

2015

Clinically Available Medicines Demonstrating *Anti-Toxoplasma* Activity

Andrew J. Neville

University of Nebraska-Omaha

Sydney J. Zach

University of Nebraska-Omaha

Xiaofang Wang

University of Nebraska Medical Center, Omaha, Nebraska

Joshua J. Larson

University of Nebraska-Omaha

Abigail K. Judge

University of Nebraska-Omaha

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/publichealthresources>


Neville, Andrew J.; Zach, Sydney J.; Wang, Xiaofang; Larson, Joshua J.; Judge, Abigail K.; Davis, Lisa A.; Vennerstrom, Jonathan L.; and Davis, Paul H., "Clinically Available Medicines Demonstrating Anti-*Toxoplasma* Activity" (2015). *Public Health Resources*. 422. <http://digitalcommons.unl.edu/publichealthresources/422>

This Article is brought to you for free and open access by the Public Health Resources at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Public Health Resources by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Andrew J. Neville, Sydney J. Zach, Xiaofang Wang, Joshua J. Larson, Abigail K. Judge, Lisa A. Davis, Jonathan L. Vennerstrom, and Paul H. Davis

Clinically Available Medicines Demonstrating Anti-*Toxoplasma* Activity

Andrew J. Neville,^a Sydney J. Zach,^a Xiaofang Wang,^b Joshua J. Larson,^a Abigail K. Judge,^a Lisa A. Davis,^{c,d,e} Jonathan L. Vennerstrom,^b  Paul H. Davis^a

Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska, USA^a; College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA^b; Rheumatology, Denver Health Medical Center, Denver, Colorado, USA^c; University of Colorado School of Medicine, Aurora, Colorado, USA^d; Department of Internal Medicine, Denver Veterans Affairs Medical Center (VAMC), Denver, Colorado, USA^e

***Toxoplasma gondii* is an apicomplexan parasite of humans and other mammals, including livestock and companion animals. While chemotherapeutic regimens, including pyrimethamine and sulfadiazine regimens, ameliorate acute or recrudescent disease such as toxoplasmic encephalitis or ocular toxoplasmosis, these drugs are often toxic to the host. Moreover, no approved options are available to treat infected women who are pregnant. Lastly, no drug regimen has shown the ability to eradicate the chronic stage of infection, which is characterized by chemoresistant intracellular cysts that persist for the life of the host. In an effort to promote additional chemotherapeutic options, we now evaluate clinically available drugs that have shown efficacy in disease models but which lack clinical case reports. Ideally, less-toxic treatments for the acute disease can be identified and developed, with an additional goal of cyst clearance from human and animal hosts.**

Known for more than 100 years, the apicomplexan parasite *Toxoplasma gondii* is distributed throughout the world in a great variety of mammalian hosts, including humans (1). Initial exposure to the parasite leads to lifelong chronic infection that is established within cells of the central nervous system (CNS) and that, until recently, has been considered largely asymptomatic in otherwise healthy human populations (2). Recent data from studies in humans and in model organisms now suggest that chronic infection by *T. gondii* may be capable of inducing behavioral changes, such as impaired response times (3) or impaired learning (4), and is associated with psychiatric disorders such as schizophrenia (5). More classically, *Toxoplasma* infection is known as a leading cause of birth defects, brought about when the woman receives a primary infection during pregnancy. These trans-uterine infections often cause life-threatening encephalitis and/or other lifelong neurological or ocular illnesses in congenitally infected newborns (6). In addition, people with weakened immune systems, such as AIDS patients or organ transplant recipients who undergo lifelong immunosuppression, are at significant risk of developing life-threatening toxoplasmic encephalitis from primary or recrudescent infection.

It is estimated that approximately 30% to 50% of adults worldwide are infected with *T. gondii*, which is acquired most frequently from eating infected, undercooked meats or via exposure to infected cat fecal matter. Studies report that toxoplasmosis causes the highest disease burden of food-borne pathogens in developed countries and, ultimately, is the second leading cause of death due to food-borne illness (7, 8). Other than fully cooking meats and changing cat litter frequently, there are few interventions that can impede human infection: the only available vaccine is not licensed in North America and is approved solely for sheep (9). Indeed, the vaccine's primary effect is to reduce spontaneous fetal abortion in agricultural mammals, a common outcome of *Toxoplasma* infection in some livestock species. It is estimated that the associated disease has an annual economic impact of \$7.7 billion in the United States alone (10). Similarly, feline companion animals can

be tested for *Toxoplasma* infection, but treatment to eliminate feline cyst-shedding ability is unavailable.

Despite the well-established maladies resulting from infection by this parasite and the recent associations of chronic infection with altered host behavior, only nonideal treatment options exist (11). For example, in the United States, there are no approved therapies for maternal and fetal infections. Moreover, common medications used synergistically for the treatment of acute toxoplasmosis (i.e., toxoplasmic encephalitis) have well-known side effects: pyrimethamine induces bone marrow toxicity, and many patients are hypersensitive to sulfadiazine (12). The relatively well-tolerated drug atovaquone is increasingly used in acute infections, but only as an adjunctive therapy. Finally, no therapeutic agent or regimen evaluated to date is capable of clearing the chronic infection in humans or in livestock animals.

In an effort to spur the development of treatment regimens with reduced toxicity and/or the capacity to clear chronic infection in human or animal populations, the purpose of this review is to comprehensively profile clinically available drugs that have shown promising activity against *T. gondii* *in vivo* and *in vitro* but that lack clinical case reports. Such medications may be repurposed for effective use against *Toxoplasma* infections (13). Historically, the chronic infection was not associated with frank disease in otherwise healthy human patients, so therapeutic attempts at clearance were not undertaken. However, there is growing awareness of the value in eradicating chronic infection: in addition to interrupting the infectious route (if regimens were to be applied to

Accepted manuscript posted online 21 September 2015

Citation Neville AJ, Zach SJ, Wang X, Larson JJ, Judge AK, Davis LA, Vennerstrom JL, Davis PH. 2015. Clinically available medicines demonstrating anti-*Toxoplasma* activity. *Antimicrob Agents Chemother* 59:7161–7169. doi:10.1128/AAC.02009-15.

Address correspondence to Paul H. Davis, pdavis@unomaha.edu.

A.J.N. and S.J.Z. contributed equally to this article.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

This document is a U.S. government work and is not subject to copyright in the United States.

TABLE 1 A list of drugs commonly used against toxoplasmosis^a

Drug(s)	References	Mechanism of action	<i>In vitro</i> IC ₅₀	Murine lethal challenge(s) and outcome (reference[s])
Pyrimethamine	15–20	Antifolate	0.4 μM	10 mg/kg/day, 20–90% survival, depending on parasite strain (18)
Dapsone, sulfadiazine, sulfadoxine, sulfamethoxazole	17, 19, 21–27	Antifolate	Dapsone, 1.2 μM; sulfadiazine, 1.6 μM; sulfamethoxazole, 395 μM	Dapsone, 100 mg/kg/day, 100% survival (24); sulfadiazine, 375 mg/kg/day, 100% survival (17); sulfamethoxazole, 600 mg/kg/day, 100% survival (26)
Clindamycin	17, 27–30	Protein synthesis inhibitor	0.005 μM	300 mg/kg/day, increased survival by 11 days (17)
Trimethoprim (typically combined with sulfamethoxazole)	21, 25, 26, 31	Antifolate	17.2 μM	70 mg/kg/day, 20% survival (26)
Atovaquone	27, 32, 33	Mitochondrial electron transfer chain inhibitor	0.82 μM	5 mg/kg/day, 20% survival (19)
Doxycycline, minocycline	34, 35	Apicoplast division inhibitor	Doxycycline, 14.4 μM	Doxycycline, 300 mg/kg/day, 100% survival (34)
Acetylspiramycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin, telithromycin, tylosin	18, 29, 36–41	Protein synthesis inhibitor	Azithromycin, 11.5 μM; clarithromycin, 401 μM; erythromycin, 19.6 μM; roxithromycin, 32.3 μM; spiramycin, 17.8 μM; telithromycin, 0.20 μM	Azithromycin, 200 mg/kg/day, 40–100% survival, depending on parasite strain (38); clarithromycin, 200 mg/kg/day, 80% survival (40); clarithromycin, 300 mg/kg/day, 100% survival (18); roxithromycin, 10 mg/day, 80–100% survival (36, 37); spiramycin, 400 mg/kg/day, no survival (38)

^a The lowest observed IC₅₀ values are reported in cases in which multiple values were found in the literature. Moreover, wide variations exist in the method of murine lethal challenge between studies: differences include infectious dose, parasite strain, drug delivery route, time postinfection before treatment initiation, treatment length, and time of observed survival.

companion and/or livestock animals), it would reduce the risk of reactivation in immunocompromised patients, and the possibility exists that clearing infection from the brain might reverse any parasite-induced behavioral phenotypes. Despite its known side effects, no treatment has been as effective against the acute stage as the synergistic combination of pyrimethamine and sulfadiazine, which target the folate synthesis pathway of the parasite. As an addition to or a replacement of the clinical use of pyrimethamine and sulfadiazine against acute infection, the following are commonly employed: clindamycin, doxycycline, dapsone, and an erythromycin derivative (e.g., clarithromycin or azithromycin) (14). These are summarized briefly in Table 1.

The characteristics of an ideal anti-*Toxoplasma* drug or combination would be severalfold. First, treatment should be effective against both stages of parasite growth in all mammals: the fast-growing tachyzoite stage associated with acute disease and rapid cell invasion and division (and easily cultivated *in vitro*) and the chronic bradyzoite stage associated with parasite formation of an intracellular chemoresistant cyst wall within infected brain and muscle cells. Often, measurements in model organisms to evaluate tachyzoite susceptibility to the drug treatment do so by quantifying parasite counts (“parasite burden”) of internal organs (heart, liver, and/or spleen) typically 3 to 10 days following the initial infection (notably, where the degree of parasite clearance is positively correlated with the length of treatment) or by providing a lethal challenge and counting mice spared due to drug treatment. In contrast, bradyzoite cyst reduction resulting from drug treatment is typically evaluated by quantifying cysts that form in the brain by at least 30 days postinfection. Although not uncommon, trials evaluating cyst levels when the drug is administered early in

the infective process (<30 days after infection) do not accurately measure the effect on the cyst stage but instead measure the reduction of the count of parasites entering the brain—a process that is known to take several days, depending on the parasite and host species/strain. Second, the ideal treatment would be parasitocidal against these two stages, but parasitostatic capability against the tachyzoite stage, which has greater difficulty resisting the host adaptive immune response, may be sufficient. Mice are used almost universally in these evaluations, which is quite appropriate and natural for this parasite: carnivorousness of infected mice is likely the most common infection route for cats, the definitive host of *Toxoplasma gondii*. For compounds to effectively eliminate parasitic brain cysts, they would likely need to penetrate the blood-brain barrier. Lastly, an ideal treatment would show high efficacy and low toxicity across a range of hosts, including humans but also livestock and companion animals.

CLINICALLY AVAILABLE MEDICATIONS WITH ANTI-TOXOPLASMA ACTIVITY

Table 2 represents a comprehensive review of the literature accessed from PubMed using a search with the keywords “toxoplasma* AND (drug* OR treatment*).” Reviewed publication dates were limited to 1 January 1980 to 4 July 2015. A total of 5,222 items were filtered for primary literature evaluating the *in vitro* or *in vivo* efficacy of clinically available compounds, excluding publications evaluating any drug with a 50% inhibitory concentration (IC₅₀) that was >10 μM or that was determined solely by the less reliable enzyme-linked immunosorbent assay (ELISA) method (81) and those already listed in Table 1. Treatments for ocular infection were not included, as these have been recently reviewed

TABLE 2 A comprehensive list of clinically available human or veterinary drugs evaluated against the parasite *Toxoplasma gondii*, ordered by common clinical uses^a

Drug or drug class	Common clinical use	Reference(s)	<i>In vitro</i> IC ₅₀	<i>In vivo</i> lethal challenge(s)	<i>In vivo</i> parasite burden for indicated tissue or animal and treatment (reference(s))
Miltefosine	Antimicrobial	42	NID	<i>In vivo</i> lethal challenge: 20 mg/kg/day, 5% increased survival*	78% significant reduction of brain cyst count; treatment lasted 15 days; starting 60 days postinfection
Niclosamide	Antimicrobial	43	~0.25 μM	ND	ND
Triclosan	Antimicrobial	44-48	0.02 μM	200-300 mg/day, no difference in survival compared to control (45, 46)	Significant reduction in parasite burden (45, 47, 48); treatment lasted 4 days (45)
Artemisinins	Antiprotozoal	49-52	Artemisinin, 0.64 μM; artesunate, 0.213 μM; artemether, 0.31 μM; dihydroartemisinin, 0.35 μM	Artemisinin, 10 mg/kg/day; 20% survival (49)	ND
Veterinary antioxiocidals	Antiprotozoal	53-60	Halofuginone, 0.00094 μM; montenism, 0.0015 μM; dichlazuril, 0.006 μM; decoquinate, 0.011 μM; apripinocid N-oxide, 0.05 μM; salinomycin, 0.053 μM; robenidine, 0.09 μM; toltrazuril, 0.94 μM; clopridol, 5.21 μM; apripinocid, 7.2 μM	Apripinocid, 100 μg/day, 100% survival (53); ponazuril, 10 mg/kg/day, 100% survival (58); dichlazuril, 1 mg/kg/day, 100% survival (56, 57)	Toltrazuril (sheep); significant reduction in muscle and heart cyst burden (59); treatment lasted 14 days
Fusidic acid	Antibacterial	61	7.74 μM	60 mg/kg/day, no difference in survival	No reduction in parasite burden; treatment lasted 5 days
Pristinamycins	Antibacterial	62	Synercid, 1.57 μM	Synercid, 200 mg/kg/day, 100% survival	ND
Rifamycins	Antibacterial	32, 40, 63, 64	NID	Rifabutin, 300 mg/kg/day, 100% survival (63); rifampin, 50-200 mg/kg/day, 100% survival (32)	ND
Quinolones	Antibacterial	65-67	Gatifloxacin, 0.56 μM; trovafloxacin, 1.85-2.35 μM; enrofloxacin, <69.5 μM	Gatifloxacin, 400 mg/kg/day, 20% survival (66); enrofloxacin, 3 mg/kg/day, 17% survival (67); trovafloxacin, 100 or 200 mg/kg/day, 100% survival (65)	Enrofloxacin, 68% parasite cyst reduction (67); treatment lasted 25 days
Azoles	Antifungal	68-70	Itraconazole, 0.05 μM; fluconazole, 3.1 μM	Itraconazole, 10 mg/kg/day, 12% increased survival* (69); fluconazole, 20 mg/kg/day, 37% increased survival (69)	Itraconazole, significant reduction of brain cysts (69); treatment lasted 10 days; fluconazole, no significant reduction of brain cysts (69); treatment was carried out for 10 consecutive days, starting 4 days postinfection
Antiretrovirals	Antiretroviral	71-74	Didanosine, 0.21 μM; zalcitabine, 1.97 μM; saquinavir, 3.88 μM; fosamprenavir, 5.29 μM	Didanosine, ~1 mg/day, 100% survival (71)	Didanosine, <70% reduction in cerebral cysts (71); treatment lasted 30 days
5-Fluorouracil	Anticancer	75	0.08-0.77 μM	ND	ND
Crizotinib	Anticancer	76	0.4-4 μM	ND	ND
Gefitinib	Anticancer	76	5-10 μM	ND	ND
Methotrexate	Anticancer	15	0.83 μM	150 mg/kg/day, treatment led to earlier time of death (78)	Variable effects (77)
Cyclosporine	Immunosuppression	16, 77, 78	0.83 μM	150 mg/kg/day, treatment led to earlier time of death (78)	Variable effects (77)
Purine nucleoside analogues	Immunosuppression	77, 79	Adenine arabinoside, 1.5 μM	Azathioprine, 10 mg/kg/day, 100% survival (77)	Azathioprine, no significant reduction in parasite burden (77) after treatment for 7-100 days
Auranofin	Immunosuppression	80	0.28 μM	ND	Significantly reduced parasite burden in chicken embryo; one dose was administered
Antischizophrenic, antipsychotic, or mood-stabilizing agents	Psychiatric disorder treatment	81-83	Thioridazine, 1.2 μM; fluphenazine, 1.7 μM, trifluoperazine, 3.8 μM, chlorpromazine, 7.3 μM; zuclopentixol, 8 μM	ND	ND

^a The lowest observed IC₅₀ values are reported when multiple values were found in the literature. With one exception (didanosine), none of these drugs have been described in published toxoplasmosis clinical case reports*. *, not statistically significant; ND, not determined. The term “*in vivo* lethal challenge” used here refers to an experiment where model organisms are exposed to a 100% lethal infectious dose of parasite, often via intraperitoneal injection. The strain of the parasite and the recipient host affect the absolute number of parasites required to produce a lethal infection, which often manifests 7 to 15 days after infection. “*In vivo* parasite burden” refers to the tissue or fluid count of parasites isolated from a host following a nonlethal infection (typically accomplished by administration of a low dose of parasites and/or delivery of parasites via the oral route and/or the use of a low-virulence strain). Notably, parasite burden measurements are performed in a manner which typically quantifies both tachyzoite and bradyzoite parasite stages (most often by PCR). However, studies which aim to evaluate bradyzoite “cyst” counts (usually determined >30 day postinfection, since by this time the tachyzoites have been cleared by the immune system) are noted in the parasite burden column as such.

elsewhere (84). Articles not available in English were also excluded. The following includes a brief discussion on the demonstrated activity of these drugs against *Toxoplasma*.

ANTIMICROBIAL AGENTS

A number of relatively distinct compounds used clinically against an array of predominantly eukaryotic pathogens have shown some efficacy against *T. gondii*. Miltefosine is an analog of the ubiquitous compound phosphatidyl choline found in eukaryotic cell membranes and was initially developed to treat tumors. Subsequently, it was discovered to display potent efficacy against non-apicomplexan *Leishmania* protozoans, and it is now used clinically for treatment of *Leishmania* infections. Results of additional *in vitro* studies, including a recent study investigating *Toxoplasma* sensitivity, suggest that it may have much broader antimicrobial properties (42). The study showed that miltefosine had little efficacy in controlling acute infection after 5 days of treatment; however, a 15-day treatment against the established chronic stage led to a 78% reduction of the level of cysts in the brain. Moreover, the remaining cysts were noticeably smaller upon microscopic examination, suggesting that the drug effectively penetrates the blood-brain barrier and that extension of treatment time may produce greater effects. In an effort to provide functional options for treatment of infections by the unrelated parasite *Naegleria fowleri*, expanded investigational access to miltefosine for use against this uncommon but deadly infection has been granted by the United States Centers for Disease Control and Prevention. While the mechanism of action is not established in these antimicrobial roles, an appealing feature of miltefosine is an extended half-life of approximately 7 days in humans.

Niclosamide is a salicylanilide which is approved for treatment of parasitic worm infection, where it appears to decouple oxidative phosphorylation. Noting that niclosamide inhibits *T. gondii* growth *in vitro* at approximately 250 nM, Fomovska et al. designed a number of niclosamide derivatives and evaluated them against *T. gondii* (43). These showed efficacy against *in vitro* parasite growth; the most potent of these had an IC_{50} of 8 nM but was found to be parasitostatic, not parasitocidal. Efficacy against the cyst stage of *Toxoplasma* has not been studied.

Triclosan is a broad-spectrum agent used topically to inhibit fatty acid synthesis in susceptible organisms. Several reports indicate that triclosan (IC_{50} , 0.02 μ M) decreases *in vitro* parasite growth of not only *Toxoplasma* (see Table 2) but also *Plasmodium* (44) and *Babesia* (85). Investigators reported that the drug targets enoyl reductase, an enzyme not found in mammals. However, parasite survival studies are either lacking or disappointing; triclosan did not extend mouse survival during a lethal challenge. This hydrophobic drug may hold greater promise if it can be more effectively delivered to the target enzyme (which resides in the parasite's quadruple-membraned organelle, the apicoplast): proof-of-concept approaches investigated triclosan conjugated to octarginine or encased in liposomal nanoparticles, the latter conferring greater reductions of parasite counts in peritoneal fluid than triclosan alone (45, 46).

ANTIPROTOZOAL AGENTS

A number of antiprotozoal agents (typically folate synthesis inhibitors) approved for human use have already been leveraged for clinical use against the acute stage. It is notable, though, that an extensive pool of drug-like compounds screened against *Plasmo-*

dium falciparum followed by secondary screening against additional parasites (*T. gondii*, *Leishmania major*, and *Trypanosoma brucei*) showed that *T. gondii* was the least responsive of that group to this compound subset, suggesting that it may be more difficult to target chemotherapeutically than other tested human parasites (86).

Several structurally unrelated veterinary agents used on livestock (poultry, cattle, sheep, etc.) and companion animals have shown *in vitro* efficacy against *T. gondii*, although those agents are not commonly used for treatment of acute toxoplasmosis in these animals (87). Rather, they are used to treat or prevent a number of coccidian infections caused by organisms from the genera *Eimeria*, *Neospora*, *Hammondia*, *Sarcocystis*, and others. The following agents (along with their anticoccidial mechanism of action, if known) demonstrated *in vitro* IC_{50} values below 1 μ M but were not evaluated further for *in vivo* effectiveness: the mitochondrial inhibitors decoquinatone and robenidine; the ionophores monensin and salinomycin; and the protein synthesis inhibitor halofuginone (see Table 2). Arprinocid (IC_{50} , 7 μ M; unknown mechanism of action) and the mitochondrial inhibitors ponazuril and diclazuril (IC_{50} , 6 nM), after administration at doses of 10 mg/kg of body weight/day or lower, each showed 100% survival of mice groups challenged with *T. gondii* acute lethal infection. Separately, *in vivo* testing of the mitochondrial inhibitor toltrazuril (IC_{50} , 0.94 μ M) in sheep showed reductions in levels of microscopically counted tissue and brain cysts.

Isolated from the plant *Artemisia annua*, artemisinin and its semisynthetic derivatives have become part of a mainstay combination therapy for malaria infections. Although the mechanism of action of these compounds is not completely clear, their activity is hemoglobin digestion dependent (88). A recent study identified the malaria parasite's phosphatidylinositol-3-kinase as another possible target (89). While *Toxoplasma* is sensitive to artemisinin, with an IC_{50} value of 0.64 μ M, this is more than 100-fold higher than the corresponding IC_{50} against *Plasmodium falciparum* (68). Additionally, treatment with artemisinin during mouse lethal challenges increased survival by only 20%, whereas treatment with artemisone, a synthetic derivative with reduced side effects that is undergoing clinical trials, permitted 50% mouse survival in the same study (49). Studies evaluating the effect of these compounds on the bradyzoite stage are lacking.

ANTIBACTERIAL AGENTS

T. gondii is a single-cell eukaryote, which obviously limits the likely efficacy of many prokaryotic-specific drugs. However, a number of antibacterial agents have shown *in vitro* efficacy against *T. gondii*. Almost all antibacterials used in the clinic to treat acute or recrudescing *Toxoplasma* patients are macrolides; clarithromycin and azithromycin are among the macrolides often used (see Table 1). Clindamycin, a lincosamide antibiotic, has also been used for this purpose. While the mechanism of action against *Toxoplasma* is not established for these agents, they are known to inhibit the ribosome in target organisms. Fusidic acid, a bacteriostatic compound used outside the United States to treat skin, bone, and joint infections by inhibiting microbial protein synthesis, shows relatively weak activity *in vitro* (IC_{50} , 7 μ M) and no efficacy against *Toxoplasma in vivo*. Administration of the potent combination drug quinupristin-dalfopristin (Synercid) used to treat antibiotic-resistant *Enterococcus* infections via protein synthesis inhibition resulted in 100% survival in the acute infection model. Notably, when administered alone, each drug was much

less effective, thus mimicking observations in bacteria where treatment using the combination is thought to be bactericidal but treatment using each of the individual drugs is thought to be bacteriostatic. However, no cyst reduction studies were conducted using quinupristin-dalfopristin or its components.

Another group of potent antibiotics are the fluoroquinolones, which function by inhibiting prokaryotic topoisomerase II, leading to DNA fragmentation. Several newer fluoroquinolone derivatives have shown efficacy against experimental fungal infections (90); however, their routine clinical use in toxoplasmosis cases is uncommon. A number of fluoroquinolone derivatives showed *in vitro* and *in vivo* efficacy: trovafloxacin permitted 100% survival of infected mice in an acute infection model.

The drugs in the rifamycin group of antibiotics work against prokaryotic organisms by inhibiting DNA-dependent RNA synthesis, and rifamycin derivatives are particularly effective against *Mycobacterium* infections. Moreover, rifamycins typically operate as bactericidal agents and show some ability to penetrate the blood-brain barrier (91). Although the classic drug rifampin showed no efficacy against *Toxoplasma in vitro* (92), a number of derivatives demonstrated growth inhibition. At relatively high (300 mg/kg) doses in mice, rifabutin protected 100% of mice during a lethal challenge with the hypervirulent RH strain; notably, lower (50 to 100 mg/kg) doses used in combination with known anti-*Toxoplasma* drugs such as pyrimethamine, sulfadiazine, and, especially, clindamycin showed potential synergistic effects (63). Another rifamycin derivative, rifapentine, is known for its long half-life in mice and humans and, evaluated against *T. gondii* acute lethal challenge in a mouse model, was 90 and 100% effective at doses of 100 and 200 mg/kg, respectively. Due to its half-life, rifapentine would be an exciting drug to evaluate against the cyst stage; however, such studies evaluating any of the drugs in the rifamycin group are lacking. Why rifampin is ineffective compared to other rifamycin derivatives is unknown, suggesting that this group of antibiotics may be affecting one or more novel targets in the parasite.

ANTIFUNGAL AGENTS

Antifungal agents effectively target a broad range of eukaryotic fungal pathogens of humans; chief among these agents are the azoles, which were first used clinically in the 1980s (93). Ketoconazole, fluconazole, and itraconazole work by inhibiting ergosterol synthesis, a key component of the fungal cell membrane. Though fluconazole and itraconazole have IC_{50} values of 3 and 0.5 μ M, respectively, the mechanism responsible for their effect against *Toxoplasma* is unknown. While both antifungals, at doses of up to 20 mg/kg/day, increased survival only marginally (<40%) in an acute mouse infection model, they significantly reduced cyst levels, albeit the effect was seen when administration began 5 days following the initial infection.

ANTIRETROVIRAL AGENTS

Beyond the classic association of *Toxoplasma* with fetal infections via primary infection of the mother, *Toxoplasma* infections have become a leading cause of mortality among HIV-positive individuals with AIDS. Symptomatic infection can occur in immunocompromised individuals via primary infection or by reactivation of a latent, chronic infection. To prevent this, patients are often prescribed the trimethoprim-sulfadiazine combination prophylactically. Surprisingly, even patients who have been on this pro-

phylactic antiparasitic regimen for more than a decade still retain viable parasite tissue cysts, which typically reassert, often profoundly, when prophylactic therapy is removed. Frequently, coincident with *T. gondii* prophylaxis is antiretroviral therapy against HIV, thus ameliorating a primary concern of *Toxoplasma* symptomatic disease. However, observant researchers hypothesized that some antiretroviral therapies were more directly affecting the *Toxoplasma gondii* parasite. Indeed, studies show that multiple-antiretroviral therapies (using both protease inhibitors and nucleic acid analogues) appear to inhibit parasite growth *in vitro* through mechanisms that are not established. Single 100 mg/kg oral doses of didanosine (a reverse transcriptase inhibitor) reduced levels of chronic brain cysts by approximately 65%; notably, a single clinical review showed a reduction in reactivation of disease in HIV patients treated with didanosine, suggesting that it may reduce cyst levels in the human brain (94). No *in vivo* studies have been conducted using antiretroviral protease inhibitors, which may target parasite proteases or may modulate host proteases required for parasite egress (95).

ANTICANCER AGENTS

Fluorouracil (5-FU) is a pyrimidine antimetabolite analog used to treat ranges of malignancies. 5-FU undergoes conversion to 5-fluorodeoxyuridylate and interacts covalently with thymidylate synthetase and N⁵,N¹⁰-methylene tetrahydrofolate, thus forming a block for DNA synthesis (75). 5-FU is effective against *T. gondii in vivo* at doses as low as 0.08 μ M. Preliminary work by Harris et al. indicated that 5-FU may be effective against *T. gondii* in doses 10-fold lower than those used for malignancies. It is assumed that 5-FU has the capacity to transit the blood-brain barrier, as it associated with CNS toxicity (96).

Crizotinib is a kinase inhibitor targeting multiple receptor tyrosine kinases, including anaplastic lymphoma kinase (ALK), which interferes with tumor cell proliferation and survival. It is approved for use in cases of ALK-positive, metastatic, non-small-cell lung cancer. Crizotinib inhibited *T. gondii* at 4.0 μ M; however, the host HeLa cells detached from the plate, indicating host toxicity. Lower doses of crizotinib (0.4 μ M) had no effect on the parasite. Gefitinib, another kinase inhibitor, is postulated to inhibit the intracellular tyrosine kinase domain of epidermal growth factor receptor (EGFR), resulting in cell cycle arrest and inhibition of angiogenesis. By inhibiting EGFR, downstream kinases such as AKT, extracellular signal-regulated kinase (ERK), Jun N-terminal protein kinase (JNK), and mitogen-activated protein kinase (MAPK) p38 are also inhibited. Whether one or more of the parasite-specific kinases are targeted is unknown (97), but this is a distinct possibility. Gefitinib at 20 μ M inhibits *T. gondii* completely, without detachment of the host HeLa cells from the plate (76). However, gefitinib concentrations of <5 μ M had little effect on the parasite. Neither kinase inhibitor was further evaluated in an *in vivo* model.

IMMUNOSUPPRESSANTS AND IMMUNOMODULATORS

Methotrexate (MTX) is an immunosuppressant folate antimetabolite analog and shows polyglutamation in the host cell, where it inhibits dihydrofolate reductase (DHFR) (98). MTX is used in various malignancy treatment protocols and is used in rheumatology as a disease-modifying antirheumatic drug (DMARD). While parasite DHFR is an essential enzyme in purine and thymidylate metabolism, mammalian cells can use leucovorin (folinic

acid), a reduced folate, to perform MTX “rescue.” Enzymatically, piritrexim (a lipid-soluble analog of MTX) is at least 10-fold more potent against the parasite than MTX and at concentrations of 0.1 to 1.0 μM was shown to inhibit replication of *T. gondii* in a mouse peritoneal macrophage. Folinic acid rescue did not diminish the efficacy of piritrexim in inhibition of *T. gondii* replication (15, 99).

Cyclosporine (CsA) is an immunosuppressive and DMARD that inhibits T lymphocytes. It is principally used in organ transplant rejection prophylaxis but may also be used to treat rheumatoid arthritis (RA) or recalcitrant plaque psoriasis. Three biochemical processes have been associated with CsA: (i) complexes form between CsA and cyclosporine-binding proteins (cyclophilins) which interfere with calcineurin and inhibit signal transduction; (ii) CsA inhibits the chaperone function of specific proteins; and (iii) CsA has been shown to inhibit P-glycoprotein, a membrane pump that confers multidrug resistance to cancer cells and parasitic protozoa (100). CsA variably affected parasite loads, depending on the time frame being investigated (77). Not surprisingly, mice undergoing an acute lethal challenge experiment perished rapidly when given CsA (78).

T. gondii lacks the ability to synthesize purines *de novo* and thus utilizes adenosine kinase (AK)-mediated phosphorylation of adenosine salvaged from the host to acquire purines (79). Biochemical assays showed that adenine arabinoside (ara-A) effectively inhibits parasite-derived AK, with an IC_{50} value of 1.5 μM . When azathioprine, another purine derivative, was used to treat mice in an acute lethal infection model, 100% survival was observed (77). Evaluated against the cyst stage, however, azathioprine (the only anticancer or immunosuppressant agent to be evaluated against this stage) showed no significant difference compared to control results.

Auranofin is a gold-containing DMARD previously used for treatment of rheumatoid arthritis (RA). Due to its toxicity, auranofin has largely been supplanted by other DMARDs and biologic medications for the treatment of RA, but it is still commercially available. The mechanism of action of auranofin against parasites is thought to be dissociation of the gold, which then targets thioredoxin reductase. Auranofin has an IC_{50} value of 0.28 μM against *T. gondii* and is effective at 1 mg/kg *in vivo* in a chicken embryo model injected with tachyzoites.

PSYCHIATRIC AGENTS

CNS-acting medications, particularly antischizophrenic or antipsychotic agents, are often prescribed to *Toxoplasma*-positive individuals due to the relatively high coincidence of these mental disorders with parasite infection (101, 102). Similarly to the hypothesis that HIV-positive individuals directly reduce parasite disease potential by taking antiretrovirals, some studies have investigated whether psychoactive drugs affect *T. gondii* growth. As a group, these drugs function against unrelated targets, but many appear to inhibit cultured *Toxoplasma* parasites. The most potent, fluphenazine (IC_{50} : 1.7 μM), is a dopamine receptor antagonist with multiple side effects; it has also shown activity against the *Leishmania* parasite (103). However, there are no animal experiments analyzing the ability of these agents to reduce acute disease spread or morbidity or to reduce activity against the cyst stage.

CONCLUSION

Among the compounds listed in Table 2, none presently possess all of the attributes of a highly promising future drug against *Tox-*

oplasma gondii. Largely, this is due to missing information regarding their efficacy against the bradyzoite stage. From the characteristics compiled, the compelling efficacy of didanosine warrants further investigation; however, this drug, a nucleoside analog of adenosine, is associated with negative and common side effects, including peripheral neuropathy. Methotrexate and related derivatives are also provocative due to their low IC_{50} values *in vitro*, warranting further *in vivo* work. However, these drugs, too, are associated with dose-dependent negative side effects. Similarly, the veterinary anticoccidial halofuginone demonstrated exceptional potency *in vitro*, warranting its further evaluation as a treatment for *Toxoplasma* infection in animals.

In summary, the need for more-effective and less-toxic anti-*Toxoplasma* drug regimens is becoming increasingly urgent with the globally growing ranks of immunocompromised patients and the continued difficulties with ensuring safe livestock food supplies. More-potent regimens may also contribute to reductions in psychiatric disorders, if *Toxoplasma* is indeed a causal factor. Current research on developing a human or animal *Toxoplasma* vaccine has been an ongoing but incredibly challenging effort (104). Recent advances in immunotherapeutics designed to boost the host response to the parasite or to parasite antigens may aid effective multispecies vaccine development, ideally resulting in disease prevention or reduction and interruption of transmission (105). Immunomodulators in combination with anti-*Toxoplasma* compounds are another area of future promise (106). While there are dozens of small molecules that have shown promise against *T. gondii* *in vitro* or *in vivo*, these are beyond the scope of this review, which addresses clinically available but uncommonly used options. Additionally, as reviewed elsewhere (107), a number of novel natural products have shown activity against the parasite. The potential exists for other apicomplexan organisms (*Plasmodium*, *Babesia*, *Eimeria*, and *Cryptosporidium*) known to cause human or livestock diseases to be similarly sensitive to the agents described in this review; thus, future studies evaluating the aforementioned drugs against a broader range of parasites may be warranted. Synergistic combinations of the anti-*Toxoplasma* drugs reviewed here may yet produce “the right mix” to completely clear *Toxoplasma* chronic infections from humans and human-raised animal species.

ACKNOWLEDGMENTS

This report was supported by the following NIH grants: GM103427 (P.H.D.) and AI116723 (J.L.V.). Additionally, the following support is acknowledged: the Rheumatology Research Foundation Scientist Development Award (L.A.D.) and the Nebraska Research Initiative (P.H.D. and J.L.V.).

REFERENCES

- Dubey JP. 2008. The history of *Toxoplasma gondii*—the first 100 years. *J Eukaryot Microbiol* 55:467–475. <http://dx.doi.org/10.1111/j.1550-7408.2008.00345.x>.
- Kamerkar S, Davis PH. 2012. *Toxoplasma* on the brain: understanding host-pathogen interactions in chronic CNS infection. *J Parasitol Res* 2012:589295. <http://dx.doi.org/10.1155/2012/589295>.
- Havlicek J, Gasová ZG, Smith AP, Zvára K, Flegr J. 2001. Decrease of psychomotor performance in subjects with latent “asymptomatic” toxoplasmosis. *Parasitology* 122:515–520.
- Piekarski G. 1981. Behavioral alterations caused by parasitic infection in case of latent toxoplasma infection. *Zentralbl Bakteriell Mikrobiol Hyg A* 250:403–406.
- Flegr J. 2013. How and why *Toxoplasma* makes us crazy. *Trends Parasitol* 29:156–163. <http://dx.doi.org/10.1016/j.pt.2013.01.007>.

6. Tenter AM, Heckerth AR, Weiss LM. 2000. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 30:1217–1258. [http://dx.doi.org/10.1016/S0020-7519\(00\)00124-7](http://dx.doi.org/10.1016/S0020-7519(00)00124-7).
7. Havelaar AH, Haagsma JA, Mangen M-JJ, Kemmeren JM, Verhoef LPB, Vijgen SMC, Wilson M, Friesema IHM, Kortbeek LM, van Duynhoven YTHP, van Pelt W. 2012. Disease burden of foodborne pathogens in the Netherlands, 2009. *Int J Food Microbiol* 156:231–238. <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.029>.
8. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 17:7–15. <http://dx.doi.org/10.3201/eid1701.P11101>.
9. Innes EA, Bartley PM, Maley S, Katzer F, Buxton D. 2009. Veterinary vaccines against *Toxoplasma gondii*. *Mem Inst Oswaldo Cruz* 104:246–251. <http://dx.doi.org/10.1590/S0074-02762009000900031>.
10. Buzby JC, Roberts T. 1997. Economic costs and trade impacts of microbial foodborne illness. *World Health Stat Q* 50:57–66.
11. el Kouni MH. 2007. Adenosine metabolism in *Toxoplasma gondii*: potential targets for chemotherapy. *Curr Pharm Des* 13:581–597. <http://dx.doi.org/10.2174/138161207780162836>.
12. Fung HB, Kirschenbaum HL. 1996. Treatment regimens for patients with toxoplasmic encephalitis. *Clin Ther* 18:1037–1056; discussion 1036. [http://dx.doi.org/10.1016/S0149-2918\(96\)80059-2](http://dx.doi.org/10.1016/S0149-2918(96)80059-2).
13. Andrews KT, Fisher G, Skinner-Adams TS. 2014. Drug repurposing and human parasitic protozoan diseases. *Int J Parasitol Drugs Drug Resist* 4:95–111. <http://dx.doi.org/10.1016/j.ijpddr.2014.02.002>.
14. Bennett JE, Dolin R, Blaser MJ. 2015. Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 8th ed. Elsevier/Saunders, Philadelphia, PA.
15. Kovacs JA, Allegra CJ, Swan JC, Drake JC, Parrillo JE, Chabner BA, Masur H. 1988. Potent antipneumocystis and antitoxoplasma activities of piritrexim, a lipid-soluble antifolate. *Antimicrob Agents Chemother* 32:430–433. <http://dx.doi.org/10.1128/AAC.32.4.430>.
16. Mack DG, McLeod R. 1984. New micromethod to study the effect of antimicrobial agents on *Toxoplasma gondii*: comparison of sulfadoxine and sulfadiazine individually and in combination with pyrimethamine and study of clindamycin, metronidazole, and cyclosporin A. *Antimicrob Agents Chemother* 26:26–30. <http://dx.doi.org/10.1128/AAC.26.1.26>.
17. Piketty C, Derouin F, Rouveix B, Pocidallo JJ. 1990. In vivo assessment of antimicrobial agents against *Toxoplasma gondii* by quantification of parasites in the blood, lungs, and brain of infected mice. *Antimicrob Agents Chemother* 34:1467–1472. <http://dx.doi.org/10.1128/AAC.34.8.1467>.
18. Alder J, Hutch T, Meulbroek JA, Clement JC. 1994. Treatment of experimental *Toxoplasma gondii* infection by clarithromycin-based combination therapy with minocycline or pyrimethamine. *J Acquir Immune Defic Syndr* 7:1141–1148.
19. Khan AA, Slifer T, Araujo FG, Polzer RJ, Remington JS. 1997. Activity of trovafloxacin in combination with other drugs for treatment of acute murine toxoplasmosis. *Antimicrob Agents Chemother* 41:893–897.
20. Sibley CH, Hyde JE, Sims PF, Plowe CV, Kublin JG, Mberu EK, Cowman AF, Winstanley PA, Watkins WM, Nzila AM. 2001. Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends Parasitol* 17:582–588. [http://dx.doi.org/10.1016/S1471-4922\(01\)02085-2](http://dx.doi.org/10.1016/S1471-4922(01)02085-2).
21. Grossman PL, Remington JS. 1979. The effect of trimethoprim and sulfamethoxazole on *Toxoplasma gondii* in vitro and in vivo. *Am J Trop Med Hyg* 28:445–455.
22. Allegra CJ, Boorman D, Kovacs JA, Morrison P, Beaver J, Chabner BA, Masur H. 1990. Interaction of sulfonamide and sulfone compounds with *Toxoplasma gondii* dihydropteroate synthase. *J Clin Invest* 85:371–379. <http://dx.doi.org/10.1172/JCI114448>.
23. Derouin F, Piketty C, Chastang C, Chau F, Rouveix B, Pocidallo JJ. 1991. Anti-*Toxoplasma* effects of dapsone alone and combined with pyrimethamine. *Antimicrob Agents Chemother* 35:252–255. <http://dx.doi.org/10.1128/AAC.35.2.252>.
24. Chang HR, Arsenijevic D, Comte R, Polak A, Then RL, Pechère JC. 1994. Activity of epiroprim (Ro 11-8958), a dihydrofolate reductase inhibitor, alone and in combination with dapsone against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 38:1803–1807. <http://dx.doi.org/10.1128/AAC.38.8.1803>.
25. van der Ven AJ, Schoondermark-van de Ven EM, Camps W, Melchers WJ, Koopmans PP, van der Meer JW, Galama JM. 1996. Anti-toxoplasma effect of pyrimethamine, trimethoprim and sulphonamides alone and in combination: implications for therapy. *J Antimicrob Chemother* 38:75–80. <http://dx.doi.org/10.1093/jac/38.1.75>.
26. Dumas JL, Pizzolato G, Pechère JC. 1999. Evaluation of trimethoprim and sulphamethoxazole as monotherapy or in combination in the management of toxoplasmosis in murine models. *Int J Antimicrob Agents* 13:35–39. [http://dx.doi.org/10.1016/S0924-8579\(99\)00073-4](http://dx.doi.org/10.1016/S0924-8579(99)00073-4).
27. Sarciron M-E, Nebois P, Pautet F, Pétavy A-F, Fillion H, Walchshofer N. 2002. Quinonic derivatives active against *Toxoplasma gondii*. *Parasitol Res* 88:969–971. <http://dx.doi.org/10.1007/s00436-002-0615-6>.
28. Araujo FG, Remington JS. 1974. Effect of clindamycin on acute and chronic toxoplasmosis in mice. *Antimicrob Agents Chemother* 5:647–651. <http://dx.doi.org/10.1128/AAC.5.6.647>.
29. Fichera ME, Bhopale MK, Roos DS. 1995. In vitro assays elucidate peculiar kinetics of clindamycin action against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 39:1530–1537. <http://dx.doi.org/10.1128/AAC.39.7.1530>.
30. Camps M, Arrizabalaga G, Boothroyd J. 2002. An rRNA mutation identifies the apicoplast as the target for clindamycin in *Toxoplasma gondii*. *Mol Microbiol* 43:1309–1318. <http://dx.doi.org/10.1046/j.1365-2958.2002.02825.x>.
31. Allegra CJ, Kovacs JA, Drake JC, Swan JC, Chabner BA, Masur H. 1987. Potent in vitro and in vivo antitoxoplasma activity of the lipid-soluble antifolate trimetrexate. *J Clin Invest* 79:478–482. <http://dx.doi.org/10.1172/JCI112837>.
32. Araujo FG, Khan AA, Remington JS. 1996. Rifapentine is active in vitro and in vivo against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 40:1335–1337.
33. McFadden DC, Tomavo S, Berry EA, Boothroyd JC. 2000. Characterization of cytochrome b from *Toxoplasma gondii* and Q(o) domain mutations as a mechanism of atovaquone-resistance. *Mol Biochem Parasitol* 108:1–12. [http://dx.doi.org/10.1016/S0166-6851\(00\)00184-5](http://dx.doi.org/10.1016/S0166-6851(00)00184-5).
34. Chang HR, Comte R, Pechère JC. 1990. In vitro and in vivo effects of doxycycline on *Toxoplasma gondii*. *Antimicrob Agents Chemother* 34:775–780. <http://dx.doi.org/10.1128/AAC.34.5.775>.
35. Dahl EL, Shock JL, Shenai BR, Gut J, DeRisi JL, Rosenthal PJ. 2006. Tetracyclines specifically target the apicoplast of the malaria parasite *Plasmodium falciparum*. *Antimicrob Agents Chemother* 50:3124–3131. <http://dx.doi.org/10.1128/AAC.00394-06>.
36. Chan J, Luft BJ. 1986. Activity of roxithromycin (RU 28965), a macrolide, against *Toxoplasma gondii* infection in mice. *Antimicrob Agents Chemother* 30:323–324. <http://dx.doi.org/10.1128/AAC.30.2.323>.
37. Luft BJ. 1987. In vivo and in vitro activity of roxithromycin against *Toxoplasma gondii* in mice. *Eur J Clin Microbiol* 6:479–481. <http://dx.doi.org/10.1007/BF02013115>.
38. Araujo FG, Shepard RM, Remington JS. 1991. In vivo activity of the macrolide antibiotics azithromycin, roxithromycin and spiramycin against *Toxoplasma gondii*. *Eur J Clin Microbiol Infect Dis* 10:519–524. <http://dx.doi.org/10.1007/BF01963942>.
39. Chamberland S, Kirst HA, Current WL. 1991. Comparative activity of macrolides against *Toxoplasma gondii* demonstrating utility of an in vitro microassay. *Antimicrob Agents Chemother* 35:903–909. <http://dx.doi.org/10.1128/AAC.35.5.903>.
40. Olliaro P, Gorini G, Jabes D, Regazzetti A, Rossi R, Marchetti A, Tinelli C, Della Bruna C. 1994. In-vitro and in-vivo activity of rifabutin against *Toxoplasma gondii*. *J Antimicrob Chemother* 34:649–657. <http://dx.doi.org/10.1093/jac/34.5.649>.
41. Araujo FG, Khan AA, Slifer TL, Bryskier A, Remington JS. 1997. The ketolide antibiotics HMR 3647 and HMR 3004 are active against *Toxoplasma gondii* in vitro and in murine models of infection. *Antimicrob Agents Chemother* 41:2137–2140.
42. Eissa MM, Barakat AMA, Amer EI, Younis LK. 2015. Could miltefosine be used as a therapy for toxoplasmosis? *Exp Parasitol* 157:12–22. <http://dx.doi.org/10.1016/j.exppara.2015.06.005>.
43. Fomovska A, Wood RD, Mui E, Dubey JP, Ferreira LR, Hickman MR, Lee PJ, Leed SE, Auschwitz JM, Welsh WJ, Sommerville C, Woods S, Roberts C, McLeod R. 2012. Salicylanilide inhibitors of *Toxoplasma gondii*. *J Med Chem* 55:8375–8391. <http://dx.doi.org/10.1021/jm3007596>.
44. McLeod R, Muench SP, Rafferty JB, Kyle DE, Mui EJ, Kirisits MJ, Mack DG, Roberts CW, Samuel BU, Lyons RE, Dorris M, Milhous WK, Rice DW. 2001. Triclosan inhibits the growth of *Plasmodium*

- falciparum and *Toxoplasma gondii* by inhibition of apicomplexan Fab I. *Int J Parasitol* 31:109–113. [http://dx.doi.org/10.1016/S0020-7519\(01\)00111-4](http://dx.doi.org/10.1016/S0020-7519(01)00111-4).
45. El-Zawawy LA, El-Said D, Mossallam SF, Ramadan HS, Younis SS. 2015. Triclosan and triclosan-loaded liposomal nanoparticles in the treatment of acute experimental toxoplasmosis. *Exp Parasitol* 149:54–64. <http://dx.doi.org/10.1016/j.exppara.2014.12.007>.
 46. El-Zawawy LA, El-Said D, Mossallam SF, Ramadan HS, Younis SS. 2015. Preventive prospective of triclosan and triclosan-liposomal nanoparticles against experimental infection with a cystogenic ME49 strain of *Toxoplasma gondii*. *Acta Trop* 141:103–111. <http://dx.doi.org/10.1016/j.actatropica.2014.09.020>.
 47. Tipparaju SK, Muench SP, Mui EJ, Ruzhenikov SN, Lu JZ, Hutson SL, Kirisits MJ, Prigge ST, Roberts CW, Henriquez FL, Kozikowski AP, Rice DW, McLeod RL. 2010. Identification and development of novel inhibitors of *Toxoplasma gondii* enoyl reductase. *J Med Chem* 53:6287–6300. <http://dx.doi.org/10.1021/jm9017724>.
 48. Stec J, Fomovska A, Afanador GA, Muench SP, Zhou Y, Lai BS, El Bissati K, Hickman MR, Lee PJ, Leed SE, Auschwitz JM, Sommerville C, Woods S, Roberts CW, Rice D, Prigge ST, McLeod R, Kozikowski AP. 2013. Modification of triclosan scaffold in search of improved inhibitors for enoyl-acyl carrier protein (ACP) reductase in *Toxoplasma gondii*. *ChemMedChem* 8:1138–1160. <http://dx.doi.org/10.1002/cmcd.201300050>.
 49. Dunay IR, Chan WC, Haynes RK, Sibley LD. 2009. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. *Antimicrob Agents Chemother* 53:4450–4456. <http://dx.doi.org/10.1128/AAC.00502-09>.
 50. Sarciron ME, Saccharin C, Petavy AF, Peyron F. 2000. Effects of artesunate, dihydroartemisinin, and an artesunate-dihydroartemisinin combination against *Toxoplasma gondii*. *Am J Trop Med Hyg* 62:73–76.
 51. Jones-Brando L, D'Angelo J, Posner GH, Yolken R. 2006. In vitro inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. *Antimicrob Agents Chemother* 50:4206–4208. <http://dx.doi.org/10.1128/AAC.00793-06>.
 52. Hencken CP, Jones-Brando L, Bordón C, Stohler R, Mott BT, Yolken R, Posner GH, Woodard LE. 2010. Thiazole, oxadiazole, and carboxamide derivatives of artemisinin are highly selective and potent inhibitors of *Toxoplasma gondii*. *J Med Chem* 53:3594–3601. <http://dx.doi.org/10.1021/jm901857d>.
 53. Luft BJ. 1986. Potent in vivo activity of arprinocid, a purine analogue, against murine toxoplasmosis. *J Infect Dis* 154:692–694. <http://dx.doi.org/10.1093/infdis/154.4.692>.
 54. Pfefferkorn ER, Eckel ME, McAdams E. 1988. *Toxoplasma gondii*: in vivo and in vitro studies of a mutant resistant to arprinocid-N-oxide. *Exp Parasitol* 65:282–289. [http://dx.doi.org/10.1016/0014-4894\(88\)90133-6](http://dx.doi.org/10.1016/0014-4894(88)90133-6).
 55. Ricketts AP, Pfefferkorn ER. 1993. *Toxoplasma gondii*: susceptibility and development of resistance to anticoccidial drugs in vitro. *Antimicrob Agents Chemother* 37:2358–2363. <http://dx.doi.org/10.1128/AAC.37.11.2358>.
 56. Lindsay DS, Blagburn BL. 1994. Activity of diclazuril against *Toxoplasma gondii* in cultured cells and mice. *Am J Vet Res* 55:530–533.
 57. Lindsay DS, Rippey NS, Blagburn BL. 1995. Treatment of acute *Toxoplasma gondii* infections in mice with diclazuril or a combination of diclazuril and pyrimethamine. *J Parasitol* 81:315–318. <http://dx.doi.org/10.2307/3283944>.
 58. Mitchell SM, Zajac AM, Davis WL, Lindsay DS. 2004. Efficacy of ponazuril in vitro and in preventing and treating *Toxoplasma gondii* infections in mice. *J Parasitol* 90:639–642. <http://dx.doi.org/10.1645/GE-250R>.
 59. Kul O, Yildiz K, Ocal N, Freyre A, Deniz A, Karahan S, Atmaca HT, Gokpinar S, Dincel GC, Uzunalioğlu T, Terzi OS. 2013. In-vivo efficacy of toltrazuril on experimentally induced *Toxoplasma gondii* tissue cysts in lambs: a novel strategy for prevention of human exposure to meat-borne toxoplasmosis. *Res Vet Sci* 94:269–276. <http://dx.doi.org/10.1016/j.rvsc.2012.08.001>.
 60. Jain V, Yogavel M, Oshima Y, Kikuchi H, Touquet B, Hakimi M-A, Sharma A. 2015. Structure of prolyl-tRNA synthetase-halofuginone complex provides basis for development of drugs against malaria and toxoplasmosis. *Structure* 23:819–829. <http://dx.doi.org/10.1016/j.str.2015.02.011>.
 61. Payne AJ, Neal LM, Knoll LJ. 2013. Fusidic acid is an effective treatment against *Toxoplasma gondii* and *Listeria monocytogenes* in vitro, but not in mice. *Parasitol Res* 112:3859–3863. <http://dx.doi.org/10.1007/s00436-013-3574-1>.
 62. Khan AA, Slifer TR, Araujo FG, Remington JS. 1999. Quinupristin-dalfopristin is active against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 43:2043–2045.
 63. Araujo FG, Slifer T, Remington JS. 1994. Rifabutin is active in murine models of toxoplasmosis. *Antimicrob Agents Chemother* 38:570–575. <http://dx.doi.org/10.1128/AAC.38.3.570>.
 64. Romand S, Della Bruna C, Farinotti R, Derouin F. 1996. In vitro and in vivo effects of rifabutin alone or combined with atovaquone against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 40:2015–2020.
 65. Khan AA, Slifer T, Araujo FG, Remington JS. 1996. Trovafloxacin is active against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 40:1855–1859.
 66. Khan AA, Slifer TR, Araujo FG, Remington JS. 2001. Activity of gatifloxacin alone or in combination with pyrimethamine or gamma interferon against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 45:48–51. <http://dx.doi.org/10.1128/AAC.45.1.48-51.2001>.
 67. Barbosa BF, Gomes AO, Ferro EAV, Napolitano DR, Mineo JR, Silva NM. 2012. Enrofloxacin is able to control *Toxoplasma gondii* infection in both in vitro and in vivo experimental models. *Vet Parasitol* 187:44–52. <http://dx.doi.org/10.1016/j.vetpar.2011.12.039>.
 68. Martins-Duarte EDS, de Souza W, Vommaro RC. 2008. Itraconazole affects *Toxoplasma gondii* endodyogeny. *FEMS Microbiol Lett* 282:290–298. <http://dx.doi.org/10.1111/j.1574-6968.2008.01130.x>.
 69. Martins-Duarte ES, Lemgruber L, de Souza W, Vommaro RC. 2010. *Toxoplasma gondii*: fluconazole and itraconazole activity against toxoplasmosis in a murine model. *Exp Parasitol* 124:466–469. <http://dx.doi.org/10.1016/j.exppara.2009.12.011>.
 70. Martins-Duarte ES, de Souza W, Vommaro RC. 2013. *Toxoplasma gondii*: the effect of fluconazole combined with sulfadiazine and pyrimethamine against acute toxoplasmosis in murine model. *Exp Parasitol* 133:294–299. <http://dx.doi.org/10.1016/j.exppara.2012.12.011>.
 71. Sarciron ME, Lawton P, Saccharin C, Petavy AF, Peyron F. 1997. Effects of 2',3'-dideoxyinosine on *Toxoplasma gondii* cysts in mice. *Antimicrob Agents Chemother* 41:1531–1536.
 72. Sarciron ME, Lawton P, Petavy AF, Peyron F. 1998. Alterations of *Toxoplasma gondii* induced by 2',3'-dideoxyinosine in vitro. *J Parasitol* 84:1055–1059. <http://dx.doi.org/10.2307/3284647>.
 73. Gherardi A, Sarciron ME, Peyron F. 2000. *Toxoplasma* encephalitis: influence of the vehicle on the efficacy of different doses of 2',3'-dideoxyinosine in mice. *Parasite* 7:39–42. <http://dx.doi.org/10.1051/parasite/2000071039>.
 74. Monzote L, Rodríguez M, Alfonso Y, Cox R. 2013. Antiretroviral activity of protease inhibitors against *Toxoplasma gondii*. *Rev Inst Med Trop Sao Paulo* 55:65–67. <http://dx.doi.org/10.1590/S0036-46652013000100012>.
 75. Harris C, Salgo MP, Tanowitz HB, Wittner M. 1988. In vitro assessment of antimicrobial agents against *Toxoplasma gondii*. *J Infect Dis* 157:14–22. <http://dx.doi.org/10.1093/infdis/157.1.14>.
 76. Yang Z, Ahn H-J, Nam H-W. 2014. Gefitinib inhibits the growth of *Toxoplasma gondii* in HeLa cells. *Korean J Parasitol* 52:439–441. <http://dx.doi.org/10.3347/kjp.2014.52.4.439>.
 77. Sumyuen MH, Garin YJ, Derouin F. 1996. Effect of immunosuppressive drug regimens on acute and chronic murine toxoplasmosis. *Parasitol Res* 82:681–686. <http://dx.doi.org/10.1007/s004360050185>.
 78. McCabe RE, Luft BJ, Remington JS. 1986. The effects of cyclosporine on *Toxoplasma gondii* in vivo and in vitro. *Transplantation* 41:611–615. <http://dx.doi.org/10.1097/00007890-198605000-00012>.
 79. Darling JA, Sullivan WJ, Carter D, Ullman B, Roos DS. 1999. Recombinant expression, purification, and characterization of *Toxoplasma gondii* adenosine kinase. *Mol Biochem Parasitol* 103:15–23. [http://dx.doi.org/10.1016/S0166-6851\(99\)00109-7](http://dx.doi.org/10.1016/S0166-6851(99)00109-7).
 80. Andrade RM, Chaparro JD, Capparelli E, Reed SL. 2014. Auranofin is highly efficacious against *Toxoplasma gondii* in vitro and in an in vivo experimental model of acute toxoplasmosis. *PLoS Negl Trop Dis* 8:e2973. <http://dx.doi.org/10.1371/journal.pntd.0002973>.
 81. Goodwin DG, Strobl JS, Lindsay DS. 2011. Evaluation of five antischizophrenic agents against *Toxoplasma gondii* in human cell cultures. *J Parasitol* 97:148–151. <http://dx.doi.org/10.1645/GE-2536.1>.
 82. Jones-Brando L, Torrey EF, Yolken R. 2003. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of

- Toxoplasma gondii*. *Schizophr Res* 62:237–244. [http://dx.doi.org/10.1016/S0920-9964\(02\)00357-2](http://dx.doi.org/10.1016/S0920-9964(02)00357-2).
83. Fond G, Macgregor A, Tamouza R, Hamdani N, Meary A, Leboyer M, Dubremetz J-F. 2014. Comparative analysis of anti-toxoplasma activity of antipsychotic drugs and valproate. *Eur Arch Psychiatry Clin Neurosci* 264:179–183. <http://dx.doi.org/10.1007/s00406-013-0413-4>.
 84. Lima GSC, Saraiva PGC, Saraiva FP. 30 July 2015, posting date. Current therapy of acquired ocular toxoplasmosis: a review. *J Ocul Pharmacol Ther* <http://dx.doi.org/10.1089/jop.2015.0059>.
 85. Bork S, Yokoyama N, Matsuo T, Claveria FG, Fujisaki K, Igarashi I. 2003. Growth inhibitory effect of triclosan on equine and bovine *Babesia* parasites. *Am J Trop Med Hyg* 68:334–340.
 86. Guiguemde WA, Shelat AA, Bouck D, Duffy S, Crowther GJ, Davis PH, Smithson DC, Connelly M, Clark J, Zhu F, Jiménez-Díaz MB, Martínez MS, Wilson EB, Tripathi AK, Gut J, Sharlow ER, Bathurst I, El Mazouni F, Fowble JW, Forquer I, McGinley PL, Castro S, Angulo-Barturen I, Ferrer S, Rosenthal PJ, Derisi JL, Sullivan DJ, Lazo JS, Roos DS, Riscoe MK, Phillips MA, Rathod PK, Van Voorhis WC, Avery VM, Guy RK. 2010. Chemical genetics of *Plasmodium falciparum*. *Nature* 465:311–315. <http://dx.doi.org/10.1038/nature09099>.
 87. Merck & Co. 2010. The Merck veterinary manual, 10th ed. Merck & Co., Inc., Whitehouse Station, NJ.
 88. Klonis N, Crespo-Ortiz MP, Bottova I, Abu-Bakar N, Kenny S, Rosenthal PJ, Tilley L. 2011. Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. *Proc Natl Acad Sci U S A* 108:11405–11410. <http://dx.doi.org/10.1073/pnas.1104063108>.
 89. Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, Rizk SS, Njimoh DL, Ryan Y, Chotivanich K, Nguon C, Ghorbal M, Lopez-Rubio J-J, Pfrender M, Emrich S, Mohandas N, Dondorp AM, Wiest O, Haldar K. 2015. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. *Nature* 520:683–687. <http://dx.doi.org/10.1038/nature14412>.
 90. Ozdek SC, Müller D, Flynn PM, Flynn HW. 2006. In vitro antifungal activity of the fourth generation fluoroquinolones against *Candida* isolates from human ocular infections. *Ocul Immunol Inflamm* 14:347–351. <http://dx.doi.org/10.1080/09273940600976953>.
 91. Nau R, Sörgel F, Eiffert H. 2010. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev* 23:858–883. <http://dx.doi.org/10.1128/CMR.00007-10>.
 92. Remington JS, Yagura T, Robinson WS. 1970. The effect of rifampin on *Toxoplasma gondii*. *Proc Soc Exp Biol Med* 135:167–172. <http://dx.doi.org/10.3181/00379727-135-35011>.
 93. Maertens JA. 2004. History of the development of azole derivatives. *Clin Microbiol Infect* 10(Suppl 1):S1–S10.
 94. Sarciron M-E, Ghéardi A, Delorme C, Peyramond D, Pétavy A-F. 2004. Prevalence of toxoplasma encephalitis in AIDS patients treated with didanosine hospitalised in a French infectious service. *Curr HIV Res* 2:301–307. <http://dx.doi.org/10.2174/1570162043351101>.
 95. Chandramohanadas R, Davis PH, Beiting DP, Harbut MB, Darling C, Velmourougane G, Lee MY, Greer PA, Roos DS, Greenbaum DC. 2009. Apicomplexan parasites co-opt host calpains to facilitate their escape from infected cells. *Science* 324:794–797. <http://dx.doi.org/10.1126/science.1171085>.
 96. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Pröschel M, Noble M. 2008. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *J Biol* 7:12. <http://dx.doi.org/10.1186/jbiol69>.
 97. Peixoto L, Chen F, Harb OS, Davis PH, Beiting DP, Brownback CS, Ouloguem D, Roos DS. 2010. Integrative genomic approaches highlight a family of parasite-specific kinases that regulate host responses. *Cell Host Microbe* 8:208–218. <http://dx.doi.org/10.1016/j.chom.2010.07.004>.
 98. De Mattia E, Toffoli G. 2009. C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation. *Eur J Cancer* 45:1333–1351. <http://dx.doi.org/10.1016/j.ejca.2008.12.004>.
 99. Piper JR, Johnson CA, Krauth CA, Carter RL, Hosmer CA, Queener SF, Borotz SE, Pfefferkorn ER. 1996. Lipophilic antifolates as agents against opportunistic infections. 1. Agents superior to trimetrexate and piritrexim against *Toxoplasma gondii* and *Pneumocystis carinii* in vitro evaluations. *J Med Chem* 39:1271–1280.
 100. Silverman JA, Hayes ML, Luft BJ, Joiner KA. 1997. Characterization of anti-*Toxoplasma* activity of SDZ 215-918, a cyclosporin derivative lacking immunosuppressive and peptidyl-prolyl-isomerase-inhibiting activity: possible role of a P glycoprotein in *Toxoplasma* physiology. *Antimicrob Agents Chemother* 41:1859–1866.
 101. Sutherland AL, Fond G, Kuin A, Koeter MWJ, Lutter R, van Gool T, Yolken R, Szoke A, Leboyer M, de Haan L. 2015. Beyond the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta Psychiatr Scand* 132:161–179. <http://dx.doi.org/10.1111/acps.12423>.
 102. Torrey EF, Bartko JJ, Lun Z-R, Yolken RH. 2007. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizoph Bull* 33:729–736. <http://dx.doi.org/10.1093/schbul/sbl050>.
 103. Pearson RD, Manian AA, Hall D, Harcus JL, Hewlett EL. 1984. Antileishmanial activity of chlorpromazine. *Antimicrob Agents Chemother* 25:571–574. <http://dx.doi.org/10.1128/AAC.25.5.571>.
 104. Verma R, Khanna P. 2013. Development of *Toxoplasma gondii* vaccine: a global challenge. *Hum Vaccin Immunother* 9:291–293. <http://dx.doi.org/10.4161/hv.22474>.
 105. Lim SS-Y, Othman RY. 2014. Recent advances in *Toxoplasma gondii* immunotherapeutics. *Korean J Parasitol* 52:581–593. <http://dx.doi.org/10.3347/kjp.2014.52.6.581>.
 106. Köksal ZŞ, Yanik K, Bilgin K, MutluYılmaz E, Hokelek M. 10 July 2015, posting date. In vivo efficacy of drugs acting on *Toxoplasma gondii* combined with immunomodulators. *Jpn J Infect Dis* <http://dx.doi.org/10.7883/yoken.JJID.2015.023>.
 107. Sepulveda-Arias JC, Veloza LA, Mantilla-Muriel LE. 2014. Anti-*Toxoplasma* activity of natural products: a review. *Recent Pat AntiInfect Drug Discov* 9:186–194.