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MINIREVIEW

Review of Experimental Compounds Demonstrating Anti-*Toxoplasma* Activity

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***Toxoplasma gondii* is a ubiquitous apicomplexan parasite capable of infecting humans and other animals. Current treatment options for *T. gondii* infection are limited and most have drawbacks, including high toxicity and low tolerability. Additionally, no FDA-approved treatments are available for pregnant women, a high-risk population due to transplacental infection. Therefore, the development of novel treatment options is needed. To aid this effort, this review highlights experimental compounds that, at a minimum, demonstrate inhibition of *in vitro* growth of *T. gondii*. When available, host cell toxicity and *in vivo* data are also discussed. The purpose of this review is to facilitate additional development of anti-*Toxoplasma* compounds and potentially to extend our knowledge of the parasite.**

Toxoplasma gondii, a common protozoan parasite, has the ability to infect nearly all warm-blooded animals, including humans, on all seven continents (1–3). This infection, while often asymptomatic in otherwise-healthy individuals, can cause severe disease or death in immunocompromised individuals, as well as severe congenital defects in prenatally infected infants. *T. gondii* is often acquired through the consumption of contaminated foods, either from undercooked meats or inadequately washed fruits and vegetables. Exposure to contaminated water is also a significant risk factor for infection, as contaminated water sources have been implicated in multiple outbreaks (4–6). Chemotherapy for treating toxoplasmosis is currently limited to the acute parasitic life stage (tachyzoite) of the infection and frequently consists of combination treatments, most often the antifolates pyrimethamine and sulfadiazine (7). Despite the ability to coadminister folinic acid orally with pyrimethamine, patients can suffer hematopoietic deficiencies, among other adverse effects, due to folate synthesis inhibition (8). Moreover, sulfadiazine has one of the highest rates of allergic reactions to an antibiotic reported in the United States, affecting 3 to 6% of the population (9, 10).

For women who become infected while pregnant, the treatment protocol is less well-defined; indeed, no regimen of treatment for expecting mothers is FDA approved (11). Often, congenitally infected infants are placed on an aggressive regimen of pyrimethamine and sulfadiazine for a period of 6 to 12 months immediately after birth, although the child remains at a high risk of later manifestations of neurological deficits regardless of the clinical symptoms presented at birth (12).

METHODS

In order to address these deficits, thousands of experimental compounds have been synthesized and investigated for activity against *T. gondii*. This work details the outcome of a comprehensive review of the literature, including articles accessed from PubMed with the following parameters: a publication date between 1 January 1980 and 28 June 2016; English as the primary language of the publication; containing the keywords "toxoplasm* AND (drug* or treatment*)". A total of 5,504 items were filtered to identify primary literature sources that evaluated the *in vitro* or *in vivo* efficacy of compounds that were not derivatives of clinically available drugs used to treat toxoplasmosis (13); thus for the purpose of this review, they were considered "experimental compounds" due to their novelty and lack of clinical availability. Compounds with 50% inhibitory concentrations (IC_{50s}) of >10 μM were not considered unless the compound had demonstrated efficacy *in vivo*. Additionally, compounds were excluded if the IC_{50} was determined based solely on the less-reliable enzyme-linked immunosorbent assay method (14). Clinically available drugs (13) and natural products (15) with activity against *T. gondii* are not considered in this review.

The experimental compounds in this review are generally divided by the methodology by which they were discovered, then by the predicted mode of action (MoA). The compounds identified as part of mid- or high-throughput screens are listed in Table 3, regardless of their MoA status. Those that have a known or suspected MoA are listed in Table 1, while those without a known or suspected MoA are listed in Table 2. All compounds described in the tables have been indexed numerically according to their order of appearance, with compound structures available in Tables S1 to S3 in the supplemental material.

For promising anti-*Toxoplasma* compounds, assessment of their ability to control *T. gondii* growth is a key step in drug development. Traditionally, successful compounds demonstrate the following: an IC_{50} under 10 μM, except in cases where the compound is used in combination with another compound; a high therapeutic index; and an ability to increase survival of an infected host animal after infection with a lethal dose of *T. gondii* (13).

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Promising compounds also ideally demonstrate marked decreases in brain and muscle parasite cyst (bradyzoite) counts, a measure of chronic infection; however, this has not been established to be clinically significant. As more is learned about the potentially deleterious effects of chronic infections, this may become a greater consideration (16). While few reviewed compounds have absorption, distribution, metabolism, and excretion (ADME) data, a lead compound should demonstrate favorable pharmacokinetic characteristics to avoid a late-stage failure during development (17).

COMPOUNDS WITH PROPOSED MODES OF ACTION

Interference with invasion or egress. Host cell invasion and egress of *T. gondii* are two distinct, complex, vital processes for the survival of the parasite. During acute infection, *T. gondii* tachyzoites require invasion of a host cell, formation of a parasitophorous vacuole, replication, and egress. Invasion is thought to be conducted through the formation of a junction between the apical end of the parasite and the host cell plasma membrane (18). The parasite uses an actin-myosin motor system to enter the host cell while surrounding itself in the newly formed vacuole or membrane arising from the host plasma membrane. Examples of compounds that specifically target invasion are pyridinylpyrroles (19) and benzophenones (20), which target cyclic GMP-dependent kinase, a parasite protein implicated in the gliding motility and microneme adhesion of *T. gondii* and other coccidians to the host cell plasma membrane (21) (Table 1). The most potent of these, pyridinylpyrrole (compound 4), had an *in vitro* IC_{50} of 0.32 μM, and increased the survival rate of mice infected with a lethal dose of parasites by 90% when the drug was administered twice a day at 50 mg/kg of body weight (19, 21, 22).

T. gondii remains in the parasitophorous vacuole for the duration of its asexual reproduction and must egress in order to maintain virulence. Egress, a less-well-understood process, requires the parasite to pass through both the established parasitophorous vacuole and the host plasma membrane (18). While this cycle has been demonstrated to be potassium and calcium dependent and may involve host coordination, specific mechanisms are not known (23). An important parasite protein for both invasion and egress, calcium-dependent protein kinase 1 (TgCDPK1), has been shown to be targeted by pyrazolopyrimidines (24) and benzoylbenzimidazoles (25) (Table 1). This parasite target is part of an important signaling pathway involved in gliding, a required motion for *T. gondii* to cross the host cell's plasma membrane (26). Other compounds that affect both invasion and egress are dinitroanilines (27) and diaryl ureas (28), which are suspected to destabilize microtubules and myosin tail interactions, respectively.

Inhibition of DNA synthesis. The ability to replicate genetic information is a central aspect of reproduction for all organisms. During this process, *T. gondii* has a number of salvage pathways and host-scavenging properties to support its nucleotide needs. This is one process in which *T. gondii* differs significantly from its apicomplexan relative, *Plasmodium falciparum*. While *P. falciparum* performs almost exclusively *de novo* pyrimidine synthesis, *T. gondii* balances salvage with *de novo* synthesis of pyrimidine nucleotides (29). Additionally, purine *de novo* synthesis is not performed in *T. gondii*; rather, the precursors are imported from the host through specialized nucleobase transporters (29).

Because of the diversity found in eukaryotic DNA synthesis, many clinically available antiparasitics are capable of targeting this process. Pyrimethamine and sulfadiazine, two of the most important clinically used drugs in the treatment of toxoplasmosis, fall within this category. These drugs target dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), respectively; both are enzymes active in the folate pathway (30). Without folate, the synthesis of new thymidine nucleotides is halted, eventually interrupting the cell cycle of the parasites. Unfortunately, specific inhibition of parasite DHFR is not possible with pyrimethamine, due to its homology with human DHFR; thus, host toxicity is high. Other inhibitors of DHFR have been extensively studied and documented elsewhere in the literature (31–34) and include 2,4-diaminopyrido 2,3-dipyrimidines (35), experimental sulfonamides (36), 2,4-diaminopteridines, and triazines. Based on available *in vitro* data, the most promising of the experimental DHFR inhibitors are the triazines; one of these, compound 14, has an IC_{50} of 0.02 μM (37).

Sulfadiazine demonstrates greater parasite specificity because of the suspected noneukaryotic origin of the DHPS enzyme; it is not found in humans or most other higher eukaryotes (38). A variety of novel sulfonamides, which target DHPS, have also demonstrated efficacy against *T. gondii*, such as compound 16, with an IC_{50} of 0.05 μM (36, 39) (Table 1). Other inhibitors of the DNA synthesis pathway in *T. gondii* generally target enzymes involved in the synthesis of individual nucleotides. Inosine-5′-monophosphate dehydrogenase, an important component of guanine synthesis, is inhibited by phthalazinones (40) through interaction at the NAD binding site (41). Additionally, parasite adenosine kinase, used in purine incorporation, binds 6-benzylthioinosine analogues as subversive substrates (42).

Most eukaryotes utilize methylation as a form of epigenetic regulation of transcription; however, in *Toxoplasma* tachyzoites, this has not been shown to be a source of control (43). Instead, *S*-adenosylmethionine (SAM)-dependent methyltransferases, which are not found in humans, are critically important in multiple parasitic metabolic pathways due to their other methylation functions (e.g., protein tagging). One compound, 1-[4-(4-nitrophenoxy)phenyl]propane-1-one (compound 21) (Table 1), is thought to affect class I SAM-dependent methyltransferases, though vacuolar ATP synthase subunit C is another potential target (44).

Inhibition of steroid synthesis. *T. gondii* is capable of synthesizing some steroids that it requires, and it is dependent on the host for others (45, 46). It has been shown that the mevalonate pathway, present and functional in most eukaryotes, including humans, does not function in *T. gondii*, likely forcing the parasite to scavenge from the host or use alternative pathways to acquire needed steroids (47). One alternative pathway is the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, which produces isopentenyl diphosphate and dimethylallyl diphosphate, both precursors to isoprenoids and essential molecules for parasite membrane support and cellular signaling (48, 49). The aryloxyphenoxy derivatives (Table 1) inhibit the synthesis of these precursors, preventing the formation of isoprenoids in the parasite, thereby disrupting multiple metabolic pathways and membrane structures within *T. gondii*. In more recent work (50), acyl coenzyme A (CoA):cholesterol transferase (ACAT) inhibitors were found to prevent cholesterol esterification by the parasite, an integral step of cholesterol uptake from the host (51) (Table 1).

Additional proposed inhibitors of *T. gondii*'s sterol synthesis have focused on therapies that target the parasite's bifunctional protein, farnesyl diphosphate/geranylgeranyl diphosphate syn-

TABLE 1 Compounds with known or suspected modes of action against *Toxoplasma gondii*[a]

MoA and compound class and no.	Reference(s)	*In vitro* IC_{50}	Host cell TD_{50}	*In vivo* survivability	*In vivo* chronic infection	Parasite burden	*In vivo* toxicity
Interference with invasion or egress							
Pyrazolopyrimidines	24, 129, 130						
Compound 1		0.003 μM (24)					
Compound 2		Not established (129)		100,000 RH strain tachyzoites injected i.p.; treatment did not increase survival (129)		1,000 RH strain tachyzoites injected i.p.; treatment decreased parasite load in liver and lungs by ~10- to 100-fold and in brain by <5-fold at 4 dpi (129)	
Compound 3		0.060 μM (130)	>10 μM HepG2 and CRL-8155 cells (130)		Unreported no. of ME49 cysts injected i.p.; treatment decreased cyst count by 88.7% (130)	"High inoculum" of RH strain tachyzoites injected i.p.; 99% reduction in spleen tachyzoites; 95% reduction in brain tachyzoites (130)	>500 mg/kg/day in mice (PK information available for rats, dogs, calves, monkeys) (130)
Pyridinylpyrroles	19, 21, 22						
Compound 4		0.32–200 μM (19, 21, 22)	>10 μM HFF cells (19, 21, 22)	1,000–3,000 RH strain tachyzoites injected i.p.; treatment increased survival by 90% (19)	ND	1,000–3,000 RH strain tachyzoites injected i.p.; no parasites detected in brain, lung, spleen, or peritoneal fluid during treatment; at 5–10 days posttreatment, parasites detected in brain, lung, spleen (19)	>50 mg/kg/day (19, 22)
Benzophenones	20, 131, 132						
Compound 5		0.14 μM (131)	0.21 μM HFF cells, 5.89 μM HeLa cells (131)	ND	ND	ND	ND
Benzoylbenzimidazoles	25						
Compound 6		>1 μM for all tested compounds	>30 μM for all tested compounds	ND	ND	ND	ND
Diaryl ureas	28						
Compound 7		<0.5 μM	>10 μM HFF cells	ND	ND	ND	ND
Dinitroanilines	27, 133						
Compound 8		0.036 μM (27)	>0.5 μM HFF cells (27)	ND	ND	ND	ND
5-Aminopyrazole-4-carboxamides	134						
Compound 9		0.089 μM	>40 μM CLR-8155 cells	ND	ND	105 RH strain tachyzoites injected i.p.; no parasites detected in peritoneal fluid, >5-fold reduction in brain tissue	>10 mg/kg/day
Inhibition of DNA synthesis							
6-Benzylthioinosines	42, 135–140						
Compound 10		9.3 μM (136)	>50 μM HFF cells (136)	200 RH or TgAK-3 strain tachyzoites injected i.p. increased survival by 2 days	ND	ND	ND
Compound 11		4.3 μM (136)	>50 μM HFF cells (136)	Increased survival by 2–3 days (136)	ND	ND	>300 mg/kg (136)
Compound 12		7.3 μM (136)	>50 μM HFF cells (136)	Increased survival by 2–3 days (136)	ND	ND	>300 mg/kg (136)
Triazines and 2,4-diaminopyrido [2,3-d]pyrimidines	37, 114, 141–148						
Compound 13		0.058 μM (143)	ND	ND	ND	ND	ND

Compound 14		0.02 μM (37)	ND	ND	ND	10,000 RH strain tachyzoites injected i.p.; 99% reduction in parasites in peritoneal fluid (37)	ND
Compound 15		~0.05 μM (114)	>100 μM HFF cells (114)	ND	ND	10,000 RH strain tachyzoites injected i.p.; significant reduction of tachyzoites in peritoneal fluid (114)	ND
Sulfonamides (exptl)	39, 149						
Compound 16		0.05 μM (39)	ND	ND	ND	ND	ND
Compound 17		ND (149)	ND	5,000 RH strain tachyzoites injected i.p.; treatment group had 10–50% survival (149)	ND	5,000 RH strain tachyzoites injected i.p. (mice that survived acute trial used for parasite burden assay); no parasites detected in heart or kidneys (149)	ND
2,4-Diaminopteridines	150						
Compound 18		0.077 μM	ND	ND	ND	ND	ND
Phthalazinone derivatives	41						
Compound 19		1 μM	ND	ND	ND	ND	ND
Urea derivatives	151						
Compound 20		0.402 μM	ND	ND	ND	ND	ND
1-[4-(4-Nitrophenoxy)phenyl]propane-1-one	152						
Compound 21		36.2 μM	67.0 μM HeLa cells	ND	ND	100,000 RH strain tachyzoites injected i.p.; 40% reduction in tachyzoite burden in peritoneal cavity at 4 dpi	ND
Inhibition of steroid synthesis							
Bisphosphonates	53, 153–158						
Compound 22		0.28 μM (154)	>826 μM KB cells (154)	5 C56 strain bradyzoites administered orally; 80% survival (154)	ND	ND	ND
Compound 23		0.55 μM (154)	>892 μM KB cells (154)	5 C56 strain bradyzoites administered orally; 80% survival (154)	ND	ND	ND
ACAT inhibitors	51						
Compound 24		~3 μM	>100 μM HFF cells	ND	ND	ND	ND
Aryloxyphenoxy derivatives	48, 159						
Compound 25		2.03 μM (55)	>50 μM Vero cells (48)	ND	ND	ND	ND
Azasterols	54, 55, 160						
Compound 26		0.12 μM (55)	ND	ND	ND	ND	ND
Quinuclidines	52						
Compound 27		0.19 μM	>15 μM LLC-MK2 cells	ND	ND	ND	ND
Fatty acid synthesis inhibition							
Acylsulfonamides	63						
Compound 28		0.02 ± 0.07 μM	>1,000 μM HFF cells	ND	ND	ND	ND
Benzimidazoles	62						
Compound 29		2.5 μM	<10 μM HFF or PC3-Luc cells	ND	ND	ND	ND
Thiolactomycin analogues	60						
Compound 30		1.6 μM	>15 μM LLC-MK2 cells	ND	ND	ND	ND

(Continued on following page)

TABLE 1 Continued

MoA and compound class and no.	Reference(s)	*In vitro* IC_{50}	Host cell TD_{50}	*In vivo* survivability	*In vivo* chronic infection	Parasite burden	*In vivo* toxicity
Inhibition of virulence factors or host interactions							
Pyridinylimidazoles	69, 70, 161						
Compound 31		0.8–5 μM (70)	ND	1,000 RH strain tachyzoites injected i.p.; 40% survival in treatment group (70)	ND	ND	~>60 mg/kg/day (70)
Compound 32		~3 μM (69)	ND		ND	1,000 RH-GFP strain tachyzoites injected i.p.; significant reduction in peritoneal exudate (69)	>10 mg/kg/day (69)
Inhibition of transcription							
Cyclic tetrapeptides	74, 75, 162						
Compound 33		0.01 μM (MIC) (75)	ND	ND	ND	ND	ND
Compound 34		0.0076 μM (74)	0.107 μM HFF cells (74)	ND	ND	ND	ND
Hydroxamic acids	77						
Compound 35		0.039 μM	>10 μM HS68 cells	ND	ND	ND	ND
Garcinol	78						
Compound 36		1.79 μM	>10 μM HFF cells	ND	ND	ND	ND
Inhibition of reproduction or differentiation							
Gossypol and derivatives	84						
Compound 37		5–10 μM	~40 μM HFF cells	ND	ND	ND	ND
Lactacystin	81						
Compound 38		2 μM (significant inhibition of intracellular tachyzoite replication)	2 μM (HFF cells showed little to no signs of toxicity)	ND	ND	ND	ND
3-Bromopyruvate	163						
Compound 39		<10 μM	>10 μM LLC-MK2 cells	ND	ND	ND	ND
Effects on ROS regulation							
Quinones	88, 164–169						
Compound 40		0.10 μM (164)	>10 μM HFF cells (164)	2,500 RH strain tachyzoites or 10 C56 strain bradyzoites given orally; 0–20% survival in treatment group (164)	ND	ND	<100 mg/kg/day (164)
Compound 41		0.11 μM (164)	>10 μM HFF cells (164)	2,500 RH strain tachyzoites or 10 C56 strain bradyzoites given orally; 40–50% survival in treatment group (164)	ND	ND	~100 mg/kg/day (164)
Compound 42		ND (168)	ND	(Coadministered with sulfadiazine) 1,000 RH strain tachyzoites injected i.p., 70% survival in treatment group; when 10 EGS strain cysts given orally, 90% survival in treatment group (168)	5 P strain cysts given orally, cyst burden reduced ~58% with treatment (168)	ND	<100 mg/kg/day (165)

Quinolines									
Compound 43	87, 170	0.0786 μM (87)	3.4 μM HS68 cells (87)	ND	ND	ND		ND	
Alkaloids									
Compound 44	86, 97, 169, 171–177	0.0007 μM (173)	0.001 μM THP-1 cells (173)	ND	ND	ND		ND	
Compound 45		0.0007 μM (173)	0.392 μM THP-1 cells (173)	ND	ND	ND		ND	
Cationic dyes									
Compound 46	85	0.26 μM	0.55 μM mouse peritoneal macrophages	ND	ND	ND		ND	

[a] Individual compound information is listed with the index number assigned to the specific molecule's structure. For cases in which multiple IC_{50} values were reported in the literature, the lowest observed IC_{50} is reported. Anti-*Toxoplasma* compounds are categorized by MoA and grouped by structure. Structures for each compound can be found in Table S1 in the supplemental material. The term *in vivo* survivability assay data refer to experiments where model organisms (mice) were exposed to a lethal infectious dose of parasite, often via i.p. injection. The strain and dose of the parasite and the recipient host differed between studies, making comparisons between studies problematic. *In vivo* chronic infection and parasite burden data refer to tissue or fluid counts of parasites isolated from a host following a nonlethal infection or bradyzoite cyst burden in brain tissue (most often determined via PCR). ND, not determined; dpi, days postinfection; HFF, human foreskin fibroblasts.

thase (TgFPPS) (47). Multiple classes of TgFPPS inhibitors have been identified, including bisphosphonates and quinuclidines (Table 1). Of these, a quinuclidine, compound 27, has the lowest IC_{50}, at 0.19 μM; however, it has not been tested *in vivo* (52, 53). Many bisphosphonates are able to act on *T. gondii* specifically; however, the host's farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase are inhibited by one subclass of bisphosphonates which also inhibits TgFPPS (47). Because of this, it has been suggested that the use of statin drugs in combination with antiparasitic treatment may be beneficial. Azasteroids do not have a defined mechanism of action in *T. gondii*, although based on data from other parasites, it is believed to be either due to the unregulated uptake of sterols, which causes damage to organelles and membranes, or due to the potential effects on the methylation status of genes related to phospholipid biosynthesis (54). One such compound, azasteroid, compound 26, has a promising IC_{50} of 0.12 μM, but it is yet to be tested for *in vivo* activity (55) (Table 1).

Fatty acid synthesis inhibition. Fatty acid synthesis (FAS) in apicomplexans is a process distinct from that of its analogue in mammals because of the presence of the apicoplast, an organelle acquired via secondary endosymbiosis (56). The fatty acids produced in the FASII pathway of the apicoplast are critical for organelle biogenesis and survival, despite the ability of *T. gondii* to scavenge some fatty acids from the host cell (57, 58). Therefore, FASII pathway enzymes are attractive drug targets. One such enzyme is β-ketoacyl-acyl carrier protein KASI/II, which plays a role in elongation of fatty acids in the FASII pathway and is specifically inhibited by thiolactomycin analogues in prokaryotes, and likely in *T. gondii* (57, 59, 60) (Table 1). Another FASII enzyme believed to be a drug target in fatty acid synthesis is enoyl acyl-carrier protein reductase, an enzyme responsible for the second reduction step in FASII (61). Inhibition of this enzyme's NAD^+-complexed form is easily achieved with triclosan, a common antibacterial found in soaps and other personal hygiene products; however, the NADH-complexed form is not targeted by this common antibiotic (62). Therefore, various benzimidazoles, which target the reduced form of the enzyme in bacterial organisms, have a potential MoA in *T. gondii*; however, none of these benzimidazoles has been shown to inhibit *T. gondii* enoyl reductase isolated protein. Instead, *in vitro* analyses of the parasite's susceptibility to the compounds have revealed that the compound has other unknown parasiticidal mechanisms. The target is still thought to involve FAS due to structural similarity to a known compound, chlormidazole, which has activity against a FAS methylase in organisms that, like *T. gondii*, lack the FASII pathway (62).

Outside of the FASII pathway, the parasite synthesizes other fatty acids not found in humans, including pantothenate. The pantothenate pathway in *T. gondii* is thought to be conserved from a common prokaryotic ancestor or to have been acquired through horizontal gene transfer (63). Due to its similarity to the prokaryotic enzyme, the parasite's pantothenate synthetase can be specifically targeted with acylsulfonamides, inhibitors originally developed for *Mycobacterium tuberculosis* (63) (Table 1). Acylsulfonamide, compound 28, inhibits *T. gondii* growth *in vitro* with an IC_{50} of 0.02 μM, and it exhibits a high therapeutic index compared to other fatty acid synthesis inhibitors, with a median toxic dose (TD_{50}) of >1 mM on HFF host cells.

Inhibition of virulence factors or host interactions. Apicomplexan parasites manipulate the host cell's signaling pathways, transcription, cytoskeletal organization, and other cellular com-

ponents in order to create an environment in which they can survive (64–68). Without this ability, the host cell's innate defense mechanisms could overcome the parasite and prevent its reproduction or egress (69). Pyridinylimidazole, compound 31, (70) inhibits *T. gondii in vitro* with an IC_{50} of 0.8 to 5 μM and inhibits the activation of activin-like kinases 4, 5, and 7, which are specific host cell signaling receptors for the activation of hypoxia-inducible factor 1, a host cell transcription factor required for *T. gondii* growth that is manipulated by the parasite in infected host cells (71) (Table 1). In addition to potently inhibiting this pathway, compound 31 also inhibits mitogen-activated protein (MAP) kinase 1, a parasite enzyme involved in invasion (72).

Inhibition of transcription. Transcriptional regulation is important for proper functioning of parasitic metabolism and is partially attained through chromatin packaging (73). Apicomplexan parasites and mammals use histone acetylation as a major source of regulation, and the histone deacetylation enzymes are nearly homologous (74). For this reason, histone hyperacetylation or deacetylation is not a parasite-specific process, which has been demonstrated with compound 33 (75), a fungal metabolite that simultaneously targets histone deacetylase in *T. gondii* and the host, as assessed by enzyme binding assays (Table 1). Despite this challenge, some investigators have identified compounds that affect histone deacetylase (HDAC) processes, such as enzyme HDAC3, in a more apicomplexan-specific manner (76). Compounds 34 and 35 are two examples of this, which have *T. gondii* IC_{50}s of 7.6 nM and 39 nM, respectively. Host cell toxicity remains a concern with compound 34; the TD_{50} for human host cells is less than 10 times greater than the IC_{50} for *T. gondii*, but compound 35 seems to fare better on host cells with a TD_{50} of >10 μM in HS68 cells (74, 77) (Table 1). Garcinol, derived from the *Garcinia indica* fruit, is another compound that affects histone acetylation, targeting a family of lysine acetyltransferases (KAT) enzymes required for *T. gondii* tachyzoites to replicate successfully (78). Additionally, other (nonhistone) substrates of these compounds may be affected, contributing to the overall host cell toxicity and to the efficacy of the compound against the parasite (79). Indeed, HDACs have various roles in many biological processes, including DNA repair and cell cycle regulation.

Inhibition of reproduction or differentiation. *T. gondii* has two distinct asexual life stages in humans: the tachyzoite, the stage associated with acute infection, and the bradyzoite, associated with the latent infection (80). Replication of the parasite within the parasitophorous vacuole or cyst is achieved through mitosis. Lactacystin, compound 38, is a recently discovered proteasome inhibitor capable of interfering with mitosis; parasites treated with this compound do not perish, but rather halt cellular replication until the compound has been metabolized or removed, even if the treatment period lasts for several days (81). Because of this, no IC_{50} could be generated for this class of inhibitor, although significant effects were noted at 2 μM. While the parasitostatic effects of compound 38 present limitations to development, no host cell toxicity was noted even at the highest compound concentration studied (2 μM).

When the parasite encounters stressful conditions, such as an immune system response or metabolic insult, the rapidly dividing tachyzoite undergoes differentiation to the slowly replicating bradyzoite (82). This shift significantly changes the genetic expression profile of *T. gondii* and leads to a slower-growing, hardier encysted form of the parasite that localizes to the brain and muscular tissue of the host. Two lactate dehydrogenase (LDH) isoforms are differentially expressed during the tachyzoite and bradyzoite stages. LDH1 is expressed mainly in the tachyzoite stage, while LDH2 is expressed during the bradyzoite stage; both are substantially different from the mammalian LDH (83). The cotton plant-derived gossypol and its derivatives (exemplified by compound 37) have been shown to be potent LDH2 inhibitors (enzyme K_i, 1.1 μM) and are somewhat active against LDH1 (enzyme K_i, 6.1 μM), with an IC_{50} of 5 to 10 μM against intracellular tachyzoites (84). However, these compounds have not been tested against the bradyzoite form.

Effects on reactive oxygen species regulation. Through the combination of the $CD8^+$ T-cell response and interferon gamma-mediated activation, a competent host immune system is capable of subduing most of the tachyzoites produced in an acute *T. gondii* infection. One facet of this response is the production of reactive oxygen species (ROS) by macrophages, a response induced by cationic dyes (85), quinoline derivatives (86), and various alkaloids (87) (Table 1). Alkaloid compounds 44 and 45 have the lowest IC_{50} values in this category (0.7 nM), but host cell toxicity is a concern: in THP-1 host cells, compound 44 demonstrated a TD_{50} of 1 nM, while compound 45 had a more favorable TD_{50} of 0.392 μM (86).

Quinones, though extensively studied, do not have an established MoA in *Toxoplasma*. A parasite-specific NADH dehydrogenase type II complex, essential to cellular respiration, binds some quinones with high affinity, indicating that this may be one target of this compound class (88). Without the ability to reduce oxygen into water, the production of ROS would be promoted, likely causing damage to parasite mitochondria (89).

COMPOUNDS WITHOUT KNOWN OR SUSPECTED MODES OF ACTION

Compounds that lack a known or suspected MoA, but demonstrate anti-*Toxoplasma* activity, present an opportunity for further exploration. Of note, pyrimethamine, atovaquone, and sulfadiazine did not have a known MoA at the time that they were FDA approved; thus, the lack of a MoA does not necessarily prevent an otherwise-promising compound from progressing in development.

Of the compounds that do not have specific mechanisms elucidated, one stands out as particularly interesting: a semisynthetic artemisinin, compound 54. Artemisinin, the precursor of all of the semisynthetic artemisinin derivatives used to treat malaria, was isolated from the plant *Artemisia annua* (90). Compound 54 has poor aqueous solubility (91); however, due to its high lipophilicity, compound 54 is suspected to have high blood-brain barrier penetration; indeed, further studies on reactivation of latent *T. gondii* infections have shown that this compound has some ability to protect mice from immunosuppression-induced reactivated infections (90). Because of the potency of this compound, as well as closely related derivatives (artemisone) passing FDA phase I trials (92), compound 54 and other artemisinin derivatives are promising candidates for further study.

Other compound groups with no known MoA include metals and metal complexes; images taken from one silver exposure study demonstrated significant morphological alterations, indicating that the MoA likely involves some form of mechanical damage to the cellular surface of the parasite (Table 2). Of these compounds, the highly effective metal ion complex, compound 50

(IC_{50}, 0.0187 μM) appears to be the most the most promising (93).

COMPOUNDS IDENTIFIED IN SCREENS

Most clinically available anti-*Toxoplasma* drugs were originally developed to treat infections caused by other protozoal or bacterial pathogens (94). Traditionally, compounds active against *Plasmodium* were often subsequently tested against *T. gondii*. More recently, *T. gondii*-specific high-throughput screens (HTS) are becoming more common for discovery of compounds active against this protozoan. However, the downside of screening is that little information other than the compound structure and IC_{50} data are readily gathered, leaving significant work to detail additional features. For the purpose of this review, *T. gondii* chemical screens were classified into 4 different groups: (i) cell-based phenotypic screens; (ii) target-based *in vitro* biochemical screens; (iii) cheminformatics-based virtual screens; (iv) drug-repurposing screens. The inherent value of such screens, as seen in Table 3, is to provide starting points for subsequent characterization and optimization.

Cell-based phenotypic screens. In cell-based phenotypic screens, compounds are evaluated against live parasites by measuring the *in vitro* parasite replication rate by using strains that express β-galactosidase or fluorescent proteins, or by employing the [^{3}H]uracil incorporation assay (95–97). As an example of the power of this approach, Table 3 presents a list of the promising compounds discovered using cell-based screens. For example, a small focused screen (98) identified 8 compounds with a biphenylimidazoazine scaffold with IC_{50}s of ≤0.6 μM against *T. gondii* (Table 3); these compounds also demonstrated broad antiparasitic effects against several apicomplexan parasites, including *P. falciparum*, *Neospora caninum*, *Eimeria tenella*, and *Besnoitia besnoiti*, suggesting that they were rather nonspecific inhibitors of *T. gondii*. In a recent high-throughput screnning (HTS) approach (99), 6,811 compounds were screened, and *N*-4-ethyl-benzoyl-2-hydroxybenzamide, compound 80, was identified, with an IC_{90} value of ~0.031 μM. Using an insertional mutagenesis approach, the authors identified a putative target gene, adaptin-3β, which is part of a secretory protein complex responsible for the secretion of micronemes, rhoptries, and dense granules. This compound was optimized to yield compound 81, which has an IC_{90} value of ~0.016 μM. Compounds 80 and 81, at respective doses of 50 and 20 mg/kg/day, protected mice against *T. gondii* acute infection. However, these compounds had low specificity for *T. gondii*, as they were also active against other protozoan parasites, including *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *P. falciparum* (100). Another phenotypic study identified endochin-like quinolone compounds 68 and 69 with *in vitro* IC_{50}s of 0.1 and 0.007 nM, respectively (101) (Table 3). These compounds are orally bioavailable and, at doses of 0.3 to 1.0 mg/kg/day given once a day for 5 days, prevented acute toxoplasmosis in a murine experimental model. Moreover, these compounds drastically reduced the cyst burden in a latent murine infection model. Their low IC_{50} combined with the ability to inhibit acute and chronic forms of infection makes these compounds promising anti-*Toxoplasma* therapeutic leads.

In addition to measuring *in vitro* growth rate, some *T. gondii* phenotypic screens identified compounds that inhibited host cell attachment and invasion. Using this approach, a focused small-molecule screen identified cysteine protease inhibitors that block invasion (102). Other screens have used dual-fluorescent image-based high-content screening (HCS) to directly observe parasite attachment and entry into the host cells (103, 104). Even though phenotypic screens are capable of identifying new chemical entities, one drawback of the phenotype-based approach is the difficulty in identifying target proteins. To circumvent this problem, Hall et al. used a combination of HCS and chemical genetics to screen a library of 1,222 inhibitors that covalently modified the target protein and then applied click chemistry along with tandem orthogonal proteolysis activity-based protein profiling to identify target proteins. With this approach, they identified compound 67, which blocks host cell invasion by inhibiting the activity of TgDJ-1, a unique regulator of microneme secretion and motility (104). These studies suggest that phenotypic screens combined with chemical genetics or forward genetic screens (69), such as drug-resistant strain generation via insertional mutagenesis or chemical mutagenesis, are powerful approaches to identify new drugs and drug targets.

Target-based *in vitro* biochemical screens. Target-based *in vitro* biochemical screens usually involve a biochemical assay with a purified recombinant protein. One of the major advantages of a target-based screen is the possibility of screening or designing drugs based on the enzymatic property or the crystal structure of a protein. These screens were carried out on *T. gondii* even before the availability of its genome sequence (105). Most of these screens were focused on developing inhibitors against known essential enzymes, such as DHFR, DHPS, and nucleoside triphosphate hydrolase (NTPase). However, due to the toxicity of clinically available antifolate drugs, many of the initial screens were focused on identifying additional compounds that specifically inhibited *T. gondii* DHFR (33, 34, 106–111) and DHPS (39, 112) while avoiding host effects. Most of these small-scale screens were performed to identify compounds that were active against these enzymes, but very few of these studies report *T. gondii in vivo* data (37, 113, 114). One of the early target-based HTS in *T. gondii* was performed by Asai et al., who screened 150,000 compounds against Tg-NTPases, which are essential proteins for *T. gondii* tachyzoite replication inside the parasitophorous vacuole (115) (Table 3). In this screen, compounds 89, 90, and 91 were identified that inhibited *T. gondii* growth *in vitro* with IC_{50}s of ≤7.0 μM and inhibited NTPase I and II enzymes with IC_{50}s ranging from 1.3 μM to 14.5 μM.

Several target-based drug screens were also performed against *T. gondii* CDPKs because, as described above, these kinases regulate essential biological processes, such as cell division, development, motility, microneme secretion, invasion, and egress (116). In addition, the ATP binding pockets of most of the apicomplexan CDPKs have a gatekeeper residue with a smaller side chain, whereas most of the ATP binding pockets in mammalian serine-threonine protein kinases have a gatekeeper amino acid with a bulky side chain. This property has been exploited to develop inhibitors specific for TgCDPK1, an essential kinase that regulates gliding motility, invasion, and egress in *T. gondii* (117). Based on structure-activity relationship (SAR) studies, a number of pyrazolopyrimidines were identified which specifically inhibit TgCDPK1 and prevent *T. gondii* growth at concentrations below 1 μM (24, 118, 119). One of these, compound 86, is orally bioavailable and can reach tachyzoites in the brains of infected mice by crossing the blood-brain barrier and reach therapeutic concentrations (120). Interestingly, compound 86 has broad-spectrum antiparasitic activity against several pathogens, such as *Cryptospo-*

TABLE 2 Compounds without known modes of action against *Toxoplasma gondii*[a]

Compound class and no.	Reference(s)	*In vitro* IC_{50}	Host cell TD_{50}	*In vivo* survivability	*In vivo* chronic infection	Parasite burden	*In vivo* toxicity
3-[{2-((E)-furan-2-ylmethylene)hydrazinyl} methylene]-1,3-dihydroindol-2-one (ATT-5126)	152						
Compound 47		19.7 μM	35.4 μM HeLa cells	ND	ND	10,000 RH strain tachyzoites injected i.p.; 19% reduction in tachyzoite burden in peritoneal cavity	ND
6-Trifluoromethyl-2-thiouracil (KH-0562)	152						
Compound 48		32.2 μM	56.3 μM HeLa cells	ND	ND	10,000 RH strain tachyzoites injected i.p.; 24% reduction in tachyzoite burden in peritoneal cavity	ND
Diamidines	178, 179						
Compound 49		0.03 μM (179)	>2 μM HFF cells (179)	ND	ND	ND	ND
Metal and metal complexes	93, 180, 181						
Compound 50		0.0187 μM (180)	2.4 μM HFF cells (180)	ND	ND	ND	ND
Compound 51		ND (93)	ND	ND	ND	3,500 RH strain tachyzoites injected i.p.; group treated 4 days preinfection had ~45%–50% reduced organ burdens; group treated 4 days postinfection had ~86% reduced spleen burden (93)	>200 μg/ml (93)
Compound 52		3.6 μM (181)	>200 μM LLC-MK2 cells (181)	ND	ND	ND	ND
Resorcinarenes	182						
Compound 53		ND	4,239 μM RAW 264.7 cells	ME49 strain, unspecified no. or life stage of parasites; led to 50% increase in survival in treatment group	25 ME49 strain bradyzoites delivered orally; no reduction in brain cysts in treatment group	ND	>500 mg/kg
Semisynthetic artemisinins	90, 183–187						
Compound 54		108 μM (90)	ND	1,000,000 PRU-Luc-GFP strain tachyzoites injected i.p.; 60% survival in treatment group (90)	(Coadministered with sulfadiazine until 23dpi) 1,000,000 PRU-Luc-GFP strain tachyzoites injected i.p.; significant reduction in brain cysts in treatment group (90)	ND	ND
Compound 55		ND (186)	ND	50 RH strain tachyzoites injected i.p.; treatment increased survival by 6–7 days (186)	18 ME49 strain bradyzoites injected i.p.; ~40% reduction of cyst burden in treatment group (186)	ND	<10 mg/kg (186)
Compound 56		0.25 μM	>320 μM HFF cells	ND	ND	ND	ND
Aculeatins	188						
Compound 57		0.173 μM	0.173 μM K562 cells	ND	ND	ND	ND
Clodinafop and derivatives	189						
Compound 58		10 μM led to 70% growth inhibition; IC_{50} not established	>400 μM HFF cells	ND	ND	ND	ND
Indirubin analogues	174						
Compound 59		0.18 μM	20 μM HFF cells	ND	ND	ND	ND

Quinones (exptl)	190, 191				ND	ND		ND
Compound 60		1.74 μM (191)	>6 μg/ml THP-1 cells (191)	ND				
Compound 61		3.37 μM (191)	>6 μg/ml THP-1 cells (191)	ND	ND	ND		ND
Compound 62		8.36 μM led to 96.5% growth inhibition; IC_{50} not established (190)	>2 μg/ml in THP-1 cells (190)	ND	ND	ND		ND
Compound 63		8.73 μM led to 92% growth inhibition; IC_{50} not established (190)	>2 μg/ml in THP-1 cells (190)	ND	ND	ND		ND
Thiazolidinones	192							
Compound 64		0.9 μM	35 μM HFF cells	ND	ND	ND		ND
Thioureides	193							
Compound 65		0.3 μM	No noted effect on HFF or Caco2 cells; exact numbers not reported	ND	ND	ND		ND
Indole-1,2-diones	194							
Compound 66		0.3 μM	6.4 μM HFF cells	ND	ND	ND		ND

[a] Individual compound information is listed with the index number assigned to the specific molecule's structure. For cases in which multiple IC_{50} values were found in the literature, the lowest observed IC_{50} is reported. Anti-*Toxoplasma* compounds are grouped by structure. Structures for each compound can be found in Table S2 in the supplemental material. The *in vivo* survivability assay data refer to experiments where model organisms (mice) were exposed to a lethal infectious dose of parasite, often via i.p. injection. The strain and dose of the parasite and the recipient host differed between studies, making comparisons between studies problematic. *In vivo* chronic infection and parasite burden data refer to the tissue or fluid counts of parasites isolated from a host following a nonlethal infection or bradyzoite cyst burden in brain tissue (most often determined via PCR). ND, not determined; dpi, days postinfection; HFF, human foreskin fibroblasts.

ridium parvum (121), *Neospora caninum* (122), and *Plasmodium falciparum* (123).

Cheminformatics-based virtual screens. Cheminformatics-based virtual screens are being performed to identify novel anti-*Toxoplasma* compounds (87, 124, 125). The availability of crystal structures for some of the *T. gondii* proteins and the improvement in computational molecular modeling approaches facilitate this approach. For example, a chemoinformatic virtual screen against rhoptry protein 18 (ROP18) led to the identification of compound 93, which inhibits *T. gondii* growth *in vitro* with an IC_{50} of ~2 nM (Table 3). In this screen, the authors performed *in silico* chemical searches on 45,384 commercially available compounds and identified 17 of these, based on their expected activity and ADME characteristics. From these 17 compounds, those authors identified compound 86 to be most potent against *T. gondii in vitro*. Even though compound 93 was identified as a ROP18 inhibitor, this compound has additional unidentified effects on the parasite.

Drug-repurposing screens. Drug-repurposing screens are common in *T. gondii* drug discovery because drugs active against one apicomplexan parasite are often active against other apicomplexans, due to the conservation of biochemical pathways between these parasites. As a result, compounds active against *Plasmodium falciparum* are often screened against *T. gondii*. Many compounds now in clinical use are a result of these investigations (13). Screening of the open-access Medicines for Malaria Venture (MMV) Malaria Box led to the identification of 7 anti-*Toxoplasma* compounds with novel chemical scaffolds (126) (Table 3), the most potent of which was compound 94, with an IC_{50} of 0.19 μM (126). In another screen, approximately 15 broad-spectrum anti-*Toxoplasma* compounds, with IC_{50}s below 10 μM, were identified by screening a library of 309,474 unique compounds against *P. falciparum* and testing a set of representative compounds against *T. gondii*, *Leishmania major*, and *Trypanosoma brucei*.

CONCLUSION

Within this review, more than 50 chemotypes have been described. Based on the identified characteristics, a number of specific compounds stand out as particularly notable. Pyridinylpyrrole, compound 3, demonstrated 90% increased survival and low organ parasite loads during an *in vivo* trial, with no sign of recrudescence 12 months after the completion of treatment (19, 21) (Table 1). Another promising compound class which inhibits fatty acid synthesis are the acylsulfonamides, the molecular target for which is not present in the host cell (63) (Table 1). Compound 28, an acylsulfonamide, does not have any reported *in vivo* data, but it has very low host cell toxicity in HFF cells (TD_{50}, >1 mM) compared to its *T. gondii* IC_{50} (0.02 μM), indicating it may have a relatively high therapeutic index. One of the most promising compounds identified via a screening assay was compound 86, a semisynthetic artemisinin (120). This compound is orally bioavailable and capable of crossing the blood-brain barrier, with a survival increase of 60% in treated mice (90). The endochin-like quinolones (compounds 68 and 69), also identified via screening, additionally demonstrate promising characteristics, such as a high therapeutic index and efficacy against the acute and chronic stages *in vitro* (Table 3).

Inhibition of translation is common against prokaryotic targets, but due to homology to host ribosomal components, it is rarely utilized in antiparasitic modes of action. Future work that

TABLE 3 Compounds with demonstrated activity against *Toxoplasma gondii* that were identified via various screen types[a]

Screen type and compound no.	Reference(s)	IUPAC name	Targeted protein	Predicted or demonstrated MoA	*In vitro* IC_{50} (μM)	Host cell TD_{50} (μM)	*In vivo* (mouse) therapeutic dose (mg/kg/day)	*In vivo* toxicity (mg/kg/day)
Phenotypic screens								
67	104	Benzyl ((S)-1-oxo-1-(((S)-3-oxopent-4-en-2-yl)amino)-3-phenylpropan-2-yl)carbamate	TgDJ-1	Prevents attachment and invasion	~2–6	ND	ND	ND
68	101	2-Methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl) quinolin-4(1H)-one	Cytochrome bc_1 complex	Inhibits mitochondrial respiratory chain	0.0001	9.3	>1	>50
69	101	6-Fluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)- quinolin-4(1H)-one	Cytochrome bc_1 complex	Inhibits mitochondrial respiratory chain	0.000007	>50	>0.33	>50
70	195	Ethyl 6-fluoro-7-methoxy-3-(4-(4-(trifluoromethoxy)phenoxy) phenyl)quinoline-2-carboxylate	Cytochrome bc_1 complex	Inhibits mitochondrial respiratory chain	5	>320	ND	ND
71	98	2-(4-Fluorophenyl)-3-(20-hydroxybiphen-3-yl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine	ND	ND	~0.61	>50	ND	ND
72	98	3-(20-Hydroxybiphen-3-yl)-2-*tert*-butyl-6-(thien-3-yl)imidazo[1,2-a]pyridine	ND	ND	~0.08	>50	ND	ND
73	98	2-(2-Methoxyphenyl)-3-(2'-hydroxybiphen-3-yl)-6-(thien-3-yl)imidazo[1,2-b]pyridazine	ND	ND	~0.36	~32.0	ND	ND
74	98	3-(20-Hydroxybiphen-3-yl)-2-(2-methoxyphenyl)-6-(thien-3-yl)imidazo[1,2-a]pyridine	ND	ND	~0.27	>50	ND	ND
75	98	3-((Biphen-3-yl)-2-(2-methoxyphenyl)imidazo [1,2-a]pyridin-6-yl)methanol	ND	ND	~0.63	~42.2	ND	ND
76	196	*N*-[(E)-(1-propan-2-ylbenzimidazol-2-yl) methylideneamino]-1H-benzimidazol-2-amine	ND	Inhibits motility and invasion	~1.36	>10	ND	ND
77	196	(5E)-5-(2,4-dinitrophenoxy)iminoquinolin-8-one	ND	ND	~1.34	>10	4.4	ND
78	196	3-Benzyl-6-[1-(2-ethoxyphenyl)-5-methyltriazol-4-yl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole	ND	ND	~0.57	>10	ND	ND
79	196	4-*N*-Benzyl-2-*N*-(2-methylphenyl)quinazoline-2,4-diamine hydrochloride	ND	Inhibits motility	~1.12	>10	ND	ND
80	99	*N*-4-Ethyl-benzoyl-2-hydroxybenzamide	Adaptin-3β	Inhibits secretion of micronemes, rhoptries, and dense granules	I0.031[b]	>10	50	ND
81	100	*N*-[4-(Diethylamino)benzoyl]-2-hydroxybenzamide	Adaptin-3β	Inhibits secretion of micronemes, rhoptries, and dense granules	0.016[b]	>10	20	ND
Targeted screens								
82	119	1-(*tert*-Butyl)-3-(3-methylbenzyl)-4-amino-1H-pyrazolo[3,4-d]pyrimidine	TgCDPK1	Inhibits invasion, gliding motility, microneme secretion, and egress	0.11	ND	5	ND
83	119	1-(tert-Butyl)-3-(3-chlorobenzyl)-4-amino-1H-pyrazolo[3,4-d]pyrimidine	TgCDPK1	Inhibits invasion, gliding motility, microneme secretion, and egress	0.03	ND	5	ND
84	119	3-(2,3-Dichlorobenzyl)-1-isopropyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine	TgCDPK1	Inhibits invasion, gliding motility, microneme secretion, and egress	0.25	ND	5	ND
85	119	1-(*tert*-Butyl)-3-(3,5-difluorobenzyl)-4-amino-1H-pyrazolo[3,4-d]pyrimidine	TgCDPK1	Inhibits invasion, gliding motility, microneme secretion, and egress	0.61	ND	5	ND
86	120, 197	1-((1-Methylpiperidin-4-yl)methyl)-3-(6-ethoxynaphthalen-2-yl)- 4-amino-1H-pyrazolo[3,4-d]pyrimidine	TgCDPK1	Inhibits invasion, gliding motility, microneme secretion, and egress	0.14	ND	40	>100
87	113	2,4-Diamino-5-methyl-6-(2¢,6¢-dimethylphenylthio) pyrrolo[2,3-d]pyrimidine	TgDHFR	Growth inhibition	1.92	>260	50	ND
88	113	2,4-Diamino-5-methyl-6-(2¢,6¢-dimethylphenylthio) pyrrolo[2,3-d]pyrimidine hydrochloride salt	TgDHFR	Growth inhibition	2.15	>260	50	ND
89	115	2-Phenylthio-indole	TgNTPase-I, TgNTPase-II	Growth inhibition	~7	>50	ND	ND
90	115	2-(2-Naphthalenylthio)-1H-indole	TgNTPase-I, TgNTPase-II	Growth inhibition	~3.6	>50	ND	ND
91	115	2-(1-Naphthalenylthio)-1H-indole	TgNTPase-I, TgNTPase-II	Growth inhibition	~3.2	>50	ND	ND

Cheminformatic screens								
92	87	5-Nitroso-8-hydroxyquinoline	ND	Oxidative stress	0.08	ND	ND	ND
93	124	3-[(E)-2-(1,3-Benzodioxol-5-yl)ethenyl]-1H-quinoxalin-2-one	ROP18	Destruction of parasitophorous vacuole	0.002	ND	ND	ND
Drug repurposing screens								
94	126	*N*-(2-Ethoxyphenyl)-2-[4-(furan-2-carbonyl)piperazin-1-yl]acetamide	ND	ND	0.19	>30	ND	ND
95	126	*N*-[4-(Dibutylsulfamoyl)phenyl]furan-2-carboxamide	ND	ND	1.07	>30	ND	ND
96	126	7-Chloro-4-pyrrolidin-1-ylquinoline	ND	ND	1.49	>30	ND	ND
97	126	*N*′,*N*′-Dimethyl-*N*-(2-phenylquinolin-4-yl)ethane-1,2-diamine	ND	ND	1.95	>30	ND	ND
98	126	6-Bromo-2-methyl-3-propyl-1H-quinolin-4-one	ND	ND	3.05	>30	ND	ND
99	126	*N*-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-1H-pyrrolo[3,2-h]quinoline-2-carboxamide	ND	ND	3.85	>30	ND	ND
100	126	2-(2-Methoxyanilino)-3-piperidin-1-ylnaphthalene-1,4-dione	ND	ND	4.54	>30	ND	ND
101	127	2-[(E)-2-(4-Ethoxyphenyl)ethenyl]-1,3-benzoxazin-4-one	ND	ND	0.27	>39.8	ND	ND
102	127	2-Amino-4-(3,5-ditert-butyl-4-hydroxyphenyl)-5-oxo-4,6,7,8-tetrahydrochromene-3-carbonitrile	ND	ND	1.33	>36.2	ND	ND
103	127	Methyl *N*-[(E)-[phenyl(pyridin-2-yl)methylidene]amino]carbamodithioate	ND	ND	1.63	>42.9	ND	ND
104	198	3-(4-Chlorophenyl)1-(1,1-dimethylethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine	ND	ND	0.2	>5	ND	ND

[a] The lowest observed IC_{50}s are reported in cases in which multiple values were found in the literature. Structure data for each compound in the table can be found in Table S3 in the supplemental material. *In vivo* therapeutic dose data refer to the best concentration tested in the literature for toxicity and efficacy in a model organism. ND, not determined.

[b] The IC_{90} value (rather than IC_{50}) is reported for this compound.

targets mechanisms of translational control may offer additional avenues for therapy (128).

In summary, with the expanding number of immunocompromised individuals at high risk, the demand for new toxoplasmosis treatment options is rising. Some of the novel compounds reviewed here may represent good starting points for the discovery of effective new drugs against *T. gondii*. Increasing the medicinal arsenal of anti-*Toxoplasma* therapeutics may yield a potent and less toxic option for future patients.

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