HHS Public Access

Author manuscript

Pharmacol Res. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Pharmacol Res. 2017 March ; 117: 192–217. doi:10.1016/j.phrs.2016.11.012.

Artemisinin and its derivatives in treating protozoan infections beyond malaria

Cecilia Shi Ni Loo[a,#], **Nelson Siu Kei Lam**[a,#], **Deying Yu**[a,#], **Xin-zhuan Su**[b,c], and **Fangli Lu**[d,e,*]

[a]Bachelor of Medicine & Bachelor of Surgery, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, Guangdong, China

[b]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

[c]State Key Laboratory of Cellular Stress Biology and School of Life Science, Xiamen University, Xiamen 361005, Fujian, China

[d]Department of Parasitology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, Guangdong, China

[e]Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education, Guangzhou 510080, Guangdong, China

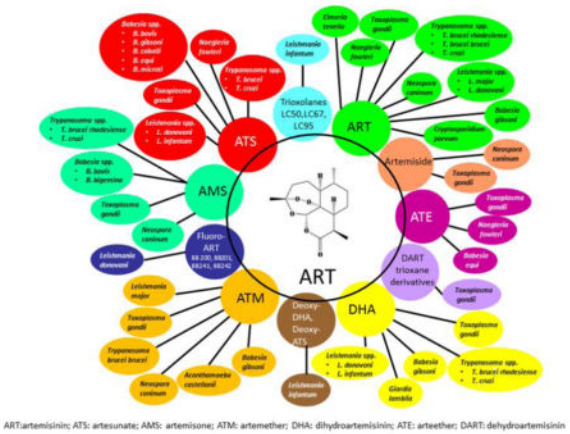
Abstract

Many parasitic protozoan diseases continue to rank among the world's greatest global health problems, which are also common among poor populations. Currently available drugs for treatment present drawbacks, urging the need for more effective, safer, and cheaper drugs. Artemisinin (ART) and its derivatives are some of the most important classes of antimalarial agents originally derived from *Artemisia annua* L. However, besides the outstanding antimalarial and antischistosomal activities, ART and its derivatives also possess activities against other parasitic protozoa. In this paper, we reviewed the activities of ART and its derivatives against protozoan parasites in *in vitro* and *in vivo*, including *Leishmania* spp., *Trypanosoma* spp., *Toxoplasma gondii*, *Neospora caninum*, *Eimeria tenella*, *Acanthamoeba castellanii*, *Naegleria fowleri*, *Cryptosporidium parvum*, *Giardia lamblia*, and *Babesia* spp. We concluded that ART and its derivatives may be good alternatives for treating other non-malarial protozoan infections in developing countries, although more studies are necessary before they can be applied clinically.

Graphical abstract

[*]Corresponding authors. Fangli Lu; fanglilu@yahoo.com.
[#]Contributed equally.

AUTHOR CONTRIBUTIONS (need to be replaced): FL conceived, supervised, and wrote the manuscript. CSNL, NSKL, and DY participated in the writing. X-zS was writing and editing. All authors read and approved the final version of the manuscript.



ART:artemisinin; ATS: artesunate; AMS: artemisone; ATM: artemether; DHA: dihydroartemisinin; ATE: arteether; DART: dehydroartemisinin

Keywords

artemisinin; antiprotozoan activity; *Leishmania* spp; *Trypanosoma* spp; *Toxoplasma gondii*; *Neospora caninum*

1. Introduction

The World Health Organization recognizes 17 major parasitic and related infections as neglected tropical diseases (NTDs) that affect many countries in Africa, Asia, and Latin America. However, the lack of commercial interest in developing new pharmaceutical compounds for combating these diseases has impaired efforts to eliminate these diseases [1,2]. Therefore, discovery of new, safe, effective, and affordable active drugs is urgently needed. Artemisinin (ART) and its derivatives is one of the most important classes of antimalarial drugs. Like many other natural sesquiterpenes, ART displays a range of different biological and pharmacological properties. Except for its antimalarial and antischistosomal activities, ART has also been shown to have antimicrobial [3] and antiviral activities [4]. In addition, the antiparasitic function of ART against non-malarial parasites cannot be ignored. Here we screened the literature through extensive searches of PubMed, ResearchGate, Elsevier ScienceDirect, Wiley Online Library, and the Springer-Link Journals database using the search term “artemisinin” for publications in English with no date limits as well as manual review of some related journals. We outline the use of ART and its derivatives in treating parasitc diseases or parasitic infections caused by protozoan parasites including *Leishmania* spp., *Trypanosoma* spp., *Toxoplasma gondii*, *Neospora caninum*, *Eimeria tenella*, *Acanthamoeba castellanii*, *Naegleria fowleri*, *Cryptosporidium parvum*, *Giardia lamblia*, and *Babesia* spp.; only drugs with activities against these protozoan parasites are reported. Studies have demonstrated good efficacies of ART and its derivatives *in vivo* and *in vitro* towards some of the parasitic protozoan infections. Clinical trials using different semisynthetic and synthetic ART derivatives should be undertaken to develoop treatments for these parasitic diseases.

2. Chemical characteristics of ART and its derivatives

In 1967, under the instructions of Chairman Mao and Premier Zhou, a secret project called “Project 523” was launched to develop a new drug to combat drug-resistant malarial parasites [5,6]. In 1971, Youyou Tu and her team isolated a new anti-malarial drug, called qinghaosu (or ART), from *Artemisia annua* L. (Qinghao), which was shown to inhibit proliferation of *Plasmodium* parasites [6].

ART and its derivatives share a common structural feature called endoperoxide linkage [7]. In general, ART has poor solubility in water or oil. However, by reducing the C-10 carbonyl group of ART, a more water-soluble derivative dihydroartemisinin (DHA) can be obtained [5,8]. Artesunate is the water-soluble hemisuccinate ester of DHA [9]. According to Chaturvedi et al., by adding a methyl or ethyl ethers at the same carbonyl group, higher oil soluble compounds like artemether and arteether were obtained [7]. Artemisone was obtained by replacing the same carbonyl with amines in the ART molecule, which showed improved water solubility, better toxicity profile, and a longer half-life [8]. Due to the short *in vivo* half-lives and low bioavailability of ART derivatives, arterolane and artefenomel (1, 2, 4-trioxolane analogues) were created to achieve more favourable pharmacokinetics. They were synthesised by coupling symmetrical O-methyl 2-adamantanone oxime with cyclohexanones by the Griesbaum co-ozonolysis [10]. Arterolane and artefenomel have superior half-lives compared to DHA, higher bioavailability than artemether, and are comparable to artesunate [11]. However, trioxolane-like arterolane has relatively low *in vivo* stability [8]; that is why 1, 2, 4, 5-tetraoxanes with similar peroxide bridges, better stability and more potent anti-malarial activity such as RKA 216 and RKA182 were developed [12]. These compounds are currently under development [8]. The structures of artemisinin and its derivatives are shown in Fig 1.

3. ART and its derivatives on *Leishmania* spp

Leishmaniasis affects approximately 350 million people in 98 countries around the world [13]. The disease manifests primarily as three forms, namely cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis, with the last being fatal if left untreated [14]. Current anti-leishmanial drugs include pentavalent antimony, amphotericin B, paromomycin, pentamidine, and miltefosine, with toxic effects to the liver, heart, and kidney, as well as anaemia, fever, and hypokalaemia. Therefore, usage is limited by their toxicity and adverse reactions [15, 16]. Miltefosine and paromomycin are two drugs that have been introduced more recently for the treatment of leishmaniasis [16]. However, long-term therapy with miltefosine’s long half-life (about 152 h) can promote early onset of drug resistance, and potential teratogenic and abortifacient effects limit its prescription during pregnancy [17,18]. Paromomycin is an aminoglycoside antibiotic that has shown promising results in the treatment of leishmaniasis, mainly for treating cutaneous leishmaniasis [16]. However, *in vitro* tests have led to the emergence of paromomycin-resistant [19], miltefosine-resistant [19], meglumine antimoniate-resistant [20], and pentamidine-resistant [21] parasites; therefore the therapy of visceral leishmaniasis is limited by resistance, toxicity, and decreased bioavailability of the existing anti-leishmanial agents [22]. The effects of ART and its derivatives on *Leishmania* parasites have been studied in mice and *in*

vitro. They are efficient in inhibiting the parasite metabolism, while showing limited adverse effects on the host, indicating a higher safety index of the drugs [23,24]. A large number of *in vitro* or *in vivo* studies have shown that ART and its derivatives have activities in controlling the parasites, and the drugs shown effective against the protozoan are selected and summarized in Table 1.

In vitro studies

ART works by impairing the activation of host macrophages, preventing the production of lethal nitric oxide (NO) and restoring normal NO production in *L. major*-infected macrophages [25]. Similar effects have been observed for the treatment of *L. major*, *L. infantum*, *L. tropica*, *L. braziliensis*, *L. mexicana*, and *L. amazonensis* [17,26]. In addition, ART also enables externalization of phosphatidylserine and leads to the loss of mitochondrial membrane potential, cell-cycle arrest at the sub-G0/G1 phase, and programmed cell death of *L. donovani* promastigotes [23]. Iron in excess binds to haemoglobin and activates ART by aiding the formation of intra-parasitic heme-iron, which catalyses the cleavage of the endoperoxide ring and enables the transfer of an oxygen atom from the peroxide group to a chelated iron ion, generating a Fe(IV)O species. The resultant free radical intermediate or iron-ART adduct then effectively kill the promastigotes of *Leishmania* parasites by alkylation [27]. ART showed high toxicity and apoptotic effect on promastigotes and low toxicity on BALB/c macrophages [28]. Artemisinin-derived trioxanes and synthetic trioxolanes were tested against promastigotes and intramacrophage amastigotes of *L. infantum*, in which trioxolanes LC50 and LC95 had the best activity and safety profiles, showing potential for leishmanial therapy [29]. Fluoro-artemisinin derivatives (BB200, BB201, BB241, and BB242) are proven to have higher efficacy than DHA and sitamaquine, especially BB201 was shown to have higher efficacy than miltefosine, DHA, and sitamaquine against the parasite [30]. ART [23,28], artemether, and deoxydihydroartemisinin (Deoxy-DHA) [31] had anti-leishmanial effects in infected host cells. In another study, fluorophenyl-artemisinin was shown to have higher efficacy in inhibiting *L. donovani in vitro* growt than artemisinin, artemison, and comparable efficacy to amphotericin B [32].

In vivo studies

Studies have shown that the leaves and seeds of *A. annua* caused increased production of Th1 cytokines [interferon-gamma (IFN-γ)] and decreased Th2 cytokines [interleukin (IL)-4 and IL-10] in *L. donovani*-infected BALB/c mice [33]. The anti-leishmanial activity of ART was mediated by increased IL-4 and IFN-γ levels in the host cells, inducing apoptotic effects on promastigotes and reducing post-treatment lesion size in *L. major*-infected BALB/c mice. In addition, using topical ART ointment as a route of administration, a higher healing effect compared to other routes of administration was achieved [28]. ART-loaded nanoparticles and combination therapy of diminazene and ART have a therapeutic effect comparable to that of the first-line drug amphotericin B, leading to significant reduction in hepatomegaly and parasite burden in *L. donovani*-infected BALB/c mice [24,33].

Human trial

The only clinical study presently available failed to show a significant difference between the artesunate plus sulfamethoxypyrazine/pyrimethamine treatment and the placebo [34].

ART and its derivatives on *Trypanosome* spp

There are two distinct species of *Trypanosome* in humans: *Trypanosoma brucei* causes African trypanosomiasis or African sleeping sickness and *T. cruzi* causes American trypanosomiasis or Chagas' disease [39]. The only drugs now available for Chagas disease, nifurtimox and benznidazole, are relatively toxic for adult patients, and require prolonged administration [40]. The drugs available for African sleeping sickness are pentamidine isethionate, suramin, melarsoprol, eflornithine, or nifurtimox–eflornithine combination treatment, the use of which depends on the disease stage and causative pathogen with toxicity and possible resistance [41]. However, the clinical use of the current medications is compromised due to emerging strain-specific drug resistance, serious side-effects, high toxicity levels, and lengthy parental administration [42]. There is a critical need to develop new drugs for treatment of Chagas disease and sleeping sickness. The studies of ART and its derivatives on *Trypanosome* spp. are summarized in Table 2.

In vitro studies

The drugs for *in vitro* studies include artesunate [43], ART, artemisone, and 4-fluorophenyl-ART [32]; the cell lines or cells used include African green monkey kidney cell line (Vero) [44], leukaemia cell line (HL-60) [45], and peripheral blood of infected rat [43]. Artesunate can inhibit the replication of *T. cruzi* epimastigotes, amastigotes, and trypomastigotes in Vero cells *in vitro* [44]. Extracts (methanol extract and dichloromethane extract) from the leaves and aerial parts of four Artemisia species (*Artemisia absinthium*, *A. abyssinica*, *A. afra*, and *A. annua*) growing in Ethiopia have been tested against *T. brucei brucei in vitro*. The dichloromethane extract from *A. abyssinica* was most active (IC_{50} = 19.13 μg/mL), while ART from *A. annua* also showed anti-trypanosomal and cytotoxic activities (IC_{50} = 35.91 μg/mL), which may be due to lipophilic sesquiterpene lactones in the extract [45]. One of the mechanisms of trypanocidal activity is that lipophilic compounds such as lipophilic sesquiterpene lactones in the extract can increase the fluidity of the membranes leading to uncontrolled efflux of ions and metabolites resulting in cell death [45–47]. Another mechanism of trypanocidal activity is the inhibition of ATPase at the presence of calcium, e.g., the inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) by inhibiting H^+/K^+-ATPase could be related to the inhibition of trypanosome growth [32]. The combination of artesunate and diminazene aceturate showed a complete clearance of *T. brucei* on day 5 in comparison of artesunate monotherapy on day 7 [43]. *S*tudies showed that artesunate inhibits the replication of epimastigotes and intracellular amastigotes of three strains of *T. cruzi* originated in different regions of Latin America *in vitro* [44]. In addition, artemisinin compounds inhibit *T. cruzi* growth *in vitro* and inhibit calcium-dependent ATPase activity in *T. cruzi* membranes [32].

In vivo studies

The animals including mice [43, 44] and rats [48] have been used in the studies. It has been reported that 2.3 mg/kg and 4.6 mg/kg of artemether are able to suppress and reduce the number of *T. brucei brucei* parasites, and extend the lifespan of rats at the early and late stage treatment [48]. The lifespan of infected rats was extended to 19 days using a combination of halofantrine HCl with artemether compared to 14 days with artemether monotherapy [46]. Halofantrine HCl can form complex with heme-iron [49, 50], which has lytic properties towards the parasite. The half-life of halofantrine HCl ranged from 1 to 4 days, thereby giving halofantrine HCl a high bioavailability [51], which may be one of the factors that prolongs the lifespan of *T. brucei*-infected rats [48,]. The reduction in parasitaemia is probably due to the ability of artemether to form complex with heme that is believed to be toxic to the parasite [52, 53]. The clinical signs of dullness and paleness of mucous membrane on *T. brucei*-infected mice disappeared by both artemether and artesunate treatment. However, compared with artesunate, artemether is more effective as it clears the parasite completely from the body after 5 days of post-treatment [43]. In another study, artesunate alone was not effective in reducing the parasites in *T. cruzi*-infected BALB/c mice and failed to cure the infection, suggesting that artesunate may be unsuitable for *in vivo* treatment of *T. cruzi* infection [44].

4. ART and its derivatives on *T. gondii*

T. gondii infects a large number of warm-blooded animals and is estimated to chronically infect up to one-third of the world's human population. Toxoplasmic encephalitis in patients with AIDS is a life-threatening disease mostly due to reactivation of *T. gondii* cysts in the brain [54]. One of the current therapies for toxoplasmic infection is a combination of the drugs sulfadiazine and pyrimethamine [55], which cause hematological side-effects that can be controlled by the administration of folinic acid. The other treatment is the combination of trimethoprim and sulfamethoxazole (cotrimoxazole), which is well tolerated and less toxic to hematopoiesis. However, this drug can cross the placental barrier, so that its use is discouraged for pregnant women [56]. Moreover, these medicines have no effect on the tissue cysts of the parasite located predominantly in a brain and muscles [57]. It has been reported that medicinal plants can be used for toxoplasmosis as an alternative to standard drug therapy with reduced side effects. The studies of ART and its derivatives on *T. gondii* are analysed, and summarized in Table 3.

In vitro studies

RH strain of *T. gondii* and human foreskin fibroblast (HFF) cells were used in most of the *in vitro* studies. ART and its derivatives tested for their *in vitro* efficacy (IC_{50}) against *T. gondii* and cytotoxicity (TD_{50}) were measured to obtain the selectivity (SI) and/or therapeutic index (TI). Artesunate is proven to have much higher efficacy than current first-line drugs such as cotrimoxazole, pentamidine, pyrimethamine, and trimethoprim [58]. Intraparasitic heme activates ART and its derivatives to form reactive oxygen species (ROS) in the cytoplasm of host cells, which is then delivered across the parasitophorous vacuole membrane (PVM) to reach the tachyzoites within the PVM. ROS may damage the parasite by alkylating and poisoning one or more essential proteins and trigger the death of infected cells to control the

growth of tachyzoites [59]. In addition, ART has the ability to trigger calcium-dependent secretion of microneme proteins in *T. gondii* parasites, affecting calcium homeostasis and signalling in the parasite, and acts as an inhibitor of SERCA [60]. Perturbation of Ca^{2+} intake levels not only blocks parasite microneme secretion but also prevents motility and cell invasion of the parasite [61]. Arteether is lipophilic and therefore has activity against the central nervous system infection. At concentration ≥ 0.5 μg/mL, arteether mediated reduction in incorporation of radiolabeled uracil into the nucleic acids of *T. gondii* tachyzoites [62] and showed higher efficacy than cycloguanil in inhibiting the parasite. Eight thiazole derivatives and two carboxamide derivatives of deoxyhydroartemisin (DART) trioxane derivatives displayed effective growth inhibitory activities of *T. gondii* parasites, comparable to artemether in potency and over 100 times more potent than the front-line drug trimethoprim. The thiazoles were more effective than the other derivatives in inhibiting the growth of both the extracellular and intracellular parasites. Unexpectedly, two thiazole trioxanes (named compounds 5 and 6) were parasiticidal, both inhibited parasite replication irreversibly when parasites were exposed to 10 μM of the drugs for 24 h, whereas the standard trioxane drugs (ART and artemether) were not parasiticidal [63].

In vivo studies

CD1, OF1, and Kunming mice have been used in animal studies; extensive studies have shown that ART, artemether, arteether, artesunate, DHA, artimisone, artimiside, and other derivaties are active against *T. gondii*. In these studies, after treatment with ART or its derivatives, the total number of cysts per brain was counted from the research subjects to evaluate the efficacy of the drugs [64–66]. Using CD1 mice-infected with the PRU-Luc-GFP type II strain of *T. gondii*, approximately 20% of ART-treated mice survived the infection; however 60% of the artemiside-treated mice and over 50% of the artemisone-treated mice survived the infection at 25 days p.i. In addition, compared with ART-treated mice, artemiside- and artemisone-treated mice developed significantly lower parasite burdens, e.g. luciferase (Luc) values in the brain at 9 days p.i. and significantly lower cyst numbers in the brain at 25 days p.i., indicating artemiside and artemisone have better antiparasitic efficacy on *T. gondii* than that of ART [64].

ART and its derivatives on *N. caninum* and *E. tenella*

Neosporosis, caused by *N. caninum* is a serious disease of dogs and livestock worldwide. Although no case of *N. caninum* infection has been reported in humans so far, the possibility of human *N. caninum* infection cannot be excluded due to its close phylogenetic relationship with *T. gondii* and its wide range of potential hosts [70]. Currently, there is no safe and effective chemotherapy for bovine neosporosis. Studies available for effective treatment of *N. caninum* using ART [71] and its derivatives (artemisone [72–74], artemether [75], GC007, and GC012 [74]) are summarized in Table 4.

An *in vitro* study showed that at a concentration of 10 or 20 μg/mL, ART was able to reduce *N. caninum* and completely eliminated all microscopic foci of *N. caninum* in Vero cells by 11 days (third subculture) and at 1 μg/mL, ART reduced *N. caninum* and completely eliminated all microscopic foci of *N. caninum* by 14 days (fourth subculture). The

morphology and growth rate of the uninfected Vero cells treated with 20 μg/mL of ART were normal, suggesting that the toxicity of ART is low. However, pretreatment of host cells or *N. caninum* tachyzoites with ART had no effect on intracellular replication of *N. caninum* tachyzoites [71]. An *in vitro* study showed that the parasites undergo apoptosis rather than uncontrolled cell death by long-term treatments using artemisinin derivatives including artemisone, GC007, and GC012, which further proves the activity of ART derivatives in controlling *N. caninum* parasites [74]. However, artemiside and artemisone did not affect the cerebral parasite burden when assessed in a chronic infection model of *N. caninum*-infected BALB/c mice [74]. In another *in vitro* study, parasite growth inhibition can be as high as 92.1% when the artemether concentration was 100 μg/mL [75].

In vivo studies

In an animal trial using gerbils (*Meriones tristrami*), only one out of 9 treated gerbils exhibited characteristic cerebral clinical signs and died 10 days after infection, indicating that artemisone plays a role in increasing the survivability of the infected hosts [72]. In another *in vivo* experiment, parasite burden in the brains of male BALB/c mice after treatment by artemisone is lower than that by placebo, mefloquine, or artemiside treatment, suggesting possible efficacy of this drug; however the parasite burden in the lungs after artemisone treatment is higher than those of the above-mentioned drugs [73]. Thus, further *in vivo* investigations are needed for these drugs.

Avian coccidiosis is the most significant parasitic disease in the poultry industry [76]. Chickens are affected by seven different *Eimeria* spp. that infect the gut and are transmitted between birds via ingestion of infective oocysts. Several drugs such as nicarbazin, amprolium, quinolone, and ionophores, alone or in combination have proven to be an effective alternative against avian coccidiosis [77]. However, the emergence of drug-resistant strains, especially after a prolonged use of a drug, is a real problem. Based on the study on ART by del Cacho et al. (2010), there are significant reduction in oocyst output, mortality, and sporulation rate with an ART dose of 17 ppm compared to 10 ppm in *E. tenella*-infected white leghorn chicken [78]. By using immunofluorescence technique, SERCA in macrogametes was found to be inhibited by ART. Because SERCA plays a role in Ca^{2+} homeostasis [79], the inhibition of SERCA by ART impairs Ca^{2+}-dependent ATPase and causes altered secretion of the wall-forming bodies in macrogametes [78]. These findings suggested that ART can be used as an alternative anti-coccidial drug for avian coccidiosis, due to its anti-*E. tenella* activity on reducing intestinal lesions, oocyst output, and oocyst sporulation. The results from selected studies of ART and its derivatives on *E. tenella* are also summarized in Table 4.

7. ART and its derivatives on *A. castellanii* and *N. fowleri*

Acanthamoeba spp. are the causative agents of *Acanthamoeba* keratitis, fatal granulomatous amoebic encephalitis, and cutaneous infections [80]. Currently, no methods or single drug exists that can eliminate both cystic and trophozoite forms of *Acanthamoeba*. To treat the cyst form of *Acanthamoeba* that is highly resistant to therapy, a combination of agents is generally used [81]. An *in vitro* study shows that using 200 μg/mL of artemether for 24 h

against *A. castellanii* trophozoites causes a reduction in the number of trophozoites [82]. The effect toward trophozoites was minimal with slight changes of structure at 100 μg/mL of artemether; there were a decreased number of trophozoites after 6 days of cultivation with 150 μg/mL of artemether and there were no viable trophozoites after 5 days of cultivation with 200 μg/mL of artemether. Based on the results, the amoebicidal activities of artemether against *A. castelanii* trophozoites were found to be time and dose-dependent. Among ART, artesunate, and DHA, DHA had the strongest amoebicidal activity against *Acanthamoeba*, followed by artemether [82]. The inhibition of *Acanthamoeba* trophozoites by artemether may be due to its ability to downregulate phosphoglycerate dehydrogenase (PGDH) and phosphoserine aminotransferase (PSAT) in trophozoites. Based on the isobaric tags for relative and absolute quantitation (iTRAQ) analysis on *Acanthomoeba* treated with 200 μg/mL of artemether, it was shown that the levels of PGDH and PSAT were decreased through inhibition of the L-serine biosynthesis pathway [82]. Further studies are needed to verify the efficacy and mechanisms of ART and its derivatives against *Acanthamoeba* infection.

N. fowleri causes a fulminating and rapidly fatal primary amoebic meningoencephalitis (PAME) in humans [83]. The major problem of curing infections involving the pathogenic free-living amoebae is the lack of effective therapeutics [84]. ART, arteether, and artesunate have been tested against experimental PAME, and the efficacy of these compounds has been compared with amphotericin B, a standard drug for this pathogen. Compared to ART and its derivatives, 2.5 mg/kg of amphotericin B is effective in treating PAME of *N. fowleri*-infected Swiss mice. ART and artesunate showed only slight protection at 120 mg/kg by increasing the mean survival time to 1.9 and 2.6 days, respectively. Beta-arteether showed slightly better results at 120 mg/kg with an increase of the mean survival time to 5.3 days. However, all of the mice treated with the maximum tolerated dose (180 mg/kg) of ART and its derivatives suffered from PAME. This indicates that ART can increase the mean survival time but is unable to cure PAME caused by *N. fowleri* in Swiss mice [85]. Therefore, more studies are needed to confirm the effect of ART towards *N. fowleri*. The studies of ART and its derivatives on *A. castellanii* and *N. fowleri* are summarized in Table 5

8. ART and its derivatives on *C. parvum* and *G. lamblia*

Human cryptosporidiosis is mainly caused by *C. hominis* and *C. parvum*, which are responsible for most of the outbreaks of *Cryptosporidium* described so far [86]. However, treatment faces challenges because of the limited options of using macrolides, paramomycin, nitazoxanide, and mirazid that have partial efficacy in reducing disease severity in immunocompromised individuals [87]. The use of ART towards *C. parvum* infection is not effective compared to macrolides antibiotics, and ART only caused a minor decrease in the mean number of oocyts at the highest concentration (2 mg/L). Thus, the inhibitory effects of ART against *C. parvum* showed limited effectiveness [88]. So far there are few reports on ART and its derivatives in the treatment of *Cryptosporidium* spp.; further studies are needed to explore their potential in treating this disease.

Giardia lamblia (syn. *G. intestinalis*, *G. duodenalis*) is one of the most frequent human intestinal protozoa causing giardiasis and infects hundreds of millions of people every year

[89]. Metronidazole, tinidazole, mebendazole, albendazole, and furazolidone are effective anti-*Giardia* drugs, but have severe side effects and potential toxicity. Developing more effective and less toxic drugs against this protozoan parasite is necessary. The effects of DHA (LD_{50} = 200 μg/mL) at different time intervals on *G. lamblia in vitro* were investigated by using microscopy and cytometry techniques, and it was shown that DHA induced the changes in morphology and cell cycle state in *G. lamblia* [90]. The studies of ART and its derivatives on *C. parvum* and *G. lamblia* are summarized in Table 6.

9. ART and its derivatives on *Babesia* spp

Human babesiosis is a zoonotic disease caused by protozoan parasites of the *Babesia* genus. In humans, the most prevalent are infections caused by *Babesia microti* and less frequently, *B. divergens*, *B. duncani*, or *B. venatorum* [91]. Babesiosis shares many clinical features with malaria and can be fatal, particularly in the elderly and in immunocompromised individuals. Anti-malaria drugs and some antibiotics have been used in chemotherapy of babesiosis, including atovaquone, azithromycin, clindamycin, and quinine [92]. However, atovaquone-resistant genotype of *cytochrome b* gene has been detected in three cases of *B. gibsoni* infections in Japan [93]. The investigations of ART and its derivatives on *Babesia* spp. are summarized in Table 7.

In vitro studies

Different *Babesia* species (*B. bovis*, *B. bigemina*, *B. caballi*, *B. equi*, and *B. gibsoni*) have been tested with ART and its derivatives (DHA, artemisone, artesunate, and arteether). Artemisone induced dose-dependent growth inhibition of both *B. bovis* and *B. bigemina*, of which *B. bigemina* appeared to be more sensitive to artemisone than *B. bovis*, as reflected by the different corresponding IC_{50} [94]. A dose- and time-dependent inhibitory effect for artesunate was also reported against *B. bovis.* The study showed that artesunate was less effective than atovaquone and diminazene aceturate in inhibiting the growth of *B. bovis* [95]. Artesunate and pamaquine were more effective in suppressing parasitemia of *B. equi* than that of *B. cablli,* but pyrimethamine was more resistant to *B.* equi than that of *B. caballi* by comparing their IC_{50} [96]. For *B. gibsoni,* artesunate was more effective in inhibiting the parasitemia than those of artemether, ART, and DHA but not atovaquone and diminazene [95, 96, 97]. In another study, it was reported that the single use of ART derivatives may only have low effectiveness, but the addition of lumefantrine to artemether may be effective for *B. gibsoni* infection [98]. *Babesia* parasites do not produce hemozoin nor contain food vacuoles [99]. The absence of these organelles in *Barbesia* spp. may reduce the action of ART and its derivatives compared with *Plasmodium* spp. The possible mechanism of ART and its derivatives against *Barbesia* spp. may be related to the activation of the mitochondrial electron transport system resulting in ROS production [100]. The mechanism of action is still not yet fully understood.

In vivo studies

Various *Babesia* species (*B. bovis*, *B. bigemina*, *B. microti*, and *B. equi*) have been tested in different experimental animal models (calves, mice, and donkeys) for the anti-babesial effects of artemisone, artesunate, and arteether. The routes of administration are mostly

intramuscular [95, 101] but also intraperitoneal [94]. Calves infected with *B. bigemina* and *B bovis* were treated by artemisone (5 mg/kg) intraperitoneally and resulted in recovery of all animals, except for one *B. bovis*-infected calf. *B. bigemina* appeared to be more sensitive to artemisone than *B. bovis* as reflected by the differences of their IC_{50}, although the treatment did not completely eliminate *Babesia* parasites. *B. microti*-infected BALB/c mice were intramuscularly treated by artesunate, which was not only inhibited the growth of the parasite but also delayed the increase of parasitemia, indicating that it can be a potential anti-*B. microti* agent [94]. Efficacy can be improved by combination with other babesiacide. It has been reported that splenectomised donkeys infected with *B. equi* were treated by intramuscular artesunate, arteether, or intramuscular arteether plus intravenous buparvaquone. In the artesunate-treated group, initial decrease in parasitaemia was observed, but the mean maximum parasitaemia increased dramatically between 16–19 days post-treatment; no recovery was observed in either the arteether- or buparvaquone-treated group. However, in the arteether-buparvaquone combination group, recovery was observed as parasites were cleared after 4-day treatment. Arteether and buparvaquone combination regimen also seemed to be safer than imidocarb alone in this study [101].

10. Conclusion

NTDs are an extremely important issue facing global health care, which mostly affect people living in extreme poverty. ART was discovered by Youyou Tu and her colleaques in China more than 40 years ago, which contributed greatly to treatment and control of malaria, and has led to Tu's sharing the 2015 Nobel Prize in Physiology or Medicine. Except for its well-known anti-malarial effect, ART and its derivatives also have anti-parasitic activities against many other parasitic infections, including protozoan parasites that infect humans such as *Leishmania* spp., *Trypanosoma* spp., *Acanthamoeba castellanii*, *Naegleria fowleri*, and *G. lamblia*. In addition, these compounds are also active against protozoan parasites of both humans and domestic animals/livestocks such as *T. gondii*, *C. parvum*, and *Babesia* spp., and domestic animal/livestock protozoan parasites such as *N. caninum* (causing a serious disease of dogs and livestock, with the potential to infect humans) and *Eimeria* spp. (causing avian coccidiosis). The current first-line anti-protozoal drugs have limitions, including serious side-effects, toxicity, drug resistance, and even lack of effective or no chemotherapy. Thus, new drugs for the treatment of these protozoan infections are urgently needed. Greater emphasis should be placed on research and development of combination chemotherapies. ART or its derivatives are relaively low cost and low toxicity, and are promising anti-protozoal drugs alone or in combination with the current anti-protozoal drugs, which open new windows for combination therapies of parasitic protozoans. Arteether is a lipophilic compound that has good potential for therapies against protozoan infections involving the central nervous system such as *T. gondii*, *Acanthamoeba*, *N. fowleri*, and *Trypanosoma* spp. The emergence of drug-resistant strains of *Leishmania* and *Trypanosoma* make the current treatment face new challenges. So far there are no safe and effective chemotherapy for giardiasis and bovine neosporosis. Therefore, further efforts should focus on evaluation of the effectiveness of the compounds on these protozoan infections. Finally, the therapeutic mechanisms both in animals and in humans require more investigations. Therefore,

understanding of structure-activity relationship of ART and its derivatives might lead to the development of effective drugs against parasitic protozoans.

Acknowledgments

Research reported in this publication was supported in part by the Natural Science Foundation of China (nos. 81471973 and 81273643); the Division of Intramural Research at the National Institute of Allergy and Infectious Diseases, National Institutes of Health; the Science and Technology Planning Project of Guangdong Province, China (no. 2014A020212108), and the Project 111 of the State Bureau of Foreign Experts and Ministry of Education of China (B06016); the Project on Brand Professional Construction of Sun Yat-sen University, and Three Major Construction (big team, big platform, and big project) of Sun Yat-sen University. The authors thank Cindy Clark, NIH Library Writing Center, for manuscript editing assistance.

List of abbreviations

ART	Artemisinin
b.i.d	two times per day
CH2CL2	dichloromethane
CI	cell index
DART	dehydroartemisinin
Deoxy-DHA	deoxydihydroartemisinin
DHA	dihydroartemisinin
DMSO	dimethyl sulphoxide
HFF	human foreskin fibroblast
IC50	concentration that causes 50% inhibition of growth
IC90	concentration that causes 90% inhibition of growth
i.g	intragastric administration
i.m	intramuscular injection
i.p	intraperitoneal injection
iTRAQ	isobaric tags for relative and absolute quantitation
i.v	intravenous injection
Luc value	luciferase value
MeOH	methanol
NO	nitric oxide
PCV	packed cell volume
PGDH	phosphoglycerate dehydrogenase

p.i	post-infection
PI staining	propidium iodide staining
p.o	oral administration
PSAT	phosphoserine aminotransferase
q.d	one time per day
q.i.d	four times per day
RBC	red blood cell
s.c	subcutaneous injection
SEM	scanning electron micrograph
SERCA	sarco/endoplasmic reticulum Ca2+-ATPase
SI	selectivity index
TD50	median cytotoxic dose
TI	therapeutic index
TEM	transmission electron micrograph
t.i.d	three times per day
t.p	topical administration

References

1. World Health Organization. Working to overcome the global impact of neglected tropical diseases: First WHO report on neglected tropical diseases. Geneva: 2010. p. 172
2. Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N. Drug development for neglected diseases: a deficient market and a public-health policy failure. The Lancet. 2002; 359(9324):2188–2194. doi:1fr;0.1016/S0140-6736(02)09096-7.
3. Appalasamy S, Lo KY, Ch'ng SJ, Nornadia K, Othman AS, Chan L-K. Antimicrobial activity of artemisinin and precursor derived from *in vitro* plantlets of Artemisia annua L. BioMed research international. 2014; 2014doi: 10.1155/2014/215872
4. Roy S, He R, Kapoor A, Forman M, Mazzone JR, Posner GH, Arav-Boger R. Inhibition of human cytomegalovirus replication by artemisinins: effects mediated through cell cycle modulation. Antimicrobial agents and chemotherapy. 2015; 59(7):3870–3879. DOI: 10.1128/AAC.00262-15 [PubMed: 25870074]
5. Miller LH, Su X. Artemisinin: discovery from the Chinese herbal garden. Cell. 2011; 146(6):855–858. DOI: 10.1016/j.cell.2011.08.024 [PubMed: 21907397]
6. Su XZ, Miller LH. The discovery of artemisinin and the Nobel Prize in Physiology or Medicine. Science China Life Sciences. 2015; 58(11):1175–1179. DOI: 10.1007/s11427-015-4948-7 [PubMed: 26481135]
7. Chaturvedi D, Goswami A, Saikia PP, Barua NC, Rao PG. Artemisinin and its derivatives: a novel class of anti-malarial and anti-cancer agents. Chemical Society Reviews. 2010; 39(2):435–454. DOI: 10.1039/b816679j [PubMed: 20111769]

8. Barnett DS, Guy RK. Antimalarials in Development in 2014. Chemical reviews. 2014; 114(22): 11221–11241. DOI: 10.1021/cr500543f [PubMed: 25340626]
9. Cui L, Su X-z. Discovery mechanisms of action and combination therapy of artemisinin. Expert review of anti-infective therapy. 2009; 7(8):999–1013. DOI: 10.1586/eri.09.68 [PubMed: 19803708]
10. Griesbaum K, Liu X, Kassiaris A, Scherer M. Ozonolyses of O - Alkylated Ketoximes in the Presence of Carbonyl Groups: A Facile Access to Ozonides. Liebigs Annalen. 1997; 1997(7): 1381–1390. DOI: 10.1002/jlac.199719970715
11. Vennerstrom JL, Arbe-Barnes S, Brun R, Charman SA, Chiu FC, Chollet J, Dong Y, Dorn A, Hunziker D, Matile H. Identification of an antimalarial synthetic trioxolane drug development candidate. Nature. 2004; 430(7002):900–904. DOI: 10.1038/nature02779 [PubMed: 15318224]
12. Opsenica I, Opsenica D, Smith KS, Milhous WK, Šolaja BA. Chemical Stability of the Peroxide Bond Enables Diversified Synthesis of Potent Tetraoxane Antimalarials (1). Journal of medicinal chemistry. 2008; 51(7):2261–2266. DOI: 10.1021/jm701417a [PubMed: 18330976]
13. Gutiérrez V, Seabra AB, Reguera RM, Khandare J, Calderón M. New approaches from nanomedicine for treating leishmaniasis. Chemical Society Reviews. 2016; 45(1):152–168. DOI: 10.1039/c5cs00674k [PubMed: 26487097]
14. Sharma U, Singh S. Immunobiology of leishmaniasis. Indian journal of experimental biology. 2009; 47(6):412. [PubMed: 19634705]
15. Lachaud L, Bourgeois N, Plourd M, Leproho P, Bastien P, Ouellette M. Parasite susceptibility to amphotericin B in failures of treatment for visceral leishmaniasis in patients coinfected with HIV type 1 and *Leishmania infantum*. Clinical Infectious Diseases. 2009; 48(2):e16–e22. DOI: 10.1086/595710 [PubMed: 19093811]
16. Den Boer M, Davidson RN. Treatment options for visceral leishmaniasis. Expert review of anti-infective therapy. 2006; 4(2):187–197. DOI: 10.1586/14787210.4.2.187 [PubMed: 16597201]
17. Rodrigues IA, Mazotto AM, Cardoso V, Alves RL, Amaral ACF, Silva JRdA, Pinheiro AS, Vermelho AB. Natural products: insights into leishmaniasis inflammatory response. Mediators of inflammation. 2015; 501:835910.doi: 10.1155/2015/835910
18. Singh N, Kumar M, Singh RK. Leishmaniasis: current status of available drugs and new potential drug targets. Asian Pacific Journal of Tropical Medicine. 2012; 5(6):485–497. DOI: 10.1016/S1995-7645(12)60084-4 [PubMed: 22575984]
19. Hendrickx S, Boulet G, Mondelaers A, Dujardin JC, Rijal S, Lachaud L, Cos P, Delputte P, Maes L. Experimental selection of paromomycin and miltefosine resistance in intracellular amastigotes of *Leishmania donovani* and *L. infantum*. Parasitology Research. 2014; 113(5):1875–81. DOI: 10.1007/s00436-014-3835-7 [PubMed: 24615359]
20. Faraut-Gambarelli F, Piarroux R, Deniau M, Giusiano B, Marty P, Michel G, Faugère B, Dumon H. *In vitro* and *in vivo* resistance of *Leishmania infantum* to meglumine antimoniate: a study of 37 strains collected from patients with visceral leishmaniasis. Antimicrobial Agents and Chemotherapy. 1997; 41(4):827–830. [PubMed: 9087498]
21. Coelho AC, Gentil LG, da Silveira JF, Cotrim PC. Characterization of *Leishmania (Leishmania) amazonensis* promastigotes resistant to pentamidine. Experimental Parasitology. 2008; 120(1):98–102. DOI: 10.1016/j.exppara.2008.03.018 [PubMed: 18511047]
22. Islamuddin M, Chouhan G, Want MY, Ozbak HA, Hemeg HA, Afrin F. Immunotherapeutic Potential of Eugenol Emulsion in Experimental Visceral Leishmaniasis. PLoS Neglected Tropical Diseases. 2016; 24(10):e0005011.doi: 10.1371/journal.pntd.0005011
23. Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, Chatterjee M. Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. Journal of medical microbiology. 2007; 56(9):1213–1218. DOI: 10.1099/jmm.0.47364-0 [PubMed: 17761485]
24. Want MY, Islamuddin M, Chouhan G, Ozbak HA, Hemeg HA, Dasgupta AK, Chattopadhyay AP, Afrin F. Therapeutic efficacy of artemisinin-loaded nanoparticles in experimental visceral leishmaniasis. Colloids and Surfaces B: Biointerfaces. 2015; 130:215–221. DOI: 10.1016/j.colsurfb.2015.04.013 [PubMed: 25936561]

25. Nemati S, Nahrevanian H, Haniloo A, Farahmand M. Investigation on nitric oxide and C-reactive protein involvement in antileishmanial effects of artemisinin and glucantim on cutaneous leishmaniasis. Advanced Studies in Biology. 2013; 5:27–36.
26. Ebrahimisadr P, Ghaffarifar F, Hassan ZM. *In-vitro* evaluation of antileishmanial activity and toxicity of artemether with focus on its apoptotic effect. Iranian Journal of Pharmaceutical Research. 2013; 12(4):903–909. [PubMed: 24523770]
27. Sen R, Ganguly S, Saha P, Chatterjee M. Efficacy of artemisinin in experimental visceral leishmaniasis. International Journal of Antimicrobial Agents. 2010; 36(1):43–49. DOI: 10.1016/j.ijantimicag.2010.03.008 [PubMed: 20403680]
28. Ghaffarifar F, Heydari FE, Dalimi A, Hassan ZM, Delavari M, Mikaeiloo H. Evaluation of Apoptotic and Antileishmanial Activities of Artemisinin on Promastigotes and BALB/C Mice Infected with *Leishmania major*. Iranian journal of parasitology. 2015; 10(2):258. [PubMed: 26246824]
29. Cortes S, Albuquerque A, Cabral LI, Lopes L, Campino L, Cristiano ML. *In vitro* susceptibility of *Leishmania infantum* to Artemisinin derivatives and selected trioxolanes. Antimicrobial agents and chemotherapy. 2015; 59(8):5032–5035. DOI: 10.1128/AAC.00298-15 [PubMed: 26014947]
30. Chollet C, Crousse B, Bories C, Bonnet-Delpon D, Loiseau PM. *In vitro* antileishmanial activity of fluoro-artemisinin derivatives against *Leishmania donovani*. Biomedicine & Pharmacotherapy. 2008; 62(7):462–465. DOI: 10.1016/j.biopha.2008.04.003 [PubMed: 18538529]
31. Islamuddin M, Chouhan G, Farooque A, Dwarakanath BS, Sahal D, Afrin F. Th1-biased immunomodulation and therapeutic potential of Artemisia annua in murine visceral leishmaniasis. PLoS neglected tropical diseases. 2015; 9(1):e3321.doi: 10.1371/journal.pntd.0003321 [PubMed: 25568967]
32. Mishina YV, Krishna S, Haynes RK, Meade JC. Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense in vitro* growth. Antimicrobial agents and chemotherapy. 2007; 51(5):1852–1854. DOI: 10.1128/AAC.01544-06 [PubMed: 17339374]
33. Mutiso JM, Macharia JC, Barasa M, Taracha E, Bourdichon AJ, Gicheru MM. *In vitro* and *in vivo* antileishmanial efficacy of a combination therapy of diminazene and artesunate against *Leishmania donovani* in BALB/c mice. Revista do Instituto de Medicina Tropical de São Paulo. 2011; 53(3):129–132. DOI: 10.1590/S0036-46652011000300003 [PubMed: 21755234]
34. Adam I, Hagelnur AA. Artesunate plus sulfamethoxypyrazine/pyrimethamine for the treatment of cutaneous leishmaniasis: a double-blind, placebo-controlled clinical trial. International journal of antimicrobial agents. 2009; 34(4):380–381. DOI: 10.1016/j.ijantimicag.2009.03.013 [PubMed: 19409761]
35. Heydari FE, Ghaffarifar F, Soflaei S, Dalimi A. Comparison between *in vitro* effects of aqueous extract of *Artemisia seiberi* and artemisinin on *Leishmania major*. Jundishapur Journal of Natural Pharmaceutical Products. 2013; 8(2):70. [PubMed: 24624191]
36. Yang D, Liew F. Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. Parasitology. 1993; 106(01):7–11. [PubMed: 8479804]
37. Islamuddin M, Farooque A, Dwarakanath B, Sahal D, Afrin F. Extracts of *Artemisia annua* leaves and seeds mediate programmed cell death in *Leishmania donovani*. Journal of medical microbiology. 2012; 61(12):1709–1718. DOI: 10.1099/jmm.0.049387-0 [PubMed: 22956747]
38. Sen R, Saha P, Sarkar A, Ganguly S, Chatterjee M. Iron enhances generation of free radicals by Artemisinin causing a caspase-independent. apoptotic death in Leishmania donovani promastigotes. Free Radical Research. 2010; 44(11):1289–95. DOI: 10.3109/10715762.2010.498475 [PubMed: 20815780]
39. Steverding D. The history of Chagas disease. Parasites & vectors. 2014; 7(1):1.doi: 10.1186/1756-3305-7-317 [PubMed: 24411014]
40. Bermudez J, Davies C, Simonazzi A, Real JP, Palma S. Current drug therapy and pharmaceutical challenges for Chagas disease. Acta tropica. 2016; 156:1–16. DOI: 10.1016/j.actatropica.2015.12.017 [PubMed: 26747009]
41. Babokhov P, Sanyaolu AO, Oyibo WA, Fagbenro-Beyioku AF, Iriemenam NC. A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. Pathogens and

global health. 2013; 107(5):242–252. DOI: 10.1179/2047773213Y.0000000105 [PubMed: 23916333]

42. Sesti-Costa R, Carneiro ZA, Silva MC, Santos M, Silva GK, Milanezi C, da Silva RS, Silva JS. Ruthenium complex with benznidazole and nitric oxide as a new candidate for the treatment of chagas disease. PLoS Neglected Tropical Disease. 2014; 8(10):e3207.doi: 10.1371/journal.pntd.0003207
43. Akande F, Fagbemi B. *In vivo* and *in vitro* effects of artemisinin group of drugs on trypanosomosis in mice. Journal of Natural Sciences Engineering and Technology. 2011; 10(1):73–80.
44. Olivera GC, Postan M, González MN. Effects of artesunate against *Trypanosma cruzi*. Experimental parasitology. 2015; 156:26–31. DOI: 10.1016/j.exppara.2015.05.014 [PubMed: 26024969]
45. Nibret E, Wink M. Volatile components of four Ethiopian Artemisia species extracts and their *in vitro* antitrypanosomal and cytotoxic activities. Phytomedicine. 2010; 17(5):369–374. DOI: 10.1016/j.phymed.2009.07.016 [PubMed: 19683909]
46. Bhakuni R, Jain D, Sharma R, Kumar S. Secondary metabolites of *Artemisia annua* and their biological activity. Current science. 2001; 80:35–48.
47. Wink M. Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. Current Drug Metabolism. 2008; 9(10):996–1009. DOI: 10.2174/138920008786927794 [PubMed: 19075616]
48. Oluyomi SA, Justine TE, Faoziyat S. Depletion of parasitaemia by halofantrine hydrochloride and artemether in rats infected with African trypanosomes. African Journal of Pharmacy and Pharmacology. 2009; 3(9):432–438.
49. Blauer G. Interaction of ferriprotoporphyrin IX with the antimalarials amodiaquine and halofantrine. Biochemistry international. 1988; 17(4):729–734. [PubMed: 3240320]
50. de Villiers KA, Marques HM, Egan TJ. The crystal structure of halofantrine–ferriprotoporphyrin IX and the mechanism of action of arylmethanol antimalarials. Journal of inorganic biochemistry. 2008; 102(8):1660–1667. DOI: 10.1016/j.jinorgbio.2008.04.001 [PubMed: 18508124]
51. Milton K, Edwards G, Ward S, Orme M, Breckenridge A. Pharmacokinetics of halofantrine in man: effects of food and dose size. British journal of clinical pharmacology. 1989; 28(1):71–77. DOI: 10.1111/j.1365-2125.1989.tb03507.x [PubMed: 2775616]
52. Pandey AV, Tekwani BL, Singh RL, Chauhan VS. Artemisinin, an endoperoxide antimalarial, disrupts the hemoglobin catabolism and heme detoxification systems in malarial parasite. Journal of biological chemistry. 1999; 274(27):19383–19388. DOI: 10.1074/jbc.274.27.19383 [PubMed: 10383451]
53. Meshnick SR. Artemisinin and Its Derivatives. Antimalarial Chemotherapy. 2001; (2):191–201. DOI: 10.1007/978-1-59259-111-4_10
54. Abgrall S, Rabaud C, Costagliola D. Incidence and risk factors for toxoplasmic encephalitis in human immunodeficiency virus-infected patients before and during the highly active antiretroviral therapy era. Clinical Infectious Disease. 2001; 33(10):1747–1755. DOI: 10.1086/322622
55. David HMJ, Tim GW. Toxoplasmosis: a comprehensive clinical guide, Cambridge University Press Cambridge University Press. Antitoxoplasma chemotherapy. 2001:319–359.
56. Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N. Trimethoprim-sulfamethoxazole revisited. Archives of Internal Medicine. 2003; 163(4):402–410. [PubMed: 12588198]
57. Antczak M, Dzitko K, Długońska H. Human toxoplasmosis-Searching for novel chemotherapeutics. Biomedicine and Pharmacotherapy. 2016; 82:677–84. DOI: 10.1016/j.biopha.2016.05.041 [PubMed: 27470411]
58. Gomes TC, Andrade HFd Júnior, Lescano SAZ, Amato-Neto V. *In vitro* action of antiparasitic drugs, especially artesunate, against *Toxoplasma gondii*. Revista da Sociedade Brasileira de Medicina Tropical. 2012; 45(4):485–490. [PubMed: 22930046]
59. Son E-S, Song K-J, Shin J-C, Nam H-W. Molecular cloning and characterization of peroxiredoxin from *Toxoplasma gondii*. The Korean journal of parasitology. 2001; 39(2):133–141. DOI: 10.3347/kjp.2001.39.2.133 [PubMed: 11441500]

60. Nagamune K, Beatty WL, Sibley LD. Artemisinin induces calcium-dependent protein secretion in the protozoan parasite *Toxoplasma gondii*. Eukaryotic cell. 2007; 6(11):2147–2156. DOI: 10.1128/EC.00262-07 [PubMed: 17766463]
61. Lovett JL, Sibley LD. Intracellular calcium stores in *Toxoplasma gondii* govern invasion of host cells. Journal of Cell Science. 2003; 116(14):3009–3016. DOI: 10.1242/jcs.00596 [PubMed: 12783987]
62. Holfels E, McAuley J, Mack D, Milhous WK, McLeod R. *In vitro* effects of artemisinin ether, cycloguanil hydrochloride (alone and in combination with sulfadiazine), quinine sulfate, mefloquine, primaquine phosphate, trifluoperazine hydrochloride, and verapamil on *Toxoplasma gondii*. Antimicrobial agents and chemotherapy. 1994; 38(6):1392–1396. DOI: 10.1128/AAC.38.6.1392 [PubMed: 8092843]
63. Hencken CP, Jones-Brando L, Bordón C, Stohler R, Mott BT, Yolken R, Posner GH, Woodard LE. Thiazole, oxadiazole, and carboxamide derivatives of artemisinin are highly selective and potent inhibitors of *Toxoplasma gondii*. Journal of medicinal chemistry. 2010; 53(9):3594–3601. DOI: 10.1021/jm901857d [PubMed: 20373807]
64. Dunay IR, Chan WC, Haynes RK, Sibley LD. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. Antimicrobial agents and chemotherapy. 2009; 53(10):4450–4456. DOI: 10.1128/AAC.00502-09 [PubMed: 19635951]
65. Sarciron ME, Saccharin C, Petavy AF, Peyron F. Effects of artesunate, dihydroartemisinin, and an artesunate-dihydroartemisinin combination against *Toxoplasma gondii*. The American journal of tropical medicine and hygiene. 2000; 62(1):73–76.
66. Liang Z, Xu L. Studies on the enzyme cytochemistry of *Toxoplasma gondii* and the influence of artemether. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi= Chinese journal of parasitology & parasitic diseases. 1994; 13(3):182–184.
67. Ke O, Krug E, Marr J, Berens R. Inhibition of growth of *Toxoplasma gondii* by qinghaosu and derivatives. Antimicrobial agents and chemotherapy. 1990; 34(10):1961–1965. DOI: 10.1128/AAC.34.10.1961 [PubMed: 2291661]
68. Jones-Brando L, D'Angelo J, Posner GH, Yolken R. *In vitro* inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. Antimicrobial agents and chemotherapy. 2006; 50(12):4206–4208. DOI: 10.1128/AAC.00793-06 [PubMed: 17060514]
69. D'Angelo JG, Bordón C, Posner GH, Yolken R, Jones-Brando L. Artemisinin derivatives inhibit *Toxoplasma gondii in vitro* at multiple steps in the lytic cycle. Journal of antimicrobial chemotherapy. 2009; 63(1):146–150. DOI: 10.1093/jac/dkn451 [PubMed: 18988681]
70. Petersen E, Lebech M, Jensen L, Lind P, Rask M, Bagger P, Björkman C, Uggla A. *Neospora caninum* infection and repeated abortions in humans. Emerging Infectious Diseases. 1999; 5(2):278.doi: 10.3201/eid0502.990215 [PubMed: 10221883]
71. Kim J-T, Park J-Y, Seo H-S, Oh H-G, Noh J-W, Kim J-H, Kim D-Y, Youn H-J. *In vitro* antiprotozoal effects of artemisinin on *Neospora caninum*. Veterinary parasitology. 2002; 103(1):53–63. DOI: 10.1016/S0304-4017(01)00580-5 [PubMed: 11751000]
72. Mazuz ML, Haynes R, Shkap V, Fish L, Wollkomirsky R, Leibovich B, Molad T, Savitsky I, Golenser J. *Neospora caninum: in vivo* and *in vitro* treatment with artemisone. Veterinary parasitology. 2012; 187(1):99–104. DOI: 10.1016/j.vetpar.2011.12.020 [PubMed: 22260899]
73. Müller J, Aguado-Martínez A, Manser V, Wong HN, Haynes RK, Hemphill A. Repurposing of antiparasitic drugs: the hydroxy-naphthoquinone buparvaquone inhibits vertical transmission in the pregnant neosporosis mouse model. Veterinary research. 2016; 47(1):1.doi: 10.1186/s13567-016-0317-1 [PubMed: 26738942]
74. Müller J, Balmer V, Winzer P, Rahman M, Manser V, Haynes RK, Hemphill A. *In vitro* effects of new artemisinin derivatives in *Neospora caninum*-infected human fibroblasts. International journal of antimicrobial agents. 2015; 46(1):88–93. DOI: 10.1016/j.ijantimicag.2015.02.020 [PubMed: 25934265]
75. Qian W, Wang H, Shan D, Li B, Liu J, Liu Q. Activity of several kinds of drugs against *Neospora caninum*. Parasitology international. 2015; 64(6):597–602. DOI: 10.1016/j.parint.2015.08.002 [PubMed: 26264260]

76. Blake DP, Clark EL, Macdonald SE, Thenmozhi V, Kundu K, Garg R, Jatau ID, Ayoade S, Kawahara F, Moftah A. Population, genetic, and antigenic diversity of the apicomplexan *Eimeria tenella* and their relevance to vaccine development. Proceedings of the National Academy of Sciences. 2015; 112(38):E5343–E5350. DOI: 10.1073/pnas.1506468112
77. Quiroz-Castañeda RE, Dantán-González E. Control of avian coccidiosis: future and present natural alternatives. BioMed research international. 2015; 2015doi: 10.1155/2015/430610
78. del Cacho E, Gallego M, Francesch M, Quílez J, Sánchez-Acedo C. Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. Parasitology international. 2010; 59(4):506–511. DOI: 10.1016/j.parint.2010.04.001 [PubMed: 20416397]
79. Eckstein-Ludwig U, Webb R, Van Goethem I, East J, Lee A, Kimura M, O'Neill P, Bray P, Ward S, Krishna S. Artemisinins target the SERCA of *Plasmodium falciparum*. Nature. 2003; 424(6951): 957–961. DOI: 10.1038/nature01813 [PubMed: 12931192]
80. Zamora A, Henderson H, Swiatlo E. Acanthamoeba encephalitis: a case report and review of therapy. Surgical neurology international. 2014; 5:68.doi: 10.4103/2152-7806.132239 [PubMed: 24991471]
81. Lorenzo-Morales J, Khan NA, Walochnik J. An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment. Parasite. 2015; 22:10.doi: 10.1051/parasite/2015010 [PubMed: 25687209]
82. Deng Y, Ran W, Man S, Li X, Gao H, Tang W, Tachibana H, Cheng X. Artemether exhibits amoebicidal activity against *Acanthamoeba castellanii* through inhibition of the serine biosynthesis pathway. Antimicrobial agents and chemotherapy. 2015; 59(8):4680–4688. DOI: 10.1128/AAC.04758-14 [PubMed: 26014935]
83. Ramanan D, Nalaganta R, Yadav Ram KS, Ramani R. Amoebic meningo-encephalitis. Indian journal of pathology & microbiology. 2010; 53(1):183–4. DOI: 10.4103/0377-4929.59230 [PubMed: 20090269]
84. Rice CA, Colon BL, Alp M, Göker H, Boykin DW, Kyle DE. Bis-Benzimidazole Hits against *Naegleria fowleri* Discovered with New High-Throughput Screens. Antimicrobial agents and chemotherapy. 2015; 59(4):2037–2044. DOI: 10.1128/AAC.05122-14 [PubMed: 25605363]
85. Gupta S, Ghosh PK, Dutta G, Vishwakarma R. *In vivo* study of artemisinin and its derivatives against primary amebic meningoencephalitis caused by *Naegleria fowleri*. The Journal of parasitology. 1995; 81(6):1012–1013. [PubMed: 8544041]
86. Ryan U, Hijjawi N. New developments in Cryptosporidium research. International journal for parasitology. 2015; 45(6):367–373. DOI: 10.1016/j.ijpara.2015.01.009 [PubMed: 25769247]
87. Abd-Ella OH. Diagnosis and treatment of cryptosporidiosis: an update review. Journal of the Egyptian Society of Parasitology. 2014; 44(2):455–466. [PubMed: 25597160]
88. Giacometti A, Cirioni O, Scalise G. *In-vitro* activity of macrolides alone and in combination with artemisin, atovaquone, dapsone, minocycline or pyrimethamine against *Cryptosporidium parvum*. Journal of Antimicrobial Chemotherapy. 1996; 38(3):399–408. DOI: 10.1093/jac/38.3.399 [PubMed: 8889715]
89. Centers for Disease Control and Prevention. [Last time accessed: June 27th] Parasites—Giardia. 2016. https://www.cdc.gov/parasites/giardia/
90. Tian XF, Shen HE, Li J, Chen Y, Yang ZH, Lu SQ. The effects of dihydroartemisinin on *Giardia lamblia* morphology and cell cycle in vitro. Parasitology Research. 2010; 107(2):369–75. DOI: 10.1007/s00436-010-1872-4 [PubMed: 20422219]
91. Leiby DA. Transfusion-transmitted Babesia spp.: bull's-eye on *Babesia microti*. Clinical microbiology reviews. 2011; 24(1):14–28. DOI: 10.1128/CMR.00022-10 [PubMed: 21233506]
92. Rozej-Bielicka W, Stypulkowska-Misiurewicz H, Golab E. Human babesiosis. Przeglad epidemiologiczny. 2015; 69(3):489–94. 605–8. [PubMed: 26519845]
93. Sakuma M, Fukuda K, Takayama K, Kobayashi Y, Miyama TS, Setoguchi A, Endo Y. Molecular epidemiological survey of the *Babesia* gibsoni cytochrome b gene in western Japan. Journal of Veterinary Medical Science. 2012; 74(10):1341–1344. DOI: 10.1292/jvms.12-0140 [PubMed: 22672842]

94. Mazuz ML, Golenser J, Fish L, Haynes RK, Wollkomirsky R, Leibovich B, Shkap V. Artemisone inhibits *in vitro* and *in vivo* propagation of *Babesia bovis* and *B. bigemina* parasites. Experimental parasitology. 2013; 135(4):690–694. DOI: 10.1016/j.exppara.2013.10.006 [PubMed: 24184077]

95. Goo Y-K, Terkawi MA, Jia H, Aboge GO, Ooka H, Nelson B, Kim S, Sunaga F, Namikawa K, Igarashi I. Artesunate, a potential drug for treatment of Babesia infection. Parasitology international. 2010; 59(3):481–486. DOI: 10.1016/j.parint.2010.06.004 [PubMed: 20541037]

96. Nagai A, Yokoyama N, Matsuo T, Bork S, Hirata H, Xuan X, Zhu Y, Claveria FG, Fujisaki K, Igarashi I. Growth-inhibitory effects of artesunate, pyrimethamine, and pamaquine against *Babesia equi* and *Babesia caballi* in *in vitro* cultures. Antimicrobial agents and chemotherapy. 2003; 47(2): 800–803. DOI: 10.1128/AAC.47.2.800-803.2003 [PubMed: 12543697]

97. Matsuu A, Yamasaki M, Xuan X, Ikadai H, Hikasa Y. *In vitro* evaluation of the growth inhibitory activities of 15 drugs against *Babesia gibsoni* (Aomori strain). Veterinary parasitology. 2008; 157(1):1–8. DOI: 10.1016/j.vetpar.2008.07.023 [PubMed: 18771856]

98. Iguchi A, Matsuu A, Matsuyama K, Hikasa Y. The efficacy of artemisinin, artemether, and lumefantrine against *Babesia gibsoni in vitro*. Parasitology international. 2015; 64(2):190–193. DOI: 10.1016/j.parint.2014.12.006 [PubMed: 25523292]

99. Kawai S, Igarashi I, Abgaandorjiin A, Miyazawa K, Ikadai H, Nagasawa H, Fujisaki K, Mikami T, Suzuki N, Matsuda H. Ultrastructural characteristics of *Babesia caballi* in equine erythrocytes *in vitro*. Parasitology research. 1999; 85(10):794–799. [PubMed: 10494804]

100. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiology Review. 1996; 60(2):301–315.

101. Kumar S, Gupta AK, Pal Y, Dwivedi SK. *In-vivo* therapeutic efficacy trial with artemisinin derivative, buparvaquone and imidocarb dipropionate against *Babesia equi* infection in donkeys. Journal of veterinary medical science. 2003; 65(11):1171–1177. [PubMed: 14665744]

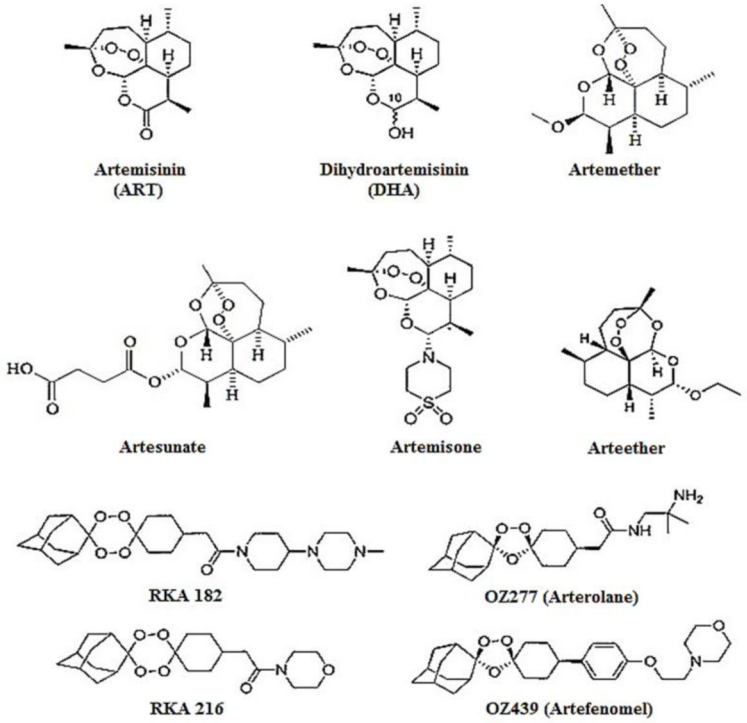


Fig. 1. Chemical structures of ART and its derivatives

ART (parent drug), DHA (active metabolite), arteether (oil-soluble derivative), artemether (oil-soluble derivative), artesunate (water soluble derivative), arterolane and artefenomel (trioxolane), and RKA 216 and 182 (tetraoxanes).

Table 1

Treatment of artemisinin and its derivatives against *Leishmania* spp.

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro						
	L. major (MRHO/IR/75/ER)	Promastigotes	Artemisinin	10, 25, 50, and 100 μg/mL. incubated for 24, 48, and 72 h	IC_{50} = 25 μg/mL. Apoptotic promastigotes were 12%, 25.90%, 40.52%, and 68.16%, respectively, in comparison of control that was 0.02%	Ghaffarifar et al. [28]
	L. major (MRHO/IR/75/ER)	Promastigotes	Artemisinin	5, 10, 25, 50, and 100 μg/mL, incubated for 24, 48, and 72 h	IC_{50} = 50 μg/mL. The viabilities of promastigote were ~48%, ~40%, and ~36% after 24, 48, and 72 h of treatment with 100 μg/mL	Heydari et al. [35]
	L. major (LV39, MRHO/SU/59/P)	Promastigotes	Artemisinin in Me_2SO diluent	100, 33.5, 9.6, 2.7, 0.75, and 0.2 μM incubated for 48 h	Estimated by the incorporation of ^{3}H-thymidine, the viability of parasites post-treatment was ~1, 2.4, 3.4, 4.5, 4.8, and 9 cpm × 10^{-3}, respectively. IC_{50} = 7.5 × 10^{-7} M. No surviving promastigotes were observed in 48 h cultures containing > 10^{-5} M artemisinin or 10^{-6} M artemether	Yang et al. [36]
	L. major (MRHO/IR/75/ER)	Promastigotes	Artemether	5, 10, 25, 50, and 100 μg/mL, incubated for 72 h	IC_{50} = 25 μg/mL. Apoptotic promastigotes were 2.44%, 42.28%, and 71.95 %, respectively	Ebrahimisadr et al. [26]
	L. major (MRHO/IR/75/ER)	Promastigotes	Aqueous extract of *Artemisia sieberi* has similar component as artemisinin	5, 10, 25, 50, and 100 μg/mL, incubated for 24, 48, and 72 h	IC_{50} = 25 μg/mL. After 24, 48, and 72 h of treatment with 100 μg/mL, the viabilities of promastigotes were ~34%, ~30%, and ~18%, respectively, which had higher parasite growth inhibitory effect on promastigotes vs. ~32% of treatment with artemisinin at the same concentration and duration at 72 h	Heydari et al. [35]
	L. major (MRHO/IR/75/ER)	Amastigotes in macrophages of BALB/c mice	Artemisinin	5, 10, 25, 50, and 100 μg/mL, incubated for 24, 48, and 72 h	IC_{50} = 25 μg/mL. Apoptotic macrophages after 48 h of treatment were 0.64%, 1.43%, 1.96%, and 9.39%, respectively, in comparison of control that was 0.6%	Ghaffarifar et al. [28]
	L. major (MRHO/IR/75/ER)	Amastigotes in macrophages of BALB/c mice	Artemisinin	5, 10, 25, 50, and 100 μg/mL, incubated for 24, 48, and 72 h	IC_{50} = 50 μg/mL, effective elimination of amastigotes from macrophages and decreasing the amastigote burden, with the cell viabilities of 2%, 1.8%, and 1.6%	Heydari et al. [35]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
					at 100 μg/mL, incubated for 24, 48, and 72 h, respectively.	
	L. major (LV39, MRHO/SU/59/P)	Amastigotes	Artemisinin	123, 41, 13, 4.5, and 1.5 μM, incubated for 48 h	Estimated by the incorporation of ^{3}H-thymidine, the viability of the parasites post-treatment was ~3, 3.5, 6, 6.1, and 7.5 cpm $\times 10^{-3}$. $IC_{50} = 3 \times 10^{-5}$ M. Using 10 times higher concentration to kill promastigotes than that to kill intracellular amastigotes	Yang et al. [36]
	L. major (MRHO/IR/75/ER)	Amastigotes in macrophages of BALB/c mice	Artemether	5, 10, 25, 50, and 100 μg/mL, incubated for 72 h	The mean number of amastigotes per macrophage after adding artemether was 0.78, 0.64, 0.49, 0.30, and 0.21, respectively; vs. 0.96 (before adding artemether), and vs. 1.6 in control	Ebrahimisadr et al. [26]
	L. major (LV39, MRHO/SU/59/P)	Amastigotes	Artemether	123, 41, 13, 4.5, and 1.5 μM, incubated for 48 h	Viability of the parasites post-treatment was ~1, 2.4, 3.4, 4.5, 4.8, 9 cpm $\times 10^{-3}$, and 3×10^{-6} M respectively. Using 10 times higher concentration to kill promastigotes than that to kill intracellular amastigotes.	Yang et al. [36]
	L. major (MRHO/IR/75/ER)	Amastigotes in macrophages of BALB/c mice	*Artemisia sieberi*	5, 10, 25, 50, and 100 μg/mL, incubated for 24, 48, and 72 h	IC_{50} = 100 μg/mL, lower cytotoxic effects on macrophages compared to artemisinin. Effectively eliminated amastigotes from macrophages and decreased amastigote burden, with the cell viabilities of 1.2%, 0.8%, and 0.4% at 100 μg/mL, incubated for 24, 48, and 72 h, respectively	Heydari et al. [35]
	L. donovani (MHOM/IN/83/AG83)	Promastigotes	Artemisinin	10 and 25 μM, incubated for 48 h	In infected macrophages, 10 μM artemisinin increased NO level to (5.52 ± 0.45) μM, and 25 μM artemisinin significantly increased NO level to (6.78 ± 0.43) μM. The NO levels achieved were comparable to that generated by uninfected macrophages, which restores its ability to eliminates the parasite	Sen et al. [27]
	L. donovani (MHOM/IN/83/AG83)	Promastigotes	Artemisinin	160 μM, incubated for 24 h	Proportion of cells in sub-G0/G1 phase increased to 10.86% compared with 3.62% of controls. Nuclear DNA fragmentation as the dUTP–FITC binding after treatment for 24 h was increased from a baseline mean fluorescence	Sen et al. [23]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
					intensity of 35.96 in untreated cells to 110.27	
	L. donovani (MHOM/IN/83/AG84)	Promastigotes	Artemisinin	160 μM, incubated for 48 h	Proportion of cells in the sub-G0/G1 phase increased to 33.11% compared with 3.03% in control cells, accompanied with a decreased number of cells in the G2/M phase compared with controls, values of 27.45% vs. 41.03% at 24 h and 12.03% vs. 29.65% at 48 h	Sen et al. [23]
	L. donovani (NLB-065)	Promastigotes	Artemisinin	Serial dilutions: 100–0.049 μg/mL, incubated for 72 h	IC_{50} = (4.64 ± 0.48) μg/mL, and lower efficacy than amphotericin B (0.16 ± 0.32) μg/mL	Mutiso et al. [33]
	L. donovani (strain not stated)	Promastigotes	Artemisinin	Dose information not available, 72 h of drug exposure	IC_{50} = (30.8 ± 1.4) μM	Mishina et al. [32]
	L. donovani (NLB-065)	Promastigotes	Diminazene + artemisinin	Serial dilutions: 100–0.049 μg/mL, incubated for 72 h	IC_{50} = (2.28 ± 0.24) μg/mL, and higher efficacy than artemisinin but lower efficacy than amphotericin B	Mutiso et al. [33]
	L. donovani (LV9 WT)	Promastigotes	BB200 (OOH group substituted at C-10 position of fluoroartemisinin)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (2.5 ± 0.3) μM, and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (LV9 WT)	Promastigotes	BB201 (paramethoxy-aniline group substituted at the C-10 position of fluoroartemisinin)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (1.5 ± 0.2) μM; most active against the parasite and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (LV9 WT)	Promastigotes	BB241 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (6.25 ± 0.8) μM, and higher efficacy than DHA and sitamaquine	Chollet et al. [30]
	L. donovani (LV9 WT)	Promastigotes	BB242 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (5.5 ± 0.6) μM, and higher efficacy than DHA and sitamaquine	Chollet et al. [30]
	L. donovani (LV9 WT)	Promastigotes	DHA	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (20.2 ± 1.9) μM, and higher efficacy than sitamaquine	Chollet et al. [30]
	L. donovani (HePC-R, miltefosine-resistant line)	Promastigotes	BB200 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (8.2 ± 0.8) μM, and higher efficacy than sitamaquine	Chollet et al. [30]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	L. donovani (HePC-R)	Promastigotes	BB201 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (1.1 ± 0.1) μM, and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (HePC-R)	Promastigotes	BB241 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (2.8 ± 0.3) μM, and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (HePC-R)	Promastigotes	BB242 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (6.3 ± 0.7) μM, and higher efficacy than sitamaquine	Chollet et al. [30]
	L. donovani (HePC-R)	Promastigotes	DHA	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (4.1 ± 0.3) μM, and higher efficacy than miltefosine and sitamaquine	Chollet et al. [30]
	L. donovani (SITA-R, sitamaquin e-resistant line)	Promastigotes	BB200 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (12.5 ± 1.3) μM, and higher efficacy than sitamaquine	Chollet et al. [30]
	L. donovani (SITA-R)	Promastigotes	BB201 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (0.38 ± 0.05) μM, and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (SITA-R)	Promastigotes	BB241 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (1.6 ± 0.2) μM, and higher efficacy than miltefosine, DHA, and sitamaquine	Chollet et al. [30]
	L. donovani (SITA-R)	Promastigotes	BB242 (fluoroartemisinin derivative)	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (12.4 ± 1.4) μM, and higher efficacy than sitamaquine	Chollet et al. [30]
	L. donovani (SITA-R)	Promastigotes	DHA	Serial dilutions: 2 μL of stock solution dissolved in DMSO, incubated for 3 days	IC_{50} = (6.0 ± 0.7) μM, and higher efficacy than sitamaquine	Chollet et al. [30]
	L. donovani (AG83)	Promastigotes	AAL (n-hexane fraction of *A annua* leaves)	Serial dilutions started from100–0 μg/mL. 100 μg AAL = 1.447 μg artemisinin, incubated for 96 h	IC_{50} = 14.4 μg/mL, no viable parasites were observed after 2-, 3-, or 5-day incubation with 100 μg AAL	Islamuddin et al. [37]
	L. donovani (AG83)	Promastigotes	AAS (n-hexane fraction of *A. annua* seeds)	Serial dilutions started from 100– 0 μg/mL. 100 μg AAS = 1.336 μg artemisinin, incubated for 96 h	IC_{50} = 14.6 μg/mL, no viable parasites were observed after 2-, 3-, or 5-day incubation with 100 μg AAS	Islamuddin et al. [37]

Pharmacol Res. Author manuscript; available in PMC 2018 March 01.

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	L. donovani (strain not stated)	Promastigotes	Artemisone	Dose information not available, 72 h of drug exposure	IC_{50} = (12.9 ± 6.3) μM	Mishina et al. [32]
	L. donovani (strain not stated)	Promastigotes	4-Fluorophenyl-artemisinin	Dose information not available, 72 h of drug exposure	IC_{50} = (3.0 ± 1.1) μM. Highest efficacy compared to artemisinin and artemisone, comparable to amphotericin B (IC_{50} = 2.8 ± 0.1 μg/mL)	Mishina et al. [32]
	L. donovani (MHOM/IN/83/AG83)	Amastigotes in a human leukaemia cell line (U937)	Artemisinin + iron	0.5 mM, incubated with Fe^{2+} (0.2 mM) for 48 h	Increased production of reactive oxygen species following incubation of artemisinin-treated cells with Fe^{2+}, measured using Fe^{2+}-artemisinin 200 geometric mean fluorescence channel vs. 100 with artemisinin alone	Sen et al. [38]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Trioxolane LC50	At 6 different concentrations (0.90–35.93 μM), incubated for 48 h	IC_{50} = 9.35 μM and SI = 768.44, higher selectivity than pentamidine and milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Trioxolane LC67	At 6 different concentrations (14.86–445.87 μM), incubated for 48 h	IC_{50} = 145.98 μM and SI = 28.24	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Trioxolane LC95	At 6 different concentrations (1.70–20.45 μM), incubated for 48 h	IC_{50} = 3.51 μM and SI = 1684.74, the highest selectivity. Higher selectivity than pentamidine, milfetosine, and amphotericin B; higher efficacy than pentamidine and milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	DHA	At 6 different concentrations (27.49–3,519.14 μM), incubated for 48 h	IC_{50} = 123.06 μM and SI = 2.04	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Deoxy-DHA	At 6 different concentrations (29.13–3,728.98 μM), incubated for 48 h	IC_{50} = 1249.33 μM and SI = 1.07	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Artesunate	At 6 different concentrations (2.03–260.29 μM), incubated for 48 h	IC_{50} = 40.68 μM and SI = 7.32	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Promastigote	Deoxy-artesunate	At 6 different concentrations (21.2–2,716.06 μM), incubated for 48 h	IC_{50} = 747.73 μM and SI = 1.28	Cortes et al. [29]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	L. infantum (MHOM/PT/88/IMT151)	Amastigote in the human acute monocytic leukemia cell line THP-1 (ATCC TIB-202)	Trioxolane LC50	At 3 different concentrations (11.68–1,077.82 μM), incubated for 48 h	IC_{50} = 79.76 μM and SI = 90.08, the highest selectivity. Higher selectivity than pentamidine, milfetosine, and amphotericin B	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote THP1 cell line	Trioxolane LC67	At 3 different concentrations (27.87–3,566.97 μM), incubated for 48 h	IC_{50} = 1202.81 μM and SI = 3.43. Higher selectivity than milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote in THP1 cell line	Trioxolane LC95	At 3 different concentrations (1.07–409.00 μM), incubated for 48 h	IC_{50} = 107.87 μM and SI = 54.82. Higher selectivity than pentamidine and milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote in THP1 cell line	DHA	At 3 different concentrations (27.49–219.95 μM), incubated for 48 h	IC_{50} = 104.06 μM and SI = 2.41. Higher selectivity than milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote in THP1 cell line	Deoxy-DHA	At 3 different concentrations (29.13–932.24 μM), incubated for 48 h	IC_{50} = 457.36 μM and SI = 2.91. Higher selectivity than milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote THP1 cell line	Artesunate	At 3 different concentrations (20.34–650.74 μM), incubated for 48 h	IC_{50} = 122.52 μM and SI = 2.43. Higher selectivity than milfetosine	Cortes et al. [29]
	L. infantum (MHOM/PT/88/IMT151)	Amastigote in THP1 cell line	Deoxy-artesunate	At 3 different concentrations (21.22–679.02 μM), incubated for 48 h	IC_{50} = 80.27 μM and SI = 11.95. Higher selectivity than pentamidine and milfetosine	Cortes et al. [29]
In vivo studies						
	L. major (MRHO/IR/75/ER)	BALB/c mice	Artemisinin	1 mg was dissolved in 50% ethanol and 50% distilled water, i.p., q.d. for 150 days	Decreased lesion size and increased IL-4 and IFN-γ levels. Survival rate at day 150 was ~34%	Ghaffarifar et al. [28]
	L. major (MRHO/IR/75/ER)	BALB/c mice	Artemisinin	0.1 mL of ointment (25 μg/mL)/mouse, t.p., b.i.d. for 150 days	Largest decreased lesion size and increased IL-4 and IFN-γ levels. Survival rate at day 150 was ~66%	Ghaffarifar et al. [28]
	L. major (MRHO/IR/75/ER)	BALB/c mice	Artemisinin	1 mg was dissolved in 50% ethanol and 50% distilled water. t.p., q.d. for 150 days	Decreased lesion size and survival rate at day 150 was 0%	Ghaffarifar et al. [28]
	L. major (MRHO/IR/75/ER)	BALB/c mice	Artemisinin	10 mg/kg/day, i.p. for 3 weeks	NO production was slightly restored (naive: ~13 μM/mL,	Nemati et al. [25]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
					without treatment: ~8 μM/mL, and artemisinin: ~9 μM/mL)	
	L. major (LV39, MRHO/SU/59/P)	BALB/c mice	Artemether	50 mg/kg/day, intra-lesion for 15 days	Developed significantly smaller footpad lesions compared to the controls (approximately 20 mm vs. 30 mm); parasite burden (4500 cpm vs. 30520 cpm)	Yang et al. [36]
	L. major (LV39, MRHO/SU/59/P)	BALB/c mice	Artemether	50 mg/kg/day, i.m. for 15 days	Developed significantly smaller lesion compared to controls; however, lesion size was not reduced further by increasing the dose of artemether	Yang et al. [36]
	L. major (LV39, MRHO/SU/59/P)	BALB/c mice	Artemether	50 mg/kg/day, p.o. for 15 days	Lesion size was indistinguishable from the controls; however, the parasite burdens were lower compared to controls	Yang et al. 36]
	L. major (LV39, MRHO/SU/59/P)	BALB/c mice	Artemether	200 mg/kg/day, p.o. for 15 days	Lesion size was significantly smaller compared to controls	Yang et al. [36]
	L. donovani (MHOM/IN/1983/AG83)	BALB/c mice	Artemisinin	10 mg/kg, i.p. for 10 days	Reduction in hepatosplenomegaly [(53.0 ± 2.0)% in liver vs. control]	Want et al. [24]
	L. donovani (MHOM/IN/1983/AG83)	BALB/c mice	Artemisinin	20 mg/kg, i.p. for 10 days	Reduction in hepatosplenomegaly [(70.3 ± 0.6)% in liver and (62.7 ± 3.7)% in spleen vs. control]	Want et al. [24]
	L. donovani (MHOM/IN/83/AG83)	BALB/c mice	Artemisinin	10 mg/kg. p.o. for 2 weeks	Splenic weight decreased to (94.0 ± 7.7) mg vs. control [(107.0 ± 8.4) mg] in the beginning and [(220.0 ± 26.0) mg] at the end; final parasite removal of 82.6%	Sen et al. [27]
	L. donovani (MHOM/IN/83/AG83)	BALB/c mice	Artemisinin	25 mg/kg. p.o. for 2 weeks	Splenic weight decreased to (110.0 ± 2.7) mg vs. control [(107.0 ± 8.4) mg] in the beginning and [(220.0 ± 26.0) mg] at the end; final parasite removal of 86.0% (higher efficacy)	Sen et al. [27]
	L. donovani (MHOM/IN/1983/AG83)	BALB/c mice	Artemisinin-loaded nanoparticles	10 mg/kg, i.p. for 10 days	Significant reduction of hepatosplenomegaly; the percentages of parasite inhabition were (68.1 ± 2.8)% in liver and (66.3 ± 2.4)% in spleen vs. artemisinin alone	Want et al. [24]
	L. donovani (MHOM/IN/1983/AG83)	BALB/c mice	Artemisinin-loaded nanoparticles	20 mg/kg, i.p. for 10 days	Significant reduction of hepatosplenomegaly; the percentages of parasite inhabition were (85.4 ± 5.4)% in liver and (82.0 ± 2.4)% in spleen vs.	Want et al. [24]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
					amphotericin B (98.6 ± 0.2)% in liver and ~90% in spleen	
	L. donovani (NLB-065)	BALB/c mice	Artesunate	12.5 mg/kg. q.d. for 28 consecutive days (oral and parenteral formulas were designed; delivery route was not stated)	Parasite burden of post-treatment (~110 amastigote/500 splenic cell nuclei) vs. amphotericin B (~10 amastigotes/500 nuclei)	Mutiso et al. [33]
	L. donovani (NLB-065)	BALB/c mice	Diminazene + artesunate granules	12.5 mg/kg + 12.5 mg/kg, q.d. for 28 consecutive days	Parasite burden of post-treatment (~30 amastigote/500 splenic cell nuclei) vs. amphotericin B (~10 amastigotes/500 nuclei)	Mutiso et al. [33]

b.i.d.: two times per day

DHA: dihydroartemisinin

DMSO: Dimethyl sulphoxide

IC_{50}: concentration that causes 50% inhibition of growth

i.m.: intramuscular injection

i.p.: intraperitoneal injection

p.o.: oral administration

q.d.: one time per day

SI: selectivity index

t.p.: topical administration

* The IC50 is either in μg/mL or μM as in the original papers.

Table 2

Treatment of artemisinin and its derivatives against *Tryponosome spp.*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro	*T. brucei rhodesiense* (strain not stated)	Trypomastigotes, human U-937 monocytes	Artemisinin	Dose information not available, 72 h of drug exposure	IC_{50} = (20.4 ± 0.3) μM and IC_{90} = (49.1 ±1.3) μM	Mishina et al. [32]
	T. brucei rhodesiense (strain not stated)	Trypomastigotes, human U-937 monocytes	Artemisone	Dose information not available, 72 h of drug exposure	IC_{50} = (22.5 ± 3.0) μM and IC_{90} = (44.8 ± 0.8) μM	Mishina et al. [32]
	T. brucei rhodesiense (strain not stated)	Trypomastigotes, human U-937 monocytes	4-fluorophenyl-artemisinin	Dose information not available, 72 h of drug exposure	IC_{50} = (15.7 ± 5.6) μM and IC_{90} = (46.9 ± 0.6) μM	Mishina et al. [32]
	T. brucei rhodesiense (strain not stated)	Trypomastigotes, human U-937 monocytes	DHA	Dose information not available, 72 h of drug exposure	IC_{50} = (24.6 ± 2.4) μM and IC_{90} = (49.6 ± 1.0) μM	Mishina et al. [32]
	T. brucei brucei (TC221)	Trypomastigotes, human leukemia cell line (HL-60)	MeOH and CH_2CL_2 crude extracts of *Artemisia absinthium* (aerial part)	Serial dilution into 7 different conocentrations: 250–3.91 μg/mg, incubated for 48 h	IC_{50} = 27.90 μg/ml (MeOH) and IC_{50} = 27.05 μg/ml (CH_2CL_2)	Nibret et al. [45]
	T. brucei brucei (TC221)	Trypomastigotes, HL-60	MeOH and CH_2CL_2 crude extracts of *A. abyssinica* (aerial part)	Serial dilution into 7 different conocentrations: 250–3.91 μg/mg, incubated for 48 h	IC_{50} = 41.76 μg/ml (MeOH) and the extracts (CH_2CL_2) showed the best anti-trypanosomal activity of IC_{50} = 19.13 μg/ml (CH_2CL_2)	Nibret et al. [45]
	T. brucei brucei (TC221)	Trypomastigotes, HL-60	MeOH and CH_2CL_2 crude extracts of *A. afra* (leaves)	Serial dilution into 7 different conocentrations: 250–3.91 μg/mg, incubated for 48 h	IC_{50} = 77.54 μg/ml (MeOH) and IC_{50} = 25.27 μg/ml (CH_2CL_2).	Nibret et al. [45]
	T. brucei brucei (TC221)	Trypomastigotes, HL-60	Artemisinin	Serial dilution into 7 different conocentrations: 250–3.91 μg/mg, incubated for 48 h	IC_{50} = 35.91 μg/ml	Nibret et al. [45]
	T. brucei (Lafia strain)	Peripheral blood of infected rat (parasite stage and rat species not mentioned)	Artesunate	50 mg/mL ampoule, incubated for 300 min	120 min of treatment: reduced motility of the trypanosome, and parasitemia reduced to moderate (++); 180 min of treatment: complete clearance of parasitaemia	Akande et al. [43]
	T. brucei (Lafia strain)	Peripheral blood of infected rat	Artemether	80 mg/mL ampoule, incubated for 300 min	60 min of treatment: reduced motility of the trypanosome, and parasitemia reduced to moderate (++)	Akande et al. [43]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	T. cruzi (strain not stated)	Epimastigotes in peripheral blood of infected rat (rat species not mentioned)	Artemisinin	Dose information not available, 72 h of drug exposure (assays were performed 2–5 times with 6 drug concentrations)	IC_{50} = (13.4 ± 2.3) μM and IC_{90} = (46.5 ± 1.2) μM	Mishina et al. [32]
	T. cruzi (strain not stated)	Epimastigotes in peripheral blood of infected rat	Artemisone	Dose information not available, 72 h of drug exposure	IC_{50} = (23.3 ± 2.7) μM and IC_{90} = (50.5 ± 0.8) μM	Mishina et al. [32]
	T. cruzi (strain not stated)	Epimastigotes in peripheral blood of infected rat	4-fluorophenyl-artemisinin	Dose information not available, 72 h of drug exposure	IC_{50} = (17.9 ± 3.9) μM and IC_{90} = (50.8 ± 0.9) μM	Mishina et al. [32]
	T. cruzi (strain not stated)	Epimastigotes in peripheral blood of infected rat	DHA	Dose information not available, 72 h of drug exposure	IC_{50} = (12.8 ± 0.3) μM and IC_{90} = (49.2 ± 1.5) μM	Mishina et al. [32]
	T. cruzi (AR-SE23C)	Epimastigotes, Vero cell (African green monkey kidney)	Artesunate	Serial dilutions: 0.01–100 μg/mL, incubated for 72 h	IC_{50} = 19.22 μg/mL and IC_{90} = 69.20 μg/mL	Olivera et al. [44]
	T. cruzi (Nicaragua strain)	Epimastigotes, Vero cell	Artesunate	Serial dilutions: 0.01–100 μg/mL, incubated for 72 h	IC_{50} = 2.35 μg/mL and IC_{90} = 14.34 μg/mL	Olivera et al. [44]
	T. cruzi (Brazil strain)	Epimastigotes, Vero cell	Artesunate	Serial dilutions: 0.01–100 μg/mL, incubated for 72 h	IC_{50} = 8.84 μg/mL and IC_{90} = 44 μg/mL	Olivera et al. [44]
	T. cruzi (AR-SE23C)	Amastigotes, Vero cell	Artesunate	Serial dilutions: 0.001–100 μg/mL, incubated for 72 h	IC_{50} = 6.63 μg/mL and IC_{90} = 19.25 μg/mL	Olivera et al. [44]
	T. cruzi (Nicaragua strain)	Amastigotes, Vero cell	Artesunate	Serial dilutions: 0.001–100 μg/mL, incubated for 72 h	IC_{50} = 0.05 μg/mL and IC_{90} = 0.70 μg/mL	Olivera et al. [44]
	T. cruzi (Brazil strain)	Amastigotes, Vero cell	Artesunate	Serial dilutions: 0.001–100 μg/mL, incubated for 72 h	IC_{50} = 2.65 μg/mL and IC_{90} = 3.84 μg/mL	Olivera et al. [44]
	T. cruzi (Brazil strain)	Trypomastigotes, Vero cell	Artesunate	Serial dilutions: 0.1–100 μg/mL, incubated for 24 h	IC_{50} = 56.9 μg/mL	Olivera et al. [44]
	T. cruzi (Brazil strain)	Trypomastigotes, from blood stream	Artesunate	Serial dilutions: 0.1–100 μg/mL, incubated for 24 h	IC50 = 31.52 μg/mL	Olivera et al. [44]
In vivo studies	*T. brucei brucei* (Lafia strain)	Rat: *Rattus novergicus*	Artemether	Early-stage treatment (started 3 days before infection): 2.3 and 4.6 mg/kg, i.p., q.d.,	Fluactuation of parasitaemia, and lifespan extended from 11 to 13–14 days	Oluyomi et al. [48]
	T. brucei brucei (Lafia strain)	Rat: *Rattus novergicus*	Artemether	Late-stage treatment (started 10^{th} day p.i.): 2.3 and 4.6 mg/kg, i.p., q.d., 3.2 mg/kg, i.m. on the 1^{st} day	Reduction in parasitaemia, and lifespan extended to 14 days	Oluyomi et al. [48]
	*T. brucei (*Lafia strain*)*	Mice (species not mentioned)	Artemether	and 1.6 mg/kg/day i.m. for the next 4 days	3 days post-treatment: reduction in activity of the trypanosome; 5 days post-	Akande et al. [43]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
					treatment: complete clearance of the parasite	
	T. brucei (Lafia strain)	Mice (species not mentioned)	Artesunate	100 mg/kg on the 1st day and 50 mg/kg, p.o., q.d. for 5 days	7 days post-treatment: complete clearance of the parasite	Akande et al. [43]
Combination therapy						
	T. brucei brucei (Lafia strain)	Rat: *Rattus novergicus*	Artemether + halofantrine HCl	Early-stage treatment (the day parasite was first sighted in the blood): 2.3 mg/kg artemether and 7.1 mg/kg halofantrine HCl, i.p.	Maintained low parasitaemia and extended lifespan of rats to 15–16 days for prophylactic treatment vs. 11 days in control	Oluyomi et al. [48]
	T. brucei brucei (Lafia strain)	Rat: *Rattus novergicus*	Artemeter + halofantrine HCI	Late-stage treatment (started 10th day p.i.): 4.6 mg/kg artemether and 14.2 mg/kg halofantrine HCl rat weight, i.p.	Parasitaemia declined drastically and extended lifespan of rats to 19 days vs. 11 days in control	Oluyomi et al. [48]
	T. brucei (Lafia strain)	Mice (species not mentioned)	Artesunate + diminazene aceturate	Artesunate, p.o. 50 mg/kg on the 1st day, 25 mg/kg for next 4 days, and 1.75 mg/kg later on; 3.5 mg/kg diminazene aceturate, i.m.	After 3 days of treatment, reduction in activity of the trypanosome; 5 or 7 days of reatment, complete clearance of the parasite	Akande et al. [43]
	T. cruzi (Brazil strain)	BALB/c mice	Artesunate + benznidazole	Artesunate (125 mg/kg/d) + benznidazole (100 mg/kg/d), p.o. for 6 consecutive days	Lower number of circulating parasites, and significantly reduced parasite density and inflammation in skeletal muscle	Olivera et al. [44]
	T. cruzi (Brazil strain)	BALB/c mice	Artesunate + benznidazole	Artesunate (75 mg/kg/d) + benznidazole (50 mg/kg/d), p.o. for 6 consecutive days	Lower number of circulating parasites and reduced parasite density in skeletal muscle	Olivera et al. [44]

CH_2CL_2: dichloromethane

DHA: dihydroartemisinin

IC_{50}: concentration that causes 50% inhibition of growth

IC_{90}: concentration that causes 90% inhibition of growth

i.m.: intramuscular injection

i.p.: intraperitoneal injection

MeOH: methanol

p.i.: post-infection

p.o.: oral administration

q.d.: one time per day

* The IC50 is either in μg/mL or μM as in the original papers.

Table 3

Treatment of artemisinin and its derivatives against *Toxoplasma gondii*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro	*T. gondii* (RH strain)	Tachyzoites, Human fibroblast (HFF) cells	Artemisinin	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.64 μM, TD_{50} ≥ 320 μM, and TI = 879. Parasites/vacuole ratio after treatment was 8, the same as the vehicle control	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisinin	98% pure artemisinin 10–50 μM, incubated for 15–20 min	Affects Ca^{2+} homeostasis and signaling in the parasite, acts as an inhibitor of SERCA that was critical for intracellular survival	Nagamune et al. [60]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisinin	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Prevented parasitic growth at 1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisinin	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 8.0 μM, TD_{50} > 1,130 μM, TI ≥ 243, and >50% reduction of penetrated tachyzoites	Jones-Brando et al. [68]
	T. gondii (RH strain)	Tachyzoites, Vero cells (CRL 6318)	Artemisinin	1 μg/mL, incubated for 120 h	*Tg*Prx expression was increased as early as 30 min and reached to the plateau in 1 h by the treatment in the intracellular stage, which indicates apoptosis of the parasite	Son et al. [59]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisinin	0.01, 0.1, 1, and 10 μg/mL, incubated for 16 h	Reduction in plaque size at 0.1 μg/mL, and inhibited all parasite growth at 1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisinin	0.4, 1.3, and 4 μg/mL, incubated for 27 days	At 1.3 μg/mL, reduced *T. gondii* plaque size was observed at day 14 and the infection was completely eliminated at day 22 post-treatment; at 4.0 μg/mL, the parasites were completely eliminated at day 14 (comparable to pyrimethamine treatment at 10 μg/mL)	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemether	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.31 μM, TD_{50} ≥ 320 μM, and TI = 1814. Parasites/vacuole ratio after treatment was 9, the same as the vehicle control	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemether	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Inhibited all parasite growth at 0.1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemether	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 0.7 μM, TD_{50} = 220 μM, and TI = 1,100. Significant reductions in the number of penetrated parasites	Jones-Brando et al. [68]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemether	0.01, 0.1, 1, and 10 μg/mL, incubated for 16 h	The only compound that diminished plaque size at 0.01 μg/mL; reduced plaque size and completely inhibited parasite growth at 0.1 μg/mL	Ke et al. [67]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	T. gondii (RH strain)	Tachyzoites in peritoneal exudate cells from Swiss Webster mice	Arteether	0.02, 0.2, 0.25, 0.5, 1.0, and 2.0 μg/mL, incubated for 21 h	> 0.5 μg/mL showed the parasite growth inhibition, higher efficacy than cycloguanil (0.5 μg/mL vs. 1.0 μg/mL), and reduction in the incorporation of ^{3}H-uracil into the nucleic acids of tachyzoites	Holfels et al. [62]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Arteether	0.01, 0.1, 1, and 10 μg/mL, incubated for 16 h	Reduced *T.gondii* plaque size and inhibited all parasite growth at 0.1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Arteether	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Greatly inhibited parasitic growth at 0.1 μg/mL (barely visible plaque)	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, epithelial cell line (LLC-MK2), derived from rhesus monkey (Macaca mulatta) kidneys	Artesunate	0.1, 0.2, 0.4, 0.8, 1.6, 3.1, 6.25, 12.5, 25, 50, and 100 μg/mL (the duration of treatment was not available)	IC_{50} = 0.075 μM or 0.029 μg/mL, TD_{50} = 2.003 μM, and SI = 26.707, higher efficacy than cotrimoxazole, pentamidine, pyramidine, and trimethoprim against tachyzoites and inducing death of infected cells	Gomes et al. [58]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artesunate	0.01–1.0 μM, incubated for 72 h	IC_{50} = 0.213 μM and IC_{90} = 0.368 μM	Dunay et al. [64]
	T. gondii (DUR strain)	Tachyzoites, THP-1 cell line	Artesunate	0.1, 0.2, and 0.5 μg/mL, incubated for 12, 24, 48, and 96 h	Maximum parasite growth inhibition of 89% was 0.1 μg/mL at 24 h, subsequent decrease in inhibition at 96 h	Sarciron et al. [65]
	T. gondii (DUR strain)	Tachyzoites, THP-1 cell line	Artesunate + DHA	0.1, 0.2, and 0.5 μg/mL, incubated for 12, 24, 48, and 96 h	Parasite growth inhibition > 82% was induced at 0.2 and 0.5 μg/mL at 12 h. Combination of the two drugs did not significantly improve the parasite growth inhibition	Sarciron et al. [65]
	T. gondii (DUR strain)	Tachyzoites, THP-1 cell line	DHA	0.1, 0.2, and 0.5 μg/mL, incubated for 12, 24, 48, and 96 h	Parasite growth inhibition > 78% was induced at 0.5 μg/mL at 12 h, triggered the death of infected cells	Sarciron et al. [65]
	T. gondii (RH strain)	Tachyzoites, HFF cells	DHA	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Inhibited all parasite growth at 1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemisone	0.010–1.0 μM, incubated for 72 h	IC_{50} = 0.12 μM and IC_{90} = 0.176 μM (the highest potency). The growth of tachyzoites was inhibited	Dunay et al. [64]
	T. gondii (RH strain)	Tachyzoites, HFF cells	1-propyl-ether-artemisinin	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Inhibited all parasite growth at 1 μg/mL	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	1-butyl-ether-artemisinin	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Inhibited all parasite growth at 1 μg/m	Ke et al. [67]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	T. gondii (RH strain)	Tachyzoites, HFF cells	sec-butyl-ether-artemisinin	0.01, 0.1, 1, and 10 μg/mL, incubated for 5 days	Greatly inhibited parasite growth at 0.1 μg/mL (barely visible plaque)	Ke et al. [67]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Artemiside	0.010–1.0 μM, incubated for 72 h	IC_{50} = 0.108 μM and IC_{90} = 0.167 μM	Dunay et al. [64]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 1 (DART-1,3-thiazole, H substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 1.1 μM, $TD_{50} \geq$ 320 μM, and TI = 511. Parasites/vacuole ratio after treatment was 2	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3 (DART-1,3-thiazole, Me substituted R_1, Me substitued R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.16 μM, TD_{50} = 38 μM, and TI = 240. Parasites/vacuole ratio after treatment was 2; higher efficacy than artemisinin and artemether	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 4 (DART-1,3-thiazole, H substituted R_1, Me substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.37 μM, $TD_{50} \geq$ 320 μM, and TI = 1520. Parasites/vacuole ratio after treatment was 2; higher efficacy than artemisinin and artemether; higher TI than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 5 (DART-1,3-thiazole, Ph substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.34 μM, $TD_{50} \geq$ 320 μM, and TI = 1654. Parasites/vacuole ratio after treatment was 2; higher efficacy than artemisinin and higher TI than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 6 (DART-1,3-thiazole, *t*-Bu substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.25 μM, $TD_{50} \geq$ 320 μM, and TI = 2249. Parasites/vacuole ratio after treatment was 2; higher efficacy and TI than artemisinin and artemether	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 9 (DART-1,3-thiazole, *p*-MeOPh substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.35 μM, $TD_{50} \geq$ 320 μM, and TI = 1607. Parasites/vacuole ratio after treatment was 2; higher efficacy than artemisinin and artemether, and higher TI than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 11 (DART-1,3-thiazole, *p*-MeSPh substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.40 μM, $TD_{50} \geq$ 320 μM, and TI = 1406. Parasites/vacuole ratio after treatment was 1; higher efficacy and TI than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 12 (DART-1,3-thiazole, *p*-MeS(O_2)Ph substituted R_1, H substituted R_2)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.42 μM, $TD_{50} \geq$ 320 μM, and TI = 1339. Parasites/vacuole ratio after treatment was 2; higher efficacy and TI than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 22 (DART-carboxamide, H substituted R_4, *p*-CF_3Bn substituted R_5)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.34 μM, $TD_{50} \geq$ 320 μM, and TI = 1654. Parasites/vacuole ratio after treatment was 2; higher efficacy than artemisinin	Hencken et al. [63]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 24 (DART-carboxamide, H substituted R_4, 3,5-F_2Bn substituted R_5)	1, 5, and 10 μM, incubated for 24–26 h	IC_{50} = 0.36 μM, $TD_{50} \geq$ 320 μM, and TI = 1562. Parasites/vacuole ratio after treatment was 4; higher efficacy and TI than artemisinin	Hencken et al. [63]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3c (aldehyde substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 0.3 mg/L (the highest potency) TD_{50} = 26 mg/L, and TI = 92 (higher potency and TI than trimethoprim); significant reduction in the number of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3b (benzothiazole substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 0.4 mg/L, TD_{50} = 9 mg/L, and TI = 28 (higher potency and TI than trimethoprim); > 50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3a (2-thiazole substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 0.6 mg/L, TD_{50} > 320 mg/L, and TI = 975 (the highest TI, higher potency and TI than trimethoprim); > 50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3e (methyl ester substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 0.9 mg/L, TD_{50} = 177 mg/L, and TI = 210 (higher potency and TI than trimethoprim); > 50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3f (ethyl amide substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 1.5 mg/L, TD_{50} > 72 mg/L, and TI = 52 (higher potency and TI than trimethoprim); > 50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 3d (carboxylic acid substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 12.5 mg/L, TD_{50} > 320 mg/L, and TI = 60 (the least potency); > 50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Deoxy-compound 3a (2-thiazole substituted at C-10)	Serial dilutions: 320–0.032 mg/L, incubated for 5 days	IC_{50} = 280.6 mg/L, TD_{50} = 320 mg/L, and TI = 2; >50% reduction of penetrated parasites	D'Angelo et al. [69]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 2d (Trioxane C-10 citronelyl ether)	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 1.1 μM, TD_{50} = 160 μM, and TI = 320	Jones-Brando et al. [68]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 2c (Trioxane C-10 2-bromobenzoate ester)	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 1.2 μM, TD_{50} > 630 μM, and TI ≥ 933	Jones-Brando et al. [68]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 2b (Trioxane C-10 benzoate ester)	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 1.4 μM, TD_{50} > 740 μM, and TI ≥ 933	Jones-Brando et al. [68]
	T. gondii (RH strain)	Tachyzoites, HFF cells	Compound 2a (Trioxane C-10 primary alcohol)	Serial dilutions: 0–320 μg/mL, incubated for 94 h	IC_{50} = 8.3 μM, TD_{50} = 1560 μM, and TI = 190	Jones-Brando et al. [68]
In vivo studies						
	T. gondii (PRU strain)	CD1 mice	Artemisinin	10 mg/kg, s.c., q.d. for 8 days	20% of artemisinin-treated mice survived the infection at 25 days p.i., the Luc value (or parasite burden) was the highest (12×10^6) among all of the treated groups at day 9, developed higher cyst number in the brain (~90)	Dunay et al. [64]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	T. gondii (PRU strain)	CD1 mice	Artemiside	10 mg/kg, s.c., q.d. for 8 days	60% of artemiside-treated mice survived the infection at 25 days p.i., the Luc value was the lowest (2×10^6) among all of the treated groups at day 9, developed the lowest cyst numbers in the brain (~10)	Dunay et al. [64]
	T. gondii (PRU strain)	CD1 mice	Artemisone	10 mg/kg, s.c., q.d. for 8 days	Over 50% of artemisone-treated mice survived the infection at 25 days p.i., reduced Luc value at day 9, developed fewer cysts in the brain (~30)	Dunay et al. [64]
	T. gondii (DUR strain, isolated from the amniotic fluid of a pregnant woman)	OF1 mice	Artesunate + DHA	With ratio 1:1 of the two drugs, 100 mg/day p.o. in a 0.2-mL volume, t.i.d. for 5 days	No mice died during the experiment. Fewer *T. gondii* cysts in the brains of treated mice (267 ± 10.31) vs. control (650 ± 37.27), 75% of the cysts did not appear different from the controls after treatment. The parasite internal membranes altered with an irregular outline, and approximately 25% of the bradyzoites were damaged	Sarciron et al. [65]
	T. gondii (RH strain)	Kunming mice	Artemether	200 mg/kg, q.d. for 8 days	After treatment, the intensity of glucose-6-phosphatase activity of the parasite was decresased compared with non-treated control parasites	Liang et al. [66]

DART: dehydroartemisinin

DHA: dihydroartemisinin

IC_{50}: concentration that causes 50% inhibition of growth

i.g.: intragastric administration

p.i.: post-infection

p.o.: oral administration,

q.d.: one time per day

s.c.: subcutaneous injection

SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase

SI: selectivity index

TD_{50}: median cytotoxic dose

TI: therapeutic index

t.i.d.: three times per day

*The IC50 is either in μg/mL or μM as in the original papers.

Table 4

Treatment of artemisinin and its derivatives against *Neospora caninum* and *Eimeria tenella*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro						
	N. caninum (KB-2)	Tachyzoites in mouse peritoneal macrophages (mouse strain was not stated)	Artemisinin	0.1, 1, and 10 μg/mL, started from 12 h p.i. to 30 h	The total number of tachyzoites per 100 infected macrophages reduced with a clear dose-dependent pattern, which were about 700, 500, and 340 at 0.1, 1, and 10 μg/mL, respectively	Kim et al. [71]
	N. caninum (KB-2)	Tachyzoites, Vero cells	Artemisinin	0.01, 0.1, 1, and 10 μg/mL, started from 12 h p.i. to 30 h	The total number of tachyzoites per 100 infected Vero cells was reduced with a clear dose-dependent pattern, which were about 980, 570, 300, and 260 at 0.01, 0.1, 1, and 10 μg/mL, respectively	Kim et al. [71]
	N. caninum (KB-2)	Tachyzoites, Vero cells	Artemisinin	0.1, 1, 10, and 20 μg/mL, started from 12 h p.i. to 12 days	10 or 20 μg/mL eliminated all microscopic foci of *N. caninum* in Vero cells by 11 days (third subculture) and 1 μg/ml eliminated all microscopic foci of *N.caninum* by 14 days (fourth subculture)	Kim et al. [71]
	N. caninum (NcIS491)	Tachyzoites, Vero cells	Artemisone	0.1, 0.5, 5.0, 10.0, or 15.0 g/mL, 5 h prior to infection 25.0 or 50.0 g/mL for	The parasite inhibitory effect was dose-dependent. Percentages of infected cells at 96 h of cultivation were 8.5%, 5.7%, 3.5%, 7.8%, 4.5%, and 1.0%, respectively. Percentages of parasite inhibition at 96 h of cultivation were 90.2%, 93.4%, 95.9%, 91.0%, 94.8%, 98.8%, and 87.3%, respectively	Mazuz et al. [72]
	N. caninum (NcIS491)	Tachyzoites, Vero cells	Artemisone	24 h, added at initial 15.3% of cells infected	The parasite inhibitory effects were 50.1% and 81.9%, respectively, compared to control	Mazuz et al. [72]
	N. caninum-β-galactosidase expression	Tachyzoites, HFF and Vero cells	Artemisone	Pre-treatment with 5 μM for 1 or 3 h. After 2 h, either 500 nM or 5 μM of the drug was added. The one with initial 500 nM is then increased stepwise for a period of 40 days	IC_{50} = 3 nM. Only after 21 days of continuous treatment with 5 μM of artemisone, all tachyzoites were eliminated	Müller et al. [74]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	N. caninum-β-galactosidase expression	Tachyzoites, HFF and Vero cells	GC007 (artemisinin derivative)	Pre-treatment with 5 μM for 1 or 3 h. After 2 h, either 500 nM or 5 μM of the drug was added. The one with initial 500 nM is then increased stepwise for a period of 40 days	IC_{50} = 29 nM. Only after 21 days of continuous treatment with 5 μM of GC007, all tachyzoites were eliminated	Müller et al. [74]
	N. caninum-β-galactosidase expression	Tachyzoites, HFF and Vero cells	GC012 (artemisinin derivative)	Pre-treatment with 5 μM for 1 or 3 h. After 2 h, either 500 nM or 5 μM of the drug was added. The one with initial 500 nM is then increased stepwise for a period of 40 days	IC_{50} = 54 nM. Tachyzoites readily adapted to a gradual increase in the concentration (0.5–10 μM) within 20 days, indicating the changes in tachyzoites were less dramatic	Müller et al. [74]
	N. caninum-GFP expression	Tachyzoites, HFF cells	Artemether	1, 10, 30, 40, 50, 60, 80, and 100 μg/mL for 2 h	IC_{50} = (1.0 ± 0.05) μg/mL, parasite growth concentration was 100 μg/mL	Qian et al. [75]
In vivo studies						
	N. caninum (NcIS491)	Gerbils (*Meriones tristrami*)	Artemisone	20 mg/kg, i.p., b.i.d. for 4 days	Among 9 treated gerbils, only 1 animal exhibited clinical signs and died 10 days p.i. vs. controls: 8 out of 9 untreated gerbils died with characteristic cerebral signs	Mazuz et al. [72]
	N. caninum (Nc-Spain7)	BALB/c male mice	Artemisone	50 mg/kg/day suspended in 100μL corn oil, i.g., q.d. for 6 days	Parasite burden in brains was 1–5 × 10^3 tachyzoites per μg DNA after treatment, lower than those of placebo (0.5–14 × 10^3), mefloquine (5–7.5 × 10^3), and artemiside (1–15 × 10^3) treatments; however parasite burden in lungs (1.4–2.2 × 10^3) after treatment, higher than those of placebo, mefloquine, and artemiside treatments	Müller et al. [73]
In vivo						
	E. tenella (S98N3 strain)	White leghorn chicken	Pure artemisinin	10 ppm, fed independently in food, p.o., 7 days p.i.	Significantly reduced oocyst output (0.7 × 10^6) vs. control (4.2 × 10^6) and reduced sporulation rate of oocyte (73.2% vs. 97.4%); chicken mortality rate reduced to 2.3% and lesion score reduced to 1.2 after treatment. SERCA expression in macrogamates reduced to 77.6% vs. 98.7% in control, indicating artemisinin was effective on the parasite	del Cacho et al. [78]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
	E. tenella (S98N3 strain)	White leghorn chicken	Pure artemisinin	17 ppm in food, fed independently in food, p.o., 7 days p.i.	Significantly reduced oocyst output (0.2×10^6) vs. control (4.2×10^6), and reduced sporulation rate of oocyte (62% vs. 97.4%); the mortality rate of chickens reduced to 1.1% and lesion score reduced to 1. SERCA expression in macrogamates reduced to 66.4% vs. 98.7% in control	del Cacho et al. [78]

b.i.d.: two times per day

IC_{50}: concentration that causes 50% inhibition of growth

HFF: human foreskin fibroblast

i.g.: intragastric administration

i.p.: intraperitoneal injection

p.i.: post-infection

p.o.: oral administration

q.d.: one time per day

SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase

*The IC50 is either in μg/mL or nM as in the original papers.

Table 5

Treatment of artemisinin and its derivatives against *A. castellanii* and *N. fowleri*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro	*A. castellanii* (ATCC 30011)	Tachyzoites in peptone-yeast-glucose medium	Artemether	100 μg/mL, incubated for 4 h	The CI is slightly changed TEM: minimal effect on the trophozoites The protein levels of PGDH and PSAT were significantly decreased by western blot analysis	Deng et al. [82]
	A. castellanii (ATCC 30011)	Tachyzoites in peptone-yeast-glucose medium	Artemether	150 μg/mL, incubated for 12 h	10 min: trophozoites rounded and detached from the bottom of the well and pseudopodia disappeared 4 h: amoebae underwent cell lysis and most visible structures including the nucleus disappeared 8 h: the CI decreased by approximately 50% 12 h: the protein levels of PGDH and PSAT enzymes were markedly downregulated by western blot analysis TEM: The nuclear condensation and mitochondrial deformation of trophozoites SEM: pseudopodium retraction and trophozoites disrupted 6 days: the numbers of viable trophozoites significantly decreased	Deng et al. [82]
	A. castellanii (ATCC 30011)	Tachyzoites in peptone-yeast-glucose medium	Artemether	200 μg/mL, incubated for 24 h	10 min: trophozoites rounded and detached from the bottom of the well and psudopodia dissapeared 4 h: amoebae underwent cell lysis and nucleus disappeared 8 h: the CI decreased by approximately 50% 12 h: the protein levels of PGDH and PSAT enzymes markedly downregulated at 200 μg/mL of artemether, by western blot analysis 5 days: directly damaged trophozoites and no viable trophozoites were detected in the culture The necrotic changes in amoebae increased to 57.2% (PI staining). Apoptotic and necrotic cells increased from 2.77% to 8.89% and from 0.124% to 25.2%, respectively (by PI staining and annexin V-FITC double staining) The protein levels of PGDH and PSAT enzymes were significantly downregulated by the iTRAQ quantitative proteomic analysis TEM: the nuclear condensation and mitochondrial deformation of trophozoites SEM: pseudopodium retraction and trophozoites disrupted	Deng et al. [82]
In vivo studies	*N. fowleri* (CJ strain)	Swiss mice	Artemisinin	60–180 mg/kg, i.m. for 5 days	Not curative and showed only slight protection as indicated by extension of the mean survival time	Gupta et al. [85]
	N. fowleri (CJ strain)	Swiss mice	Beta-arteether	60–180 mg/kg, i.m. for 5 days	Not curative and showed only slight protection as indicated by extension of the mean survival time	Gupta et al. [85]
	N. fowleri (CJ strain)	Swiss mice	Artesunate	60–180 mg/kg, i.m. for 5 days	Not curative and showed only slight protection as indicated by extension of the mean survival time	Gupta et al. [85]

CI: cell index

i.m.: intramuscular injection

iTRAQ: isobaric tags for relative and absolute quantitation

PGDH: phosphoglycerate dehydrogenase

PI staining: propidium iodide staining

PSAT: phosphoserine aminotransferase

SEM: scanning electron micrograph

TEM: transmission electron micrograph

Table 6

Treatment of artemisinin and its derivatives against *Cryptosporidium parvum* and *Giardia lamblia*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and time)	Effect	Reference
In vitro	*C. parvum* (solated from stools of AIDS patients)	Sporozoites, human lung carcinoma (A549 cells)	Artemisin	0.02, 0.2, and 2 mg/L, incubated for 72 h	Minor decrease in mean numbers of oocysts (1.3%) and schizonts (1.6%) at 2 mg/L	Giacometti et al. [88]
In vitro	*G. lamblia* (isolate C2)	Trophozoites cultured in modified TYI-S-33 medium (without cells)	DHA	12.5, 25, 50, 100 and 200 μg/mL, incubated for 12 h	G_0/G1 phase: 43.70%; S phase: 0%; G2/M phase: 56.30% Mortality rate at 100 μg/mL and 200 μg/mL increased from 48.06% to 100%	Tian et al. [90]

DHA: dihydroartemisinin

Table 7

Treatment of artemisinin and its derivatives against *Barbesia spp.*

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and	Effect	Reference
In vitro	*B. bovis* (Texas T2B strain from cattle)	In bovine RBCs	Artesunate	0.26, 2.6, 26, and 260 μM, medium replaced daily, incubated for for 4 days	Less effective than atovaquone and diminazene aceturate. Growth inhibition was ≥ 2.6 μM, maintained even after withdrawal of treatment at day 4	Goo et al. [95]
	B. bovis (isolate 767 from naturally infected cows)	In Holstein-Friesian calf RBCs	Artemisone	11–300 μg/mL, incubated for 24, 48, and 72 h	Parasite inhibitory effect within 24 h was dose-dependent. Significant effect was at 300 μg/mL and IC_{50} = 180.4 μg/mL (or 449 μM). At 72 h post-treatment, all the doses significantly inhibited parasitemia	Mazuz et al. [94]
	B. bigemina (isolate 871 from naturally infected cows)	In Holstein-Friesian calf RBCs	Artemisone	11–300 μg/mL, incubated for 24, 48, and 72 h	Parasite inhibitory effect at 24 h was 61.4%–80% with IC_{50} = 8.9 μg/mL (22.5 μM). At 72 h post-treatment, the maximum percentage of parasite growth inhibition was 99.6%	Mazuz et al. [94]
	B. gibsoni (M1211 from a naturally infected Tosa dog)	In canine RBCs	Artemisinin	The concentrations for atovaquone-resistant *B. gibsoni* were 3–300 μM, medium replaced daily, incubated for 6 days	Growth inhibition at 300 μM and IC_{50} = 2210 nM	Iguchi et al. [98]
	B. gibsoni (M1211 from a naturally infected Tosa dog)	In canine RBCs	Artemether	The concentrations for atovaquone -resistant *B. gibsoni* were 1–100 μM, medium replaced daily, incubated for 6 days	Growth inhibition at 100 μM and IC_{50} = 4720 nM. The activity of artemether against *B. gibsoni* was lower than other artemisinin compounds	Iguchi et al. [98]
	B. gibsoni (NRCPD strain from dogs)	In canine RBCs	Artesunate	0.26, 2.6, 26, and 260 μM, medium replaced daily, incubated for 4 days	Growth inhibition at 26 and 260 μM from day 1 post-treatment. Significant differences of test concentrations were observed at day 3 post-treatment. Withdrawal of treatment, reemergence of the parasite failed to occur at concentration ≥ 2.6 μM	Goo et al. [95]
	B. gibsoni (from a naturally infected Tosa dog)	In canine RBCs	Artesunate	0.1–10 μM, medium replaced daily, incubated for 7 days	IC_{50} = (878.89 ± 27.13) nM	Matsuu et al. [97]
	B. gibsoni (from a naturally infected Tosa dog)	In canine RBCs	DHA	0.1–10 μM, medium replaced daily, incubated for 7 days	IC_{50} = (937.50 ± 45.21) nM	Matsuu et al. [97]
	B. caballi (USDA strain)	In equine RBCs	Artesunate	0.04–5 μg/mL, incubated for 24 h	IC_{50} = 0.18 μg/mL (or 468 nM)	Nagai et al. [96]
	B. equi (USDA strain)	In equine RBCs	Artesunate	0.04–5 μg/mL, incubated for 24 h	IC_{50} = 0.1 μg/mL (or 260 nM)	Nagai et al. [96]

Experiments	Species (strain)	Host/cell line	Drug	Treatment (dose, route, and	Effect	Reference
In vivo						
	B. bovis (isolate 767 from naturally infected cows)	Holstein-Friesian calves	Artemisone	5 mg/kg diluted in 5 mL of DMSO, i.p., b.i.d. for 4 consecutive days	Fever: 40.9°C to 41.3°C was recorded. In 4/5 animals, the minimum PCV was 17%, and maximum parasitemia ranged from 0.1% to 0.5%; 4/5 animals recovered, one did not response to treatment	Mazuz et al. [94]
	B. bigemina (isolate 871 from naturally infected cows)	Holstein-Friesian calves	Artemisone	5 mg/kg diluted in 5 mL of DMSO, i.p., b.i.d. for 4 consecutive days	No acute signs of babesiosis: no fever, the minimum PCV decreased to 27%, the parasitemia reached a maximum of 1.3%, both calves recovered, and parasitemia <0.1% during the observation period	Mazuz et al. [94]
	B. microti (strain not stated)	BALB/c mice	Artesunate	1, 10, or 50 mg/kg, i.m. for 6 consecutive days, started at 2 days p.i.	Lower parasitemia was observed in 10 and 50 mg/kg groups	Goo et al. [95]
	B. equi (Indian strain)	Splenectomised donkeys	Artesunate	2.5 mg/kg, i.m., q.d. for 4 days	No recrudescence, died between 16–19 days post-treatment; the mean survival time after treatment was 17 days	Kumar et al. [101]
	B. equi (Indian strain)	Splenectomised donkeys	Arteether	5 mg/kg, i.m., q.d. for 3 days	No recrudescence, died between 4–6 days post-treatment; the mean survival time after treatment was 5 days	Kumar et al. [101]
	B. equi (Indian strain)	Splenectomised donkeys	Arteether + buparvaquone	5 mg/kg of arteerther, i.m., q.d. for 3 days and then with 5 mg/kg of buparvaquone, i.v., q.i.d. for 4 days	Recrudescence occurred 50–54 days after treatment. Animals recovered 4 days post-treatment; the mean survival time after treatment was 66 days	Kumar et al. [101]

b.i.d.: two times per day

DHA: dihydroartemisinin

DMSO: dimethyl sulphoxide

IC_{50}: concentration that causes 50% inhibition of growth

i.m.: intramuscular injection

i.p.: intraperitoneal injection

i.v.: intravenous injection

PCV: packed cell volume

p.i.: post-infection

q.d.: one time per day

q.i.d.: four times per day

RBC: red blood cell

* The IC50 is either in μg/mL, μM, or nM as in the original papers.