µg/mL [97]. Intracellular localization of the liver stage parasites and the effect of the active extracts on the morphology and development of the liver stage parasites in Huh7 cells studied by immunofluorescence analysis. showed that, both extracts severely impaired the development of liver-stage parasites, which was similar to that observed with 15 µM (= 6.8 µg/mL) primaquine [97]. In addition, sesquiterpene avarone, its reduced form avarol, isolated from sponge *Dysidea avara* and thiazoavarone (semisynthetic thiazinoquinone), exhibited good activity against different stages of *P. falciparum* asexual and sexual forms (gametocytes stage V) (IC_{50} = 15.53 ± 5.26, 15.01 ± 3.19, and 9.30 ± 1.90 µmol/L) [98]. The above consideration towards marine algae opens the door for the investigation of marine sources for an anti-parasitic drugs search. Investigating marine sources emerges as a key tool in this direction. Till now, no studies have shown the transmission-blocking potential of marine extracts or derived compounds. Hence, marine algae could have appeared as a novel source of investigations for malaria transmission-blocking drugs search. It is evident from the various findings that natural compounds from plants and marine still need to be explored further whereas a significant case study has proven the potential role of natural compounds for malaria transmission-blocking.

Figure 6 illustrates the intervention points where natural compounds inhibit the development of Plasmodium, thereby blocking malaria transmission at fundamental stages in both human and mosquito hosts.

Table 4 provides a detailed summary of natural products derived from plants, microorganism-associated sources, and marine environments with demonstrated transmission-blocking activity against malaria.

Limitations and clinical gaps in malaria transmission-blocking drug discovery

The current limitations and clinical gaps in malaria transmission-blocking drug discovery pose significant challenges to advancing effective treatments. One of the primary limitations is the lack of comprehensive and standardized screening methods. Assays used to evaluate transmission-blocking efficacy vary widely and are often inconsistent, especially in their ability to measure drug effectiveness across all stages of *Plasmodium* development. This variability hinders comparisons across studies and laboratories, making it difficult to identify reliable transmission-blocking agents.

In addition, there is a scarcity of clinical validation for transmission-blocking compounds, as most research remains at the preclinical stage. Very few transmission-blocking agents have progressed to clinical trials, creating a gap between laboratory findings and real-world applications. Another critical gap lies in the specificity of existing drugs, which predominantly target asexual blood stages, leaving mature gametocytes, particularly stage V, less affected. These later-stage gametocytes are crucial for transmission but are difficult to target effectively without causing adverse effects in patients.

The safety of currently available transmission-blocking drugs, such as primaquine and tafenoquine, is also a concern. Both drugs are associated with haemolytic risks in individuals with glucose-6-phosphate dehydrogenase

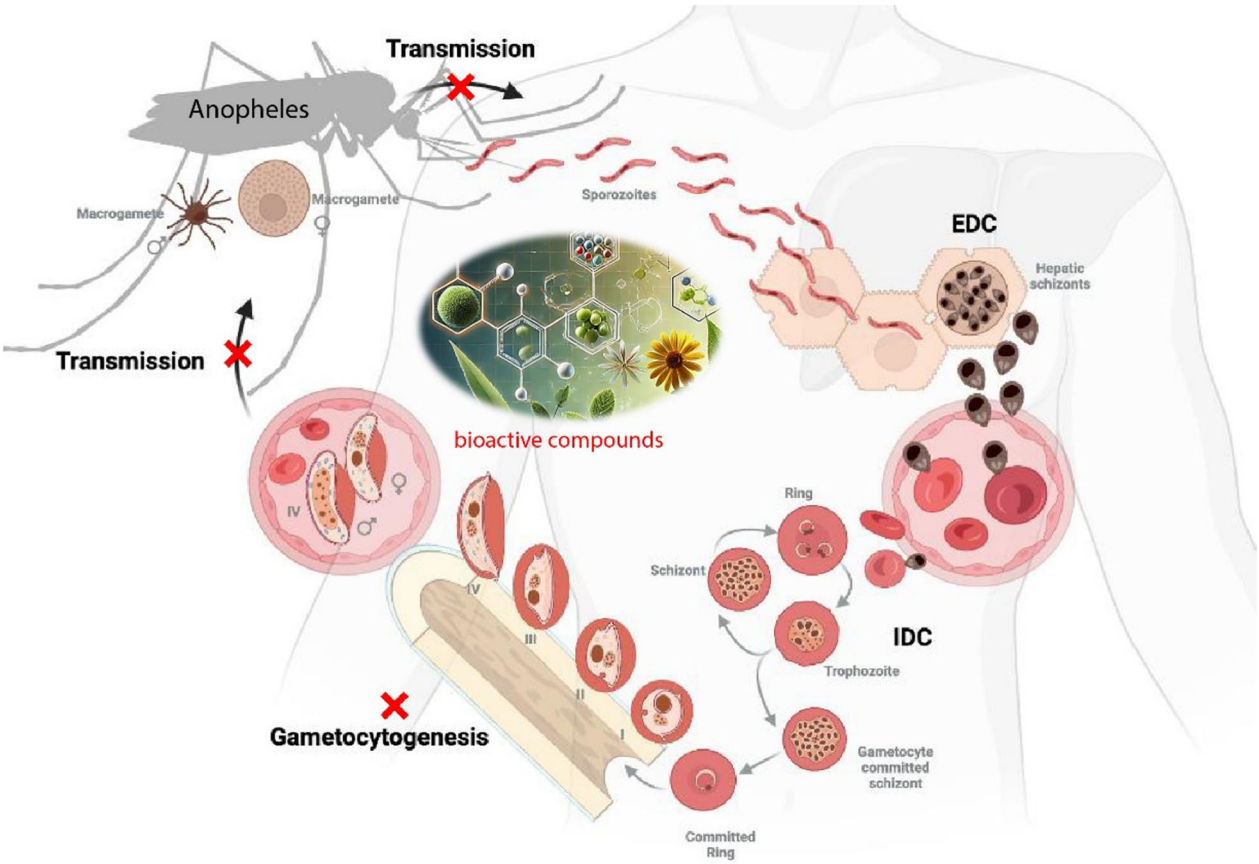


Fig. 6 Targeting malaria lifecycle stages with natural bioactive compounds for transmission blocking. Bioactive compounds derived from medicinal plants, microorganisms, and marine algae act at multiple lifecycle stages. In the hepatic (liver) stage, these compounds inhibit the maturation of hepatic schizonts, which release merozoites into the bloodstream to initiate infection. During the intraerythrocytic developmental cycle (IDC), merozoites infect red blood cells and develop through ring, trophozoite, and schizont stages, with some differentiating into gametocytes. The compounds also prevent gametocytogenesis by inhibiting the maturation of gametocytes within red blood cells, reducing the number of transmissible forms. In the mosquito vector, these compounds target the transmission stages by blocking the development of male and female gametes, thus preventing the formation of infective sporozoites and further transmission. Red X marks indicate stages where bioactive compounds interfere, blocking progression to the next stage and thereby disrupting the malaria transmission cycle

(G6PD) deficiency, which is prevalent in many malaria-endemic areas. This limitation reduces the applicability of these drugs in regions where they are most needed, emphasizing the urgent need for safer transmission-blocking alternatives.

Despite the potential of natural products from sources like plants, marine organisms, and microorganisms, there has been limited exploration of these resources for malaria transmission-blocking purposes. Much of the natural world remains untapped, and systematic screening efforts are lacking, which could otherwise identify new candidates with transmission-blocking potential. Furthermore, the emergence of resistance to artemisinin-based therapies, which are central to current malaria treatments, poses a significant threat to ongoing transmission-blocking efforts. The limited availability of alternative drugs with novel mechanisms of action exacerbates this issue, highlighting the need for new candidates that can overcome resistance.

Lastly, current research focuses primarily on human-stage gametocytes, with minimal emphasis on mosquito-stage interventions, such as targeting ookinetes and oocysts. A more comprehensive approach that includes both human and mosquito stages could enhance the overall efficacy of transmission-blocking strategies. Addressing these gaps requires developing standardized, efficient screening protocols, advancing promising preclinical findings to clinical testing, expanding research into underexplored natural sources, and addressing resistance to current therapies. By tackling these limitations, malaria eradication efforts can be significantly strengthened, bringing us closer to effective, sustainable transmission-blocking solutions.

Table 4 Overview of natural products with transmission-blocking potential against malaria

Category	Compound/source	Targeted stage(s)	Mechanism of action	IC_{50}/activity	References
Plant-derived compounds	1α,4α-Dihydroxybishopsolicepolide (*Artemisia afra*)	*P. falciparum* early-stages Gametocytes (> 85% IV–V)	Inhibit late gametocyte	IC_{50} = 2 µg/ml	[109]
		P. falciparum early-stages gametocytes (> 85% II–III)	Inhibit early gametocyte	IC_{50} = 5.6 µg/ml	
	Yomogiartemin (*Artemisia afra*)	*P. falciparum* early-stage Gametocytes (> 85% IV–V)	Inhibit late gametocyte	IC_{50} = 5.3 µg/ml	[109]
		P. falciparum early-stage gametocytes (> 85% II–III)	Inhibit early gametocyte	IC_{50} = 4.4 µg/ml	
	Vernolide, Vernodalol (*Vernonia amygdalina*)	*Plasmodium berghei* early sporogonic stages	Showed weak (Vernolide) to moderate (Vernodalol) inhibitory activity	IC_{50} = 18.7 µM (Vernodalol)	[109]
	Azadirachtin (*Azadirachta indica*)	Male gametes	Prevents motile male gamete formation; blocks ex-flagellation	IC_{50} = 3.5 µM (P. berghei); 100 µM (*P. falciparum*)	[71, 108]
	Parthenin (*Parthenium hysterophorus*)	*P. falciparum* oocysts	Reduced oocyst intensity in mosquito midgut	6.25–100 µg/ml	[73]
		P. falciparum stage V gametocytes	Inactivation, preventing exflagellation and oocyst development	100–1000 ng/ml	
		P. falciparum microgametes (Exflagellation)	Inhibition of proper exflagellation completion	1000 ng/ml	
		P. falciparum ookinetes	Abrogation of ookinete development in midgut	50 µg/ml, 500 µg/ml (used for initial tests) |	
		P. berghei zygotes/ookinete	Reduced total number of zygotes and ookinetes in vitro	Dose-dependent inhibition	
		P. falciparum oocysts	Reduced oocyst intensity in mosquito midgut	6.25–100 µg/ml	
	Parthenolide (*Tanacetum parthenium*)	*P. falciparum* oocysts	Reduced oocyst intensity in mosquito midgut	6.25–100 µg/ml	[73]
		P. falciparum stage V gametocytes	Inactivation, preventing exflagellation and oocyst development	10–1000 ng/ml	
		P. falciparum microgametes (Exflagellation)	Inhibition of proper exflagellation completion	1000 ng/ml	
		P. falciparum ookinetes	Abrogation of ookinete development in midgut	50 µg/ml, 500 µg/ml (used for initial tests) |	
		P. berghei Zygotes/Ookinete	Reduced total number of zygotes and ookinetes in vitro	Dose-dependent inhibition	
	Securinine (*From Natural Product Fragment Library*)	*P. falciparum* mature stage V gametocytes	Inhibits viability by allosterically binding to *Plasmodium falciparum* 2′-deoxyuridine 5′-triphosphate nucleotidohydrolase (PfdUTPase), enhancing enzyme activity and inhibiting viability of both asexual and sexual stages	IC_{50} = 36.7 µM (viability) IC_{50} = 33.3 µM (PfdUTPase)	[74]

Table 4 (continued)

Category	Compound/source	Targeted stage(s)	Mechanism of action	IC_{50}/activity	References
	Pellitorine (*Zanthoxylum heitzii*)	Adult mosquitoes and larvae	Toxicity on adult mosquitoes and larvae	LD_{50}: 50 ng/mg (insect)/13 µg/ml (	[76]
	Dihydronitidine/ Heitziquinone(*Zanthoxylum heitzii*)	*Plasmodium berghei* ookinete	Block ookinete conversion	IC_{50}: 0.59/6.2 µg/ml (larvae)	[76]
	Brusatol	*P. berghei* Male Gametes (Stage V)	Gametocidal	IC_{50} value of 43.0 nM	[77]
		P. berghei Ookinete Development	Complete blocked likely through inhibition of P25 expression	10 µM (ookinete); 10 nM (p25)	
		P. berghei In Vivo Transmission	Blocks transmission to mosquitoes	0.5 mg/kg	
		P. falciparum gametocyte maturation	Prevents stage progression (I to II/III, II/III to III/IV) by inhibiting development from early to late stages	100 nM, 500 nM	
		P. falciparum oocyst	Gametocidal	500 nM	
		P. falciparum Male gamete activity (Exflagellation)	Reduces activity	> 80% inhibition at 500 nM	
		P. falciparum Oocyst Development	Reduces burden & prevalence in mosquitoes	500 nM	
	Mallotojaponin C (*Mallotus japonicus*)	*Plasmodium falciparum* Late and Early gametocyte stages	Showed gametocytocidal activity Prevent gametocytogenesis	3.6 µM (late gametocyte) 0.14 µM (gemetogenisis)	[72]
Microorganism-Derived Compounds	Salinomycin, Nigericin, Monensin (*Streptomyces* sp.)	Early and late gametocytes; sporogony	Ionophore action; disrupts ion transport across membranes	IC_{50} < 200 nM	[82]
	Maduramicin (*Actinomadura rubra*)	Late gametocytes	Fast-acting; kills gametocytes and inhibits oocyst development	IC_{50} < 200 nM; > 90% viability reduction at 12 h	[83]
	Adriamycin, Plicamycin	Gametocytes	Inhibits nucleic acid synthesis	Sub-micromolar activity	[84]
	Epoxomicin	Late gametocytes	Inhibits *P. falciparum* proteasome	IC_{50} < 10 nM	[34, 50]
	Carmaphycin B	Asexual stages; gametocytes	Inhibits yeast 20 s proteasome β5 subunit	IC_{50} < 1 µM	[91, 92]
	Thiostrepton	Gametocytes; oocyst	Cyclic oligopeptide targeting gametocytes and mosquito midgut oocysts	IC_{50} = 1.82–3.4 µM	[63, 85]
	Dactinomycin, Romidepsin	Gametocytes	Inhibits transcription	Sub-micromolar activity	[93]
	P-Orlandin (*Aspergillus niger*)	Oocyst	Disrupts FREP1-Plasmodium interaction	92% inhibition of FREP1 association	[94]
	Pulixin (*Purpureocillium lilacinum*)	Mature stage V gametocyte	Block transmission of the parasite to mosquitoes	EC_{50}: 11 µM	[78]
	Sterigmatocystin (*Purpureocillium lilacinum*)	*Plasmodium* sporogonic stages	Limits sexual parasite infection in mosquitoes	IC_{50}: 48 µM	[78]

Table 4 (continued)

Category	Compound/source	Targeted stage(s)	Mechanism of action	IC_{50}/activity	References
Marine-derived compounds	Seaweed extracts (e.g., *Cladophora rupestris, Cystoseira baccata*)	Hepatic stages	Impairs liver-stage parasite development	IC_{50} = 14.9–52.9 µg/mL	[97]
	Avarone, Avarol (*Dysidea avara*)	Asexual stages; gametocytes (Stage V)	Unknown	IC_{50} = 9.30–15.53 µM	[98]

Conclusions and future perspectives

The quest for effective malaria transmission-blocking strategies remains crucial in the global effort to eradicate malaria. Natural products derived from medicinal plants, microorganisms, and marine sources offer a promising reservoir of bioactive compounds that can target various stages of the *Plasmodium* life cycle, especially the gametocyte and mosquito transmission stages. While initial studies have identified some promising candidates, there are still significant gaps in translating these findings into clinically effective drugs. In the future, expanding systematic screening of natural compounds using advanced bioassay-guided purification methods will be essential. Developing standardized protocols for assessing transmission-blocking efficacy across all stages of *Plasmodium* development can help address current inconsistencies and enhance reproducibility across laboratories. Furthermore, a focus on identifying compounds that can target mature gametocytes, particularly stage V, as well as mosquito stages (such as ookinetes and oocysts), could provide a more comprehensive approach to blocking malaria transmission. Additionally, exploring safe and effective alternatives to drugs like primaquine and tafenoquine, which have limitations due to toxicity in G6PD-deficient populations, should remain a research priority. New technologies, such as high-throughput screening and the integration of machine learning to predict active compounds, could expedite the discovery and development process. Drug repurposing, which involves identifying existing drugs with potential transmission-blocking activity, presents another valuable avenue for finding effective treatments faster. Advances in genomics and proteomics may further aid in understanding the specific mechanisms through which these natural compounds act, facilitating the development of compounds with targeted mechanisms against the malaria parasite's transmissible stages. Ultimately, a concerted effort involving multidisciplinary research, collaborations between academia, industry, and public health organizations, and increased funding will be required to move these promising findings from the laboratory to clinical use. With these advancements, the development of effective, safe, and accessible transmission-blocking agents can become a reality, strengthening global malaria eradication efforts and improving health outcomes in endemic regions.

Author contributions

PVTF, MBTT, CDJM, LRTY, JS-R, DC, R, MK, JCT, FFB made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas. That is revising or critically reviewing the article; giving final approval of the

version to be published; agreeing on the journal to which the article has been submitted; and, confirming to be accountable for all aspects of the work.

Funding
Not applicable.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biochemistry, Faculty of Science, University of Bamenda, 39, Bambili, Cameroon. [2]Antimicrobial and Biocontrol Agents Unit, Department of Biochemistry, Faculty of Science, University of Yaounde 1, Ngoa Ekelle, 812, Yaounde, Cameroon. [3]Institute for Medical Research and Medicinal Plants Studies, Yaoundé 13033, Cameroon. [4]Universidad Espíritu Santo, Samborondón, 092301, Ecuador. [5]Centro de Estudios Tecnológicos y , Universitarios del Golfo, Veracruz, Mexico. [6]Department of Medicine, College of Medicine, Korea University, Seoul 02841, Republic of Korea. [7]Department of Clinical Pharmacy, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania. [8]School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India. [9]Chemical and Biochemical Processing Division, ICAR – Central Institute for Research on Cotton Technology, Mumbai 400019, India. [10]Laboratory of Natural Products and Organic Synthesis, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon. [11]Advanced Research & Health Innovation Hub, P.O. Box 20133, Yaoundé, Cameroon.

Received: 29 November 2024 Accepted: 3 May 2025
Published online: 26 May 2025

References

1. Ashley EA, Phyo AP. Drugs in development for malaria. Drugs. 2018;78:861–79.
2. WHO. World malaria report 2011. Geneva: World Health Organization; 2011.
3. Nilsson SK, Childs LM, Buckee C, Marti M. Targeting human transmission biology for malaria elimination. PLoS Pathog. 2015;11:e1004871.
4. Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: what is the evidence? Trends Parasitol. 2014;30:183–90.
5. Oleribe OO, Osita-Oleribe P, Ekom E, Ofem O, Igwesi C, Chigozie OG, Ekweghariri M, Iyalla G, Taylor-Robinson SD. Is malaria over-diagnosed? A world malaria day 2017 experience by Excellence and Friends Management Care Centre (EFMC) and partners, Abuja Nigeria. Pan Afr Med J. 2017;28:273. https://doi.org/10.11604/pamj.2017.28.273.12732.
6. Shekalaghe S, Drakeley C, Gosling R, Ndaro A, van Meegeren M, Enevold A, et al. Primaquine clears submicroscopic *Plasmodium falciparum* gametocytes that persist after treatment with sulphadoxine-pyrimethamine and artesunate. PLoS ONE. 2007;2:e1023.
7. Wells TNC, Alonso PL, Gutteridge WE. New medicines to improve control and contribute to the eradication of malaria. Nat Rev Drug Discov. 2009;8:879–91.
8. Watson J, Taylor WRJ, Menard D, Kheng S, White NJ. Modelling primaquine-induced haemolysis in G6PD deficiency. Elife. 2017;6:e23061.
9. Genis D, Kirpichenok M, Kombarov R. A minimalist fragment approach for the design of natural-product-like synthetic scaffolds. Drug Discov Today. 2012;17:1170–4.
10. Hong SC. *Artemisia annua*, artemisinin, ACTs, and malaria control in Africa: tradition, science, and public policy. Washington DC: Politics and Prose; 2012. p. 254.
11. Moyo P, Kunyane P, Selepe MA, Eloff JN, Niemand J, Louw AI, et al. Bioassay - guided isolation and identification of gametocytocidal compounds from *Artemisia afra* (Asteraceae). Malar J. 2019;18:65.
12. Moyo P, Botha ME, Nondaba S, Niemand J, Maharaj VJ, Eloff JN, et al. *In vitro* inhibition of *Plasmodium falciparum* early and late stage gametocyte viability by extracts from eight traditionally used South African plant species. J Ethnopharmacol. 2016;185:235–42.
13. Abay SM, Lucantoni L, Dahiya N, Dori G, Dembo EG, Esposito F, et al. *Plasmodium* transmission blocking activities of *Vernonia amygdalina* extracts and isolated compounds. Malar J. 2015;14:288.
14. Amoah LE, Kakaney C, Kwansa-Bentum B, Kusi KA. Activity of herbal medicines on *Plasmodium falciparum* gametocytes: implications for malaria transmission in Ghana. PLoS ONE. 2015;10:e0142587.
15. Soré H, Alessandro SD, Sanon S, Parapini S, Tiono A, Valea I, et al. *In vitro* inhibitory activity against *Plasmodium falciparum* sexual and asexual. Int J Curr Pharm Res. 2018;4:2976–83.
16. Udeinya IJ, Brown N, Shu EN, Udeinya FI, Quakeyie I. Fractions of an antimalarial neem-leaf extract have activities superior to chloroquine, and are gametocytocidal. Ann Trop Med Parasitol. 2006;100:17–22.
17. Lucantoni L, Yerbanga RS, Lupidi G, Pasqualini L, Esposito F. Transmission blocking activity of a standardized neem (*Azadirachta indica*) seed extract on the rodent malaria parasite *Plasmodium berghei* in its vector *Anopheles stephensi*. Malar J. 2010;9:66.
18. Sinden RE. A biologist's perspective on malaria vaccine development. Hum Vaccin. 2010;6:3–11.
19. Ngotho P, Soares AB, Hentzschel F, Achcar F, Bertuccini L, Marti M. Revisiting gametocyte biology in malaria parasites. FEMS Microbiol Rev. 2019;43:401–14.
20. Smalley ME, Brown J. *Plasmodium falciparum* gametocytogenesis stimulated by lymphocytes and serum from infected Gambian children. Trans R Soc Trop Med Hyg. 1981;75:316–7.
21. Kuehn A, Pradel G. The coming-out of malaria gametocytes. J Biomed Biotechnol. 2010;2010:976827.
22. Smith RC, Vega-Rodríguez J, Jacobs-Lorena M. The *Plasmodium* bottleneck: malaria parasite losses in the mosquito vector. Mem Inst Oswaldo Cruz. 2014;109:644–61.
23. Killeen GF, Seyoum A, Gimnig JE, Stevenson JC, Drakeley CJ, Chitnis N. Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. Malar J. 2014;13:146.
24. Chen I, Clarke SE, Gosling R, Hamainza B, Killeen G, Magill A, et al. "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. PLoS Med. 2016;13:e1001942.
25. Birkholtz L-M, Coetzer TL, Mancama D, Leroy D, Alano P. Discovering new transmission-blocking antimalarial compounds: challenges and opportunities. Trends Parasitol. 2016;32:669–81.
26. WHO. World malaria report 2017. Geneva: World Health Organization; 2017.
27. Trager W. What triggers the gametocyte pathway in *Plasmodium falciparum*? Trends Parasitol. 2005;21:262–4.
28. Salcedo-Sora JE, Caamano-Gutierrez E, Ward SA, Biagini GA. The proliferating cell hypothesis: a metabolic framework for *Plasmodium* growth and development. Trends Parasitol. 2014;30:170–5.
29. Puta C, Manyando C. Enhanced gametocyte production in Fansidar-treated *Plasmodium falciparum* malaria patients: implications for malaria transmission control programmes. Trop Med Int Health. 1997;2:227–9.
30. Ono T, Ohnishi Y, Nagamune K, Kano M. Gametocytogenesis induction by berenil in cultured *Plasmodium falciparum*. Exp Parasitol. 1993;77:74–8.
31. Roncalés M, Vidal-Mas J, Leroy D, Herreros E. Comparison and optimization of different methods for the *in vitro* production of *Plasmodium falciparum* gametocytes. J Parasitol Res. 2012;2012:927148.
32. Reader J, Botha M, Theron A, Lauterbach SB, Rossouw C, Engelbrecht D, et al. Nowhere to hide: interrogating different metabolic parameters of *Plasmodium falciparum* gametocytes in a transmission blocking drug discovery pipeline towards malaria elimination. Malar J. 2015;14:213.
33. Lucantoni L, Avery V. Whole-cell in vitro screening for gametocytocidal compounds. Future Med Chem. 2012;4:2337–60.
34. Lelièvre J, Almela MJ, Lozano S, Miguel C, Franco V, Leroy D, et al. Activity of clinically relevant antimalarial drugs on *Plasmodium falciparum*

mature gametocytes in an ATP bioluminescence "transmission blocking" assay. PLoS ONE. 2012;7:e35019.
35. Lucantoni L, Duffy S, Adjalley SH, Fidock DA, Avery VM. Identification of MMV malaria box inhibitors of *Plasmodium falciparum* early-stage gametocytes using a luciferase-based high-throughput assay. Antimicrob Agents Chemother. 2013;57:6050–62.
36. D'Alessandro S, Silvestrini F, Dechering K, Corbett Y, Parapini S, Timmerman M, et al. A *Plasmodium falciparum* screening assay for anti-gametocyte drugs based on parasite lactate dehydrogenase detection. J Antimicrob Chemother. 2013;68:2048–58.
37. Tanaka TQ, Williamson KC. A malaria gametocytocidal assay using oxidoreduction indicator, alamarblue. Mol Biochem Parasitol. 2011;177:160–3.
38. Oduola AMJ, Omitowoju GO, Sowunmi A, Makler MT, Falade CO, Kyle DE, et al. *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. Exp Parasitol. 1997;87:283–9.
39. Mueller I, Zimmerman PA, Reeder JC. *Plasmodium malariae* and *Plasmodium ovale* - the "bashful" malaria parasites. Trends Parasitol. 2007;23:278–83.
40. Young JA, Fivelman QL, Blair PL, De P, Le KG, Zhou Y, et al. The *Plasmodium falciparum* sexual development transcriptome: a microarray analysis using ontology-based pattern identification. Mol Biochem Parasitol. 2005;143:67–79.
41. Tanaka TQ, Dehdashti SJ, Nguyen D, Mckew JC, Zheng W, Williamson KC. A quantitative high throughput assay for identifying gametocytocidal compounds. Mol Biochem Parasitol. 2013;188:20–5.
42. Adjalley SH, Johnston GL, Li T, Eastman RT, Ekland EH, Eappen AG. Quantitative assessment of *Plasmodium falciparum* sexual development reveals potent transmission-blocking activity by methylene blue. Proc Natl Acad Sci USA. 2011;108:E1214–23.
43. De Niz M, Stanway RR, Wacker R, Keller D, Heussler VT. An ultrasensitive NanoLuc-based luminescence system for monitoring *Plasmodium berghei* throughout its life cycle. Malar J. 2016;15:232.
44. Azevedo R, Markovic M, Machado M, Franke-Fayard B, Mendes AM, Prudêncio M. Bioluminescence method for *in vitro* screening of *Plasmodium* transmission-blocking compounds. Antimicrob Agents Chemother. 2017;61:e02699-e2716.
45. Crouch SPM, Kozlowski R, Slater KJ, Fletcher J. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J Immunol Methods. 1993;160:81–8.
46. Wilson AL, Taskforce I. A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment of malaria in children. PLoS ONE. 2011;6:e16976.
47. Vos MW, Stone WJR, Koolen KM, Van Gemert GJ, Van Schaijk B, Leroy D, et al. A semi-automated luminescence based standard membrane feeding assay identifies novel small molecules that inhibit transmission of malaria parasites by mosquitoes. Sci Rep. 2015;5:18704.
48. Lucantoni L, Fidock DA, Avery VM. Luciferase-Based, High-throughput assay for screening and profiling transmission-blocking compounds against *Plasmodium falciparum* gametocytes. Antimicrob Agents Chemother. 2016;60:2097–107.
49. Bezrukov AV. Romanowsky staining, the Romanowsky effect and thoughts on the question of scientific priority. Biotech Histochem. 2017;92:29–35.
50. Bolscher JM, Koolen KMJ, Van Gemert GJ, Van de Vegte-Bolmer MG, Bousema T, Leroy D, et al. A combination of new screening assays for prioritization of transmission-blocking antimalarials reveals distinct dynamics of marketed and experimental drugs. J Antimicrob Chemother. 2014;70:1357–66.
51. Wadi I, Nath M, Anvikar AR, Singh P, Sinha A. Recent advances in transmission-blocking drugs for malaria elimination. Future Med Chem. 2019;11:3047–88.
52. Maguire JD, Lederman ER, Barcus MJ, Prudhomme O'Meara WA, Jordon RG, Duong S, et al. Production and validation of durable, high-quality standardized malaria microscopy slides for teaching, testing and quality assurance during an era of declining diagnostic proficiency. Malar J. 2006;5:18704.
53. Malleret B, Claser C, Ong ASM, Suwanarusk R, Sriprawat K, Howland SW, et al. A rapid and robust tri-color flow cytometry assay for monitoring malaria parasite development. Sci Rep. 2011;1:1–10.
54. Buchholz K, Burke TA, Williamson KC, Wiegand RC, Wirth DF, Marti M. A high-throughput screen targeting malaria transmission stages opens new avenues for drug development. J Infect Dis. 2011;203:1445–53.
55. Saul A, Myler P, Mangan T, Kidson C. *Plasmodium falciparum*: automated assay of erythrocyte invasion using flow cytofluorometry. Exp Parasitol. 1982;54:64–71.
56. Chevalley S, Coste A, Lopez A, Pipy B, Valentin A. Flow cytometry for the evaluation of anti-plasmodial activity of drugs on *Plasmodium falciparum* gametocytes. Malar J. 2010;9:49.
57. Pukrittayakamee S, Chotivanich K, Chantra A, Clemens R, Looareesuwan S, White NJ. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. Antimicrob Agents Chemother. 2004;48:1329–34.
58. Coulibaly B, Pritsch M, Bountogo M, Meissner PE, Nebié E, Klose C, et al. Efficacy and safety of triple combination therapy with artesunate-amodiaquine-methylene blue for falciparum malaria in children: a randomized controlled trial in Burkina Faso. J Infect Dis. 2015;211:689–97.
59. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2014;371:411–23.
60. Charman SA, Arbe-Barnes S, Bathurst IC, Brund R, Campbell M, Charman WN, et al. Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. Proc Natl Acad Sci USA. 2011;108:4400–5.
61. Van Pelt-Koops JC, Pett HE, Graumans W, Van Der Vegte-Bolmer M, Van Gemert GJ, Rottmann M, et al. The spiroindolone drug candidate NITD609 potently inhibits gametocytogenesis and blocks *Plasmodium falciparum* transmission to anopheles mosquito vector. Antimicrob Agents Chemother. 2012;56:3544–8.
62. Upton LM, Brock PM, Churcher TS, Ghani AC, Gething PW, Delves MJ, et al. Lead clinical and preclinical antimalarial drugs can significantly reduce sporozoite transmission to vertebrate populations. Antimicrob Agents Chemother. 2015;59:490–7.
63. Delves MJ, Ruecker A, Straschil U, Lelièvre J, Marques S, López-barragán MJ. Male and female *Plasmodium falciparum* mature gametocytes show different responses to antimalarial drugs. Antimicrob Agents Chemother. 2013;57:3268–74.
64. Amedei A, Elios MMD. New therapeutic approaches by using microorganism-derived compounds. Curr Med Chem. 2012;19:3822–40.
65. Mishra BB, Tiwari VK. Natural products: an evolving role in future drug discovery. Eur J Med Chem. 2011;46:4769–807.
66. Tlhapi DB, Ramaite IDI, Van RT, Anokwuru CP, Orazio T, Hoppe HC. Isolation, chemical profile and antimalarial activities of bioactive compounds from *Rauvolfia caffra* Sond. Molecules. 2018;24:39.
67. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: role and biochemical activity in plants and human. J Med Plants Res. 2011;5:6697–703.
68. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta. 2013;1830:3670–95.
69. Udeinya JI, Shu EN, Quakyi I, Ajayi FO. An antimalarial neem leaf extract has both schizonticidal and gametocytocidal activities. Am J Ther. 2008;15:108–10.
70. Dhar R, Zhang K, Talwar GP, Garg S, Kumar N. Inhibition of the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite *Plasmodium falciparum* by neem (*Azadirachta indica*) fractions. J Ethnopharmacol. 1998;61:31–9.
71. Jones IW, Denholm AA, Ley SV, Lovell H, Wood A, Sinden RE. Sexual development of malaria parasites is inhibited in vitro by the neem extract Azadirachtin, and its semi-synthetic analogues. FEMS Microbiol Lett. 1994;120:267–73.
72. Harinantenaina L, Bowman JD, Brodie PJ, Slebodnick C, Callmander MW, Rakotobe E, et al. Antiproliferative and antiplasmodial dimeric phloroglucinols from *Mallotus oppositifolius* from the Madagascar dry forest. J Nat Prod. 2013;76:388–93.
73. Balaich JN, Mathias DK, Torto B, Jackson BT, Tao D, Ebrahimi B, et al. The nonartemisinin sesquiterpene lactones parthenin and parthenolide block *Plasmodium falciparum* sexual stage transmission. Antimicrob Agents Chemother. 2016;60:2108–17.

74. Vu H, Roullier C, Campitelli M, Trenholme KR, Gardiner DL, Andrews KT, et al. *Plasmodium* gametocyte inhibition identified from a natural-product-based fragment library. ACS Chem Biol. 2013;8:2654–9.
75. Moussavi N, Malterud KE, Mikolo B, Dawes D, Chandre F, Corbel V, et al. Identification of chemical constituents of *Zanthoxylum heitzii* stem bark and their insecticidal activity against the malaria mosquito *Anopheles gambiae*. Parasit Vectors. 2015;8:503.
76. Goodman CD, Austarheim I, Mollard V, Mikolo B, Malterud KE, McFadden GI, et al. Natural products from *Zanthoxylum heitzii* with potent activity against the malaria parasite. Malar J. 2016;15:481.
77. Cox A, Krishnankutty N, Shave S, Howick VM, Auer M, La Clair JJ, et al. Repositioning brusatol as a transmission blocker of malaria parasites. ACS Infect Dis. 2024;10:3586–96.
78. Niu G, Wang X, Hao Y, Kandel S, Niu G, Raptis RG, et al. A novel fungal metabolite inhibits *Plasmodium falciparum* transmission and infection. Parasit Vectors. 2021;14:177.
79. Moyo P, Mugumbate G, Eloff JN, Louw AI, Maharaj VJ, Birkholtz L-M. Natural products: a potential source of malaria transmission blocking drugs? Pharmaceuticals. 2020;13:251.
80. Ovchinnikov YA, Abdulaev NG, Feigina MY, Kiselev AV, Lobanov NA. The structural basis of the functioning of bacteriorhodopsin: an overview. FEBS Lett. 1979;100:219–24.
81. Pressman BC. Biological applications of ionophores. Annu Rev Biochem. 1976;45:501–30.
82. Rutkowski J, Brzezinski B. Structures and properties of naturally occurring polyether antibiotics. Biomed Res Int. 2013;2013:162513.
83. Maron MI, Magle CT, Czesny B, Turturice BA, Huang R, Zheng W, et al. Maduramicin rapidly eliminates malaria parasites and potentiates the gametocytocidal activity of the pyrazoleamide PA21A050. Antimicrob Agents Chemother. 2015;60:1492–9.
84. Sun W, Tanaka TQ, Magle CT, Huang W, Southall N, Huang R, et al. Chemical signatures and new drug targets for gametocytocidal drug development. Sci Rep. 2014;4:374.
85. Plouffe DM, Wree M, Alan Y, Scherer CA, Winzeler EA, Plouffe DM, et al. High-throughput assay and discovery of small molecules that interrupt malaria transmission. Cell Host Microbe. 2016;19:114–26.
86. Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, Elizabeth A, et al. The activities of current antimalarial drugs on the life cycle stages of *Plasmodium*: a comparative study with human and rodent parasites. PLoS Med. 2012;9:e1001169.
87. Held J, Gebru T, Kalesse M, Jansen R, Gerth K, Müller R. Antimalarial activity of the myxobacterial macrolide chlorotonil A. Antimicrob Agents Chemother. 2014;58:6378–84.
88. Alessandro SD, Camarda G, Corbett Y, Siciliano G, Parapini S, Cevenini L, et al. A chemical susceptibility profile of the *Plasmodium falciparum* transmission stages by complementary cell-based gametocyte assays. J Antimicrob Chemother. 2016;71:1148–58.
89. Almela MJ, Lozano S, Lelièvre J, Colmenarejo G, Coterón JM, Rodrigues J, et al. A new set of chemical starting points with *Plasmodium falciparum* transmission-blocking potential for antimalarial drug discovery. PLoS ONE. 2015;10:e0135139.
90. Czesny B, Goshu S, Cook JL, Williamson KC. The proteasome inhibitor epoxomicin has potent *Plasmodium falciparum* gametocytocidal activity. Antimicrob Agents Chemother. 2009;53:4080–5.
91. Aminake MN, Schoof S, Sologub L, Leubner M, Kirschner M, Arndt H, et al. Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite proteasome and apicoplast. Antimicrob Agents Chemother. 2011;55:1338–48.
92. Lamonte GM, Almaliti J, Bibo-verdugo B, Keller L, Zou BY, Yang J, et al. Development of a potent inhibitor of the *Plasmodium* proteasome with reduced mammalian toxicity. J Med Chem. 2017;60:6721–32.
93. Saraiva RG, Huitt-roehl CR, Tripathi A, Cheng Y, Bosch J, Townsend CA, et al. *Chromobacterium* spp. mediate their anti-Plasmodium activity through secretion of the histone deacetylase inhibitor romidepsin. Sci Rep. 2018;8:6176.
94. Niu G, Wang B, Zhang G, King JB, Cichewicz RH, Li J. Targeting mosquito FREP1 with a fungal metabolite blocks malaria transmission. Sci Rep. 2015;5:14694.
95. Pastrana-mena R, Mathias DK, Delves M, Rajaram K, King JG, Yee R, et al. A malaria transmission-blocking (+)-usnic acid derivative prevents *Plasmodium* zygote-to-ookinete maturation in the mosquito midgut. ACS Chem Biol. 2016;11:3461–72.
96. Alves C, Silva J, Pinteus S, Gaspar H, Alpoim MC, Botana LM, et al. From marine origin to therapeutics: the antitumor potential of marine algae-derived compounds. Front Pharmacol. 2018;9:1–24.
97. Spavieri J, Allmendinger A, Kaiser M, Itoe MA, Blunden G, Mota MM, et al. Assessment of dual life stage antiplasmodial activity of British seaweeds. Mar Drugs. 2013;11:4019–34.
98. Shao C-L, Mou X-F, Cao F, Spadafora C, Glukhov E, Gerwick L, et al. Bastimolide B, an antimalarial 24-membered marine macrolide possessing a tert -butyl group. J Nat Prod. 2018;81:211–5.
99. Peatey CL, Spicer TP, Hodder PS, Trenholme KR, Gardiner DL. A high-throughput assay for the identification of drugs against late-stage *Plasmodium falciparum* gametocytes. Mol Biochem Parasitol. 2011;180:127–31.
100. Benoit-Vical F, Lelièvre J, Berry A, Deymier C, Dechy-Cabaret O, Cazelles J, et al. Trioxaquines are new antimalarial agents active on all erythrocytic forms, including gametocytes. Antimicrob Agents Chemother. 2007;51:1463–72.
101. Duffy S, Avery VM. Identification of inhibitors of *Plasmodium falciparum* gametocyte development. Malar J. 2013;12:408.
102. Peatey CL, Skinner-Adams TS, Dixon MW, McCarthy JS, Gardiner DL, Trenholme KR. Effect of antimalarial drugs on *Plasmodium falciparum* gametocytes. J Infect Dis. 2009;200:1518–21.
103. Pybus BS, Marcsisin SR, Jin X, Deye G, Sousa JC, Li Q, et al. The metabolism of primaquine to its active metabolite is dependent on CYP 2D6. Malar J. 2013;12:212.
104. Kemirembe K, Cabrera M, Cui L, Kemirembe K, Cabrera M, Cui L. Interactions between tafenoquine and artemisinin-combination therapy partner drug in asexual and sexual stage *Plasmodium falciparum*. Int J Parasitol Drugs Drug Resist. 2017;7:131–7.
105. Bridgford JL, Xie SC, Cobbold SA, Pasaje CFA, Herrmann S, Yang T, et al. Artemisinin kills malaria parasites by damaging proteins and inhibiting the proteasome. Nat Commun. 2018;9:3801.
106. Ramsay RR, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. Br J Pharmacol. 2007;152:946–51.
107. Uhlemann AC, Wittlin S, Matile H, Bustamante LY, Krishna S. Mechanism of antimalarial action of the synthetic trioxolane RBX11160 (OZ277). Antimicrob Agents Chemother. 2007;51:667–72.
108. Yerbanga RS, Lucantoni L, Ouédraogo RK, Da DF, Yao FA, Yaméogo KB, et al. Transmission blocking activity of *Azadirachta indica* and *Guiera senegalensis* extracts on the sporogonic development of *Plasmodium falciparum* field isolates in *Anopheles coluzzii* mosquitoes. Parasit Vectors. 2014;7:185.
109. Moyo P, Shamburger W, Van Der Watt ME, Reader J, De Sousa ACC, Egan TJ, et al. Naphthylisoquinoline alkaloids, validated as hit multistage antiplasmodial natural products. Int J Parasitol Drugs Drug Resist. 2020;13:51–8.

Publisher's Note