Abstract: Toxoplasmosis is a parasitic disease caused by the globally distributed protozoan parasite *Toxoplasma gondii*, which infects around one-third of the world population. This disease may result in serious complications for fetuses, newborns, and immunocompromised individuals. Current treatment options are old, limited, and possess toxic side effects. Long treatment durations are required since the current therapeutic system lacks efficiency against *T. gondii* tissue cysts, promoting the establishment of latent infection. This review highlights the most promising drug targets involved in anti-*T. gondii* drug discovery, including the mitochondrial electron transport chain, microneme secretion pathway, type II fatty acid synthesis, DNA synthesis and replication and, DNA expression as well as others. A description of some of the most promising compounds demonstrating antiparasitic activity, developed over the last decade through drug discovery and drug repurposing, is provided as a means of giving new perspectives for future research in this field.

Keywords: anti-*Toxoplasma* agents; drug discovery; drug repurposing; drug targets; *Toxoplasma gondii*; toxoplasmosis

1. Introduction

The parasite *Toxoplasma gondii* is an obligate intracellular protozoan that infects most warm-blooded animals [1]. However, its sexual lifecycle can only occur in members of the Felidae family, known as definitive hosts, such as domestic cats and wild felids, whose global *T. gondii* seroprevalence is estimated to be 35% and 59%, respectively [2]. The rates of *T. gondii* seroprevalence in humans vary greatly among different geographical areas, ranging from approximately 30% in the American, European, and Asiatic regions, to more than 60% in the African continent. These fluctuations may result from different cultural practices, environmental conditions, or the socioeconomic status of the population. Overall, *T. gondii* is considered one of the most successful parasites worldwide, infecting about 30% of the world’s population [3]. Despite its ubiquitous distribution, several divergent strains of *T. gondii* were detected.

Most strains isolated in Europe and North America fall into one of three genotypes, referred to as types I, II and III. Type I are considered the most virulent strains and are lethal in mice, displaying enhanced migratory capacity both in vitro and in vivo, the faster growth rate being in vitro, and being capable of generating high parasite loads in the host organism. Type II and III have intermediate and low virulence, respectively, with type II being the most common strains among human infections, while type III are mostly found in non-human infections. Certain strains are typically predominant in certain geographic
locations. The three major clonal lineages are found in Asia and America, in addition to certain specific genotypes for the former area and various recombinant strains for the latter. Type II and III strains are isolated in North Africa, the Middle East, and the Arabic peninsula, while in Europe, type II strains are more common. Several African genotypes, including type II and III strains, are found in sub-Saharan Africa [4–6].

2. Toxoplasmosis

In humans, the severity of infection is mainly determined by the host immune system efficacy. In immunocompetent individuals, acute *T. gondii* infection is usually asymptomatic or may cause, in rare instances, a flu-like disease with mild symptoms. Strain virulence and several host-cell conditions—pH, heat shock, mitochondrial inhibition, and nitric oxide production—may also influence disease outcome [4,7,8]. Under host immune pressure, the parasite naturally becomes dormant. In this process, tachyzoite–bradyzoite conversion occurs, with tissue cyst formation in specific locations, such as cerebral, skeletal, and cardiac muscle tissues, reaching the chronic phase of infection. An intact immune system prevents cyst rupture and reactivation of infection [4,7]. However, in immunocompromised individuals, the parasite generally remains active, causing continuous host-cell infection, leading to acute disease. Moreover, reactivation may also occur in individuals who are initially immunocompetent but later undergo immunosuppression, including individuals with acquired immunodeficiency syndrome (AIDS), or subjected to immunosuppressive therapy, as in the case of autoimmune diseases or organ transplantation. Individuals with AIDS and not initially infected with *T. gondii* usually develop a severe primary infection, whereas individuals already infected with the parasite who become immunosuppressed are at an increased risk of disease relapse [9,10]. In fact, *T. gondii* encephalitis was frequently reported in AIDS patients, especially those with low CD4 T lymphocyte cell counts. Therefore, *T. gondii* may be regarded as an opportunistic parasite that contributes to the death of AIDS patients [10]. In these situations, where *T. gondii* infection is acquired throughout an individual’s life, the disease is referred to as acquired toxoplasmosis.

Toxoplasmosis may also be potentially dangerous in seronegative pregnant women that become primo-infected during pregnancy, as it may lead to transplacental transmission of the parasite, which may result in congenital toxoplasmosis [11]. However, the incidence of congenital toxoplasmosis varies with the trimester during which primary infection is acquired. The transmission rate is greater in the final stages of pregnancy, as placental irritation increases, allowing for a greater area of contact with the fetus. On the other hand, the severity of infection is greater in the early stages of pregnancy, due to fetal immunological immaturity [12–14]. During the first trimester, there is a higher risk of abortion, stillbirth, or premature birth. In the second trimester, the risk of miscarriage decreases, however, in more severe cases, hydrocephaly, chorioretinitis and cerebral calcification may occur, according to the parasite’s brain and ocular tropism. In the last trimester, although severe clinical manifestations in the newborn are at lower risk, there is an increased probability of congenital infection [11,15–18].

Regarding cerebral tropism, recent data suggest an association between congenital infection and the development of neurological and psychiatric disorders later in life [9,19,20], including Alzheimer’s disease [19,20], depression [19–21], schizophrenia [22–24], bipolar disease [24], and even suicidal tendencies [21,24].

3. Current Treatment Options

First-line conventional treatment for acquired and congenital toxoplasmosis generally includes a pyrimethamine-based regimen, which comprises three drugs: pyrimethamine, sulfadiazine and folinic acid (leucovorin; Table 1) [25,26]. Pyrimethamine is a folic acid antagonist as it inhibits the dihydrofolate reductase (DHFR) enzyme, blocking the synthesis of purines and pyrimidines, essential for DNA synthesis and cell multiplication. The action of this drug is enhanced when used in conjunction with sulfonamides, such as sulfadiazine, which is capable of interfering with *T. gondii’s* folic acid synthesis, by com-
petitively inhibiting the dihydropteroate synthetase (DHPS) enzyme. This combination
must not be used during the first trimester of pregnancy due to the teratogenic potential of
pyrimethamine, which also causes reversible myelosuppression, forcing combination with
folic acid, to prevent hematologic adverse reactions [25–28]. Moreover, although rare,
different severe complications were reported, such as agranulocytosis, Stevens-Johnson
syndrome, toxic epidermal necrolysis and hepatic necrosis, as well as many others [29–34].
Although several alternative treatment options are available, including pyrimethamine
combined with clindamycin, clarithromycin, azithromycin or atovaquone, and monother-
apy using cotrimoxazole (trimethoprim-sulfamethoxazole) or atovaquone, no regimen
was found to be more effective than the conventional treatment [25,35]. Despite clinical
complications, standard chemotherapy has proven to reduce the risk of development
of toxoplasmosis-related sequels and symptoms associated with congenital infection in
newborns, if it is administered immediately after diagnosis of either maternal infection or
congenital transmission [25,27,36]. Alternatively, when the maternal infection is suspected,
but not confirmed, therapy with spiramycin must be implemented. Spiramycin is a potent
macrolide antibiotic, and although it does not readily cross the placental barrier, it is greatly
accumulated in the placenta, preventing transplacental transmission of *T. gondii*. Neverthe-
less, when fetal or neonatal toxoplasmosis is confirmed, spiramycin is discontinued and
conventional treatment is applied [36]. The use of steroids is beneficial in the treatment of
ocular toxoplasmosis in combination with antimicrobial therapy. However, excessive doses
can lead to a minimal response [25]. In fact, phase II clinical trials are currently underway
to determine the optimal dose of dexamethasone to be used as adjunctive therapy to reduce
brain edema in HIV-infected patients exhibiting cerebral toxoplasmosis [37].

Table 1. Current drugs used for toxoplasmosis treatment.

| Compound     | Chemical Structure * | Mechanism of Action                     | References |
|--------------|----------------------|-----------------------------------------|------------|
| Pyrimethamine| ![Pyrimethamine](image) | Antifolate                              | [25,27]    |
| Sulfadiazine | ![Sulfadiazine](image) | Antifolate                              | [25,27]    |
| Folinic acid | ![Folinic acid](image) | Reduction of pyrimethamine side effects | [25,27]    |
Table 1. Cont.

| Compound     | Chemical Structure * | Mechanism of Action       | References |
|--------------|-----------------------|---------------------------|------------|
| Spiramycin   | ![Spiramycin Structure](image1) | Protein synthesis inhibitor | [25,35] |
| Clindamycin  | ![Clindamycin Structure](image2) | Protein synthesis inhibitor | [25,35] |
| Clarithromycin | ![Clarithromycin Structure](image3) | Protein synthesis inhibitor | [25,35] |
Current treatment options are limited and not optimal regarding the harsh profile of side effects and treatment duration (from 4–6 weeks to over 1 year), which may affect compliance [27,38]. *T. gondii*-related factors, such as the increasing drug resistance, different drug susceptibility for different strains, and the remaining unknown aspects of the parasite’s pathogenicity, also play an important role in disease progression and treatment failure [39–41]. In addition, no current drug can eliminate tissue cysts from the infected host, which remain quiescent, establishing the latent phase of infection, as long as the host’s immune system remains capable enough [27,38]. Although immunization strategies are currently being studied and developed, there is no vaccine available for human administration [42].

Thus, there is an urgent need for the development of newer, safer, and more effective treatment alternatives for toxoplasmosis, which consequently relies on rising knowledge in *T. gondii* pathophysiology and the discovery of promising drug targets.

This review highlights some of the most promising drug targets in anti-*T. gondii* drug discovery and the compounds discovered, developed, and repurposed over the last decade, while focusing on their relevant features, in vitro and in vivo activity, and future perspectives. A literature search was conducted using PubMed database and the query "((toxoplasmosis) OR (Toxoplasma gondii) OR (anti-Toxoplasma) AND (drug) OR (treatment)). Experimental compounds with established in vitro activity were primarily...
considered, of which those with in vivo activity or efficacy against bradyzoite-containing cysts were prioritized.

4. Promising Drug Targets and Strategies in Anti-T. gondii Drug Discovery

In the last decade, considerable efforts have been made in the study and development of repurposed drugs and novel compounds with new mechanisms of action. Drug screens involved various parasite targets, including mitochondrial electron transport chain, calcium-dependent protein kinases, type II fatty acid synthesis, DNA synthesis and replication, and DNA expression as well as many others. Drug targets and respective promising inhibitors with interesting mechanisms of action (Figure 1) and efficient in vitro and/or in vivo activity against T. gondii are described and discussed. Relevant experimental in vitro and in vivo results are summarized in Table 2.

Figure 1. Graphical representation of a Toxoplasma gondii tachyzoite. Organelle and pathway targets of several experimental and repurposed compounds [43–56]. AR: apical ring; Mic: microneme; R: rhoptry; DG: dense granule; A: apicoplast; M: mitochondrion, N: nucleus; BKI: bumped-kinase inhibitor; ELQ: endochin-like quinolone.
Table 2. Experimental compounds with anti-*Toxoplasma gondii* activity.

| Compound       | Chemical Structure * | Drug Target | Affected *T. gondii* Pathway | In Vitro IC₅₀/T. gondii Strain/Host Cell | In Vivo Results/T. gondii Strain/Animal Model/Infection Route | References |
|----------------|----------------------|-------------|-----------------------------|----------------------------------------|---------------------------------------------------------------|------------|
| BKI-1294       | ![Chemical Structure](chart1.png) | TgCDPK1 | Parasite microneme secretion | 140 nM/RH/ HFF                          | 93% reduction of parasite burden at 30 mg/kg. No *T. gondii* detected in peritoneal fluid of half the mice at 100 mg/kg/CF-1 mice/ Intraperitoneal Protection against abortion and vertical transmission in sheep experimentally infected with *T. gondii* tachyzoites during pregnancy | [43,44,57–59] |
| Compound 32 *  | ![Chemical Structure](chart2.png) | TgCDPK1 | Parasite microneme secretion | 60 nM/ME49/ HFF                        | 88.7% reduction in the number of brain cysts/ME49/ CBA/J mice/ Oral gavage | [44] |
| Compound 24 *  | ![Chemical Structure](chart3.png) | TgCDPK1 | Parasite microneme secretion | TgCDPK1 inhibition at 10.9 nM (enzyme activity assay) Inhibition of parasite proliferation at 0.264 μM/RH/ HFF | Decreased severity of acute infection. Delayed chronic reactivation of disease. Completely cured part of the animals/BALB/c mice and mice lacking IFN-γ receptor/ Oral gavage | [45] |
| Triclosan      | ![Chemical Structure](chart4.png) | ENR | FAS II                       | 3 μM/RH/ HFF                           | Reduction in mice mortality, parasite burden and viability. Poor solubility and oral bioavailability/RH/Swiss albino mice/Intraperitoneal | [46,47,60] |
| Triclosan-liposomal | ![Chemical Structure](chart5.png) | Liposomal | ENR | FAS II | ND | Reduction in host mortality and *T. gondii* brain burden by 98%/ME49/Swiss albino mice/Oral gavage | [46,47] |
Table 2. Cont.

| Compound          | Chemical Structure * | Drug Target      | Affected T. gondii Pathway | In Vitro IC_{50}/T. gondii Strain/Host Cell | In Vivo Results/T. gondii Strain/Animal Model/Infection Route                                                                 | References |
|-------------------|----------------------|------------------|-----------------------------|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|------------|
| Compound 16c      | ![Compound 16c](image) | ENR              | FAS II                      | 250 nM/ RH/ HFF                             | Improvement in pharmacokinetics in comparison to triclosan Decreased peritoneal burden of T. gondii/ RH/ Swiss albino mice/ Intraperitoneal | [61]       |
| Thiolactomycin (and analogs) | ![Thiolactomycin](image) | KAS I/II         | FAS II                      | 1.6–29.4 µM/ RH/ LLCMK2                    | Serious morphological alterations in treated parasites (electron microscopy)                                                   | [62]       |
| SW413             | ![SW413](image)      | Pantothenate synthetase (within FAS II) | Fatty acid chain elongation | 20 nM/ RH/ HFF                             | ND                                                                                                                             | [49]       |
| SW404             | ![SW404](image)      | Pantothenate synthetase (within FAS II) | Fatty acid chain elongation | 130 nM/ RH/ HFF                            | ND                                                                                                                             | [49]       |
| FR235222          | ![FR235222](image)   | TgHDAC3          | DNA expression              | 9.7 nM/ RH/ HFF                            | Reduced tachyzoite infection in mice. No brain cysts detected. No detectable humoral response/ PRU/ Outbred female Swiss mice/ Intraperitoneal | [53]       |
| Compound | Chemical Structure † | Drug Target | Affected *T. gondii* Pathway | In Vitro IC₅₀/ *T. gondii* Strain/Host Cell | In Vivo Results/ *T. gondii* Strain/Animal Model/Infection Route | References |
|----------|-----------------------|-------------|-------------------------------|---------------------------------------------|---------------------------------------------------------------|------------|
| W363 d   | ![Chemical Structure](image) | TgHDAC3     | DNA expression                | 10.2 nM/ RH/ HFF                            | ND                                                            | [53]       |
| W399 d   | ![Chemical Structure](image) | TgHDAC3     | DNA expression                | 11.3 nM/ RH/ HFF                            | ND                                                            | [53]       |
| ELQ-271 e| ![Chemical Structure](image) | Cytochrome bc₁ complex (Qi site) | Cell respiration              | 0.1 nM/ 2F/ HFF Human bc₁ inhibition at 800 nM | ND                                                            | [54–56,63] |
| ELQ-316 e| ![Chemical Structure](image) | Cytochrome bc₁ complex (Qi site) | Cell respiration              | 0.007 nM/ 2F/ HFF No human bc₁ inhibition Not toxic to HFF/HepG2 cells at 10 µM | 88% reduction in the number of brain cysts/ ME49/ CBA/J mice/ Intrapitoneal | [54–56,63] |
| ELQ-400 e| ![Chemical Structure](image) | Cytochrome bc₁ complex (Qi and Qo site) | Cell respiration              | 5 µM/ RH/ HFF                              | Increased mice survival and reduction in brain and spleen parasite load/ RH/ CF-1 mice/ Intrapitoneal | [56,64] |
| MMV673968 f| ![Chemical Structure](image) | DHFR        | Folate synthesis              | 0.02 µM/ RH/ Vero High selectivity towards parasite DHFR | ND                                                            | [65]       |
### Table 2. Cont.

| Compound                  | Chemical Structure * | Drug Target | Affected *T. gondii* Pathway | In Vitro IC₅₀/ Host Cell | In Vivo Results/T. gondii Strain/Animal Model/Infection Route | References |
|---------------------------|----------------------|-------------|------------------------------|--------------------------|-------------------------------------------------------------|------------|
| Buparvaquone (MMV689480) | ![Chemical Structure](image) | Mitochondrial electron transport chain enzymes (dehydrogenase enzymes) | Cell respiration          | 0.10 µM/ RH/ Vero                                             | ND         | [65]       |
| Tanshinone IIA            | ![Chemical Structure](image) | NK          | NK                           | 2.5 µM/ PLK/ HFF          | Reduced number of in vitro-induced bradyzoites at 1 µM       | ND         | [66]       |
| Hydroxyzine               | ![Chemical Structure](image) | NK          | NK                           | 1.0 µM/ PLK/ HFF          | No host cell toxicity at 25 µM                              | ND         | [66]       |

**Abbreviations**: ND: not done; NK: not known; IC₅₀: half-maximal inhibitory concentration; TgCDPK1: *T. gondii* calcium-dependent protein kinase 1; ENR: enoyl-acyl carrier protein reductase; KAS I/II: β-ketoacyl-acyl carrier protein synthase; FAS II: type II fatty acid synthesis; TgHDAC3: *T. gondii* histone deacetylase 3; DHFR: dihydrofolate reductase; HFF: human foreskin fibroblasts; IFN: interferon; LLCMK2: rhesus monkey kidney epithelial cells; HepG2: human hepatocarcinoma cells. *: Chemical structures were designed using ChemDraw 17.0 software. a: Bumped-kinase inhibitors derived from BKI-1294. b: Triclosan analogue. c: Acylsulfonamides originally developed for *Mycobacterium tuberculosis*. d: FR235222 derivatives. e: Endochin-like quinolones. f: Repurposed compounds from the pathogen box (initiative of the Medicines for Malaria Venture). g: Repurposed compounds from the compound library provided by the Drug Discovery Initiative (University of Tokyo). h: Type II strain of *T. gondii*, derived from the ME49 strain.
4.1. Drug Targets Involved in Parasite Motility and Host-Cell Invasion

*T. gondii* belongs to the Apicomplexa family, which also includes other relevant protozoans, such as *Cryptosporidium* spp., a common cause of diarrhea in children, and *Plasmodium* spp., the etiological agent of malaria. In fact, both *T. gondii* and *Plasmodium* spp. share very similar organellar organization [67]. The members of this family possess well-developed structures at the anterior end of the cell—the apical complex—responsible for host-cell invasion [68]. Unlike *Plasmodium* spp., which specifically infects erythrocytes and hepatocytes, *T. gondii* does not require a specific host receptor for cell invasion, allowing it to infect all nucleated host cells [67]. The invasion process (Figure 2) requires the participation of specific *T. gondii* secretory organelles, belonging to the apical complex: the micronemes, small rod-shaped structures accumulated in the apical third of the protozoan body, housing proteins responsible for extracellular motility and invasion; and rhoptries, long club-shaped organelles located at the apical portion of the parasite, which accommodate proteins responsible for the invasion and host-cell manipulation. Proteins segregated by micronemes (named MICs) and rhoptries (named ROPs) allow host-cell entrance while dragging cytoplasmatic membrane around the tachyzoite, forming the Parasitophorous Vacuole (PV)—an intracellular compartment in which *T. gondii* reproduces asexually. Upon the formation of the PV, the third set of proteins derived from other secretory organelles, the dense granules (GRAs), along with ROPs, decorate the PV membrane [68–71]. Other ROPs and GRAs accumulate inside PV forming a tubular network of intravacuolar structures that serve various purposes: escape from host-cell aggression, inhibit phagolysosome formation, hinder intravacuolar acidification, metabolic exchange of compounds between PV and host cytoplasm, among others [69,72]. Interaction between *T. gondii* and host cell endocytic machinery is well described, however, the infection may occur through other mechanisms, such as the clathrin-mediated endocytosis or micropinocytosis [73]. These processes are essentially controlled by the host cell, revealing a successful interplay between host cell and parasite in *T. gondii* infection [70].

![Figure 2](image-url)

*Figure 2. Toxoplasma gondii* host-cell invasion and establishment of intracellular parasitic compartments [68–72]. (A) To invade the host cell, the parasite requires the secretion of proteins from two organelles, micronemes and rhoptries. These proteins allow the parasite to enter the host cell while coating itself with the host cytoplasmatic membrane, forming the Parasitophorous Vacuole (PV). (B) Upon entry, the PV is decorated with rhoptry proteins (ROPs) and proteins derived from the third set of organelles, known as dense granules (GRAs). (C) When the parasite is established inside the PV, it replicates asexually, generating a large enough number of tachyzoites capable of rupturing the host cell and infecting surrounding cells or, if in specific tissues or under certain conditions, convert into metabolically less competent bradyzoites and form tissue cysts.
**T. gondii Calcium-Dependent Protein Kinase 1**

MICs secretion is essential for parasite motility, host cell invasion, and egress and thus constitutes a potential target for drug development. In fact, the inhibition of *T. gondii* calcium-dependent protein kinase 1 (TgCDPK1), a member of the serine/threonine-protein kinase family located in the cytosol and regulates the calcium-dependent pathway, which in turn leads to MICs secretion, impairs host-cell invasion capacity [74,75]. TgCDPK1 has thus proven to be an interesting target for drug discovery. In comparison to mammalian kinases, it presents a key structural difference at the “gatekeeper residue” in the ATP-binding pocket. TgCDPK1 contains a small glycine residue, whereas human kinases possess larger residues, providing additional space for extra interactions with the target protein, which resulted in the development of potent and selective ATP-competitive TgCDPK1 inhibitors [75–77].

Several bumped kinase inhibitors (BKI) were found to selectively inhibit TgCDPK1, being 15,000-fold more active against the parasite kinase in comparison to human tyrosine kinases [43,78]. BKI-1294, a pyrazolo-pyrimidine based compound, was a promising candidate belonging to this class. Doggett et al. described an in vitro IC$_{50}$ of 140 nM, leading to a reduction in acute *T. gondii* infection by 93% when given orally, and high efficiency against established toxoplasmosis [43]. In addition, Müller et al. demonstrated excellent activity of BKI-1294 against congenital toxoplasmosis [57]. However, despite elevated *T. gondii* specificity, BKI-1294 was found to inhibit the *Ether-à-go-go-Related Gene* (hERG), which codes for the protein Kv11.1, the alpha subunit of a potassium ion channel that is essential in cardiomyocyte repolarization. Its inhibition may ultimately result in the development of life-threatening cardiac arrhythmias, such as *torsades de pointes* [44,58,79]. This occurrence halted BKI-1294 development due to the risk of cardiotoxicity. Nevertheless, Sánchez-Sánchez et al. recently demonstrated the safety and significant protection provided by BKI-1294 against abortion and vertical transmission in sheep experimentally infected with *T. gondii* during pregnancy. Thus, although BKI-1294 advancement for human toxoplasmosis ceased, the reduction of infection rates among other intermediate hosts may be a way to indirectly reduce human infection [59].

Vidadala et al. investigated BKI-1294 modifications that maintained TgCDPK1 selectivity and efficacy while reducing interference with hERG channels. The authors developed a compound (compound 32 in their series of BKIs) with an hERG IC$_{50}$ > 10 µM, an in vitro IC$_{50}$ against *T. gondii* of 60 nM, and high in vivo efficiency regarding brain parasite load when given orally. In fact, compound 32 reduced the number of brain cysts by 88.7% [44].

Recently, Rutaganira et al. tested other pyrazolo-pyrimidine inhibitors of TgCDPK1. The resulting compound (compound 24 in their series of BKIs) exhibited in vitro inhibition of the enzyme and parasite proliferation in the nanomolar and submicromolar range, respectively. In addition, in vivo assays showed this BKI analog to exhibit excellent oral bioavailability, decreased severity of acute infection, reduced cyst burden and delayed chronic reactivation of disease in immunocompromised mice. Noteworthy, compound 24 was able to completely cure some of the immunocompromised animals [45].

**4.2. Drug Targets Involved in Fatty Acid Synthesis**

The fatty acid synthesis (FAS) pathway can provide attractive approaches in *T. gondii* drug development, especially since several drug targets include enzymes absent in the host cell. As with other metabolic pathways, FAS takes place in the apicoplast of apicomplexan parasites [80,81]. Whilst *T. gondii* can effectively scavenge host cell precursors, the fatty acids produced in the apicoplast are essential for parasite development and survival [81,82]. In fact, the parasite can sense lipid availability in the surrounding environment, allowing a proper balance between *de novo* synthesis and nutrient scavenging pathways, to maintain membrane genesis, which is important for division, growth and overall survival and pathogenesis [83]. Elongation of nascent fatty acids in the FAS II pathway is a process mediated by multiple enzymes located in the endoplasmic reticulum, which are also present in bacteria and plants. In animals and fungi, the elongation process is catalyzed by the type I FAS pathway, leading to a single large multifunctional polypeptide [80,83,84]. Since
lipid synthesis is regarded as an essential process, especially during tachyzoite intracellular development, for membrane genesis and lipid homeostasis and signaling, its inhibition is of great interest in anti-\textit{T. gondii} drug discovery [83].

4.2.1. The FAS II Enzyme Enoyl-Acetyl Carrier Protein Reductase (ENR)

The FAS II apicoplast-located enzyme enoyl-acetyl carrier protein reductase (ENR) catalyzes the last step in fatty acid synthesis [46,82]. ENR is inhibited by triclosan, an antibacterial compound, which inhibits \textit{T. gondii} in vitro growth at low micromolar to nanomolar concentrations [46]. However, triclosan has poor solubility and oral bioavailability. El-Zawawy et al. described a liposomal-based delivery system for triclosan, which was able to reduce, in vivo, both tachyzoite and cyst burden. In fact, the incorporation of triclosan within the liposomes allowed the use of lower dosages, undermining possible adverse effects, whilst maintaining its antiparasitic activity [47,60]. Stec et al. later reported several promising triclosan analogs with better anti-\textit{Toxoplasma} activity than the parent compound, such as compound 16c, of this series, presenting an in vitro IC$_{50}$ of 250 nM, compared to 3 µM of triclosan. In addition, an overall improvement in pharmacokinetics was also observed. However, despite promising in vitro results, in vivo assays only showed decreased \textit{T. gondii} proliferation in mice at much higher doses (75 mg/kg) when compared to the most effective doses used in triclosan assays (10 mg/kg). Nevertheless, compound 16c was 10–fold less toxic than triclosan in vivo [61]. Overall, the promising in vitro activity and interesting pharmacokinetic profile of compound 16c makes it a potential scaffold for further development.

4.2.2. β-Ketoacyl-Acyl Carrier Protein Synthase I and II (KAS I/II)

The β-ketoacyl-acyl carrier protein synthases I and II (KAS I/II) are other drug targets belonging to the FAS II pathway and that are essential for fatty acid elongation. In fact, mutants that lack these enzymes are deficient in unsaturated fatty acids [48]. KAS I/II are specifically inhibited by thiolactomycin and its analogs. Martins-Duarte et al. determined IC$_{50}$ values between 1.6 and 29.4 µM, and electron microscopy studies of treated parasites revealed serious morphological alterations in parasite shape and intracellular organelle organization. Treatment with these compounds resulted in swollen mitochondria with disrupted structures, enlarged Golgi complex, and expanded endoplasmic reticulum throughout the whole parasite cytoplasm. The replication process (endodyogeny) was also affected, as uncompleted division processes were observed, resulting in large multinucleated parasites. These findings are indicative of parasite toxicity and death, so thiolactomycin and analogs show a clear impact in parasite development and survival [62]. However, to the best of our knowledge, in vivo testing of these compounds has not yet been reported.

4.2.3. Pantothenate Synthetase

\textit{T. gondii} is capable of synthesizing FAS precursors outside the FAS II pathway, such as pantothenate, a coenzyme A precursor. The parasite’s pantothenate pathway includes the terminal enzyme pantothenate synthetase that converts pantoate to pantothenate. Unlike humans, \textit{T. gondii} does not require an external source of pantothenate [49]. Host cell conversion of pantothenate to coenzyme A is rather rapid, preventing \textit{T. gondii} from scavenging the precursor from the host cell [27]. Thus, de novo pantothenic acid synthesis can be an attractive target for drug discovery, as it avoids interference with host cell FAS. Mageed et al. tested several acylsulfonamides, originally developed to target \textit{Mycobacterium tuberculosis} pantothenate synthetase. In order to assess parasite growth inhibition, the commonly used drug pyrimethamine served as a positive control. Compounds SW413 and SW404 of this series demonstrated in vitro IC$_{50}$ values of 20 and 130 nM, respectively, with median toxic doses (TD$_{50}$) above 1000 µM in human foreskin fibroblasts (HFF). These inhibitors were at least as potent as pyrimethamine regarding parasite growth inhibition. To infer whether pantothenate synthetase is the possible target of the compounds, the
EC\textsubscript{50} values were assessed in the presence and absence of supplemental pantothenate. The addition of pantothenate increased the EC\textsubscript{50} values of both SW413 and SW404, indicating that \textit{de novo} pantothenate synthesis is essential for \textit{T. gondii} survival and can be effectively targeted [49]. Regardless, in vivo experiments are needed to examine the efficacy of these candidates in animals.

4.3. Drug Targets Involved in DNA Expression

\textit{T. gondii} is capable of rapidly differentiating between the active tachyzoite stage and the slow-growing bradyzoite stage. This tachyzoite–bradyzoite interconversion process requires the expression and modulation of stage-specific genes. This modulation may be performed through epigenetic mechanisms, using a post-translational modification (PTM) of histone proteins. PTMs include acetylation or deacetylation of histone residues [85,86]. Acetylation of conserved histone lysine residues by histone acetyltransferases (HATs) generates PTMs that generally lead to increased target gene expression. Instead, deacetylation of these residues by histone deacetylases (HDACs) removes the modification, resulting in decreased target gene expression [50,51]. HATs and HDACs that target stage-specific genes contribute greatly to parasite interconversion between tachyzoite and bradyzoite stages. Modulation of gene expression in this regard may contribute greatly towards the development of treatment options for chronic toxoplasmosis. Preventing bradyzoite differentiation may help to avoid chronic infection, whereas preventing tachyzoite conversion may avoid prompt reactivation of \textit{T. gondii} acute infection in immunocompromised patients [38]. In addition, this PTM is also present in other drug targets, including several proteins involved in DNA repair, including the chaperone Hsp90 and the ATM serine/threonine-protein kinase [87]. Therefore, epigenetic regulation of gene expression can be considered an attractive idea for \textit{T. gondii} drug development.

Histone Deacetylase Enzyme TgHDAC3

Histone deacetylase enzyme TgHDAC3 proved to be an effective drug target for \textit{T. gondii} inhibition [52]. The cyclopeptide FR235222 targets TgHDAC3, causing hyper-acetylation of histone H4 in \textit{T. gondii}. The compound demonstrated in vitro inhibition of intracellular parasite growth with an IC\textsubscript{50} of 9.7 nM, induced in vitro bradyzoite conversion, and was able to reach bradyzoites within ex vivo cysts, preventing tachyzoite conversion. This promising result was further confirmed as FR235222-pretreated bradyzoites were found to be incapable of infecting HFF monolayers, and unable to cause toxoplasmosis in the mouse model. However, host cell toxicity was observed in FR235222 treatment, causing HFF cell inhibition at an IC\textsubscript{50} of 128 nM. Further development of FR235222 analogs led to W363 and W399, which demonstrated higher parasite selectivity in comparison to the parent compound while maintaining equivalent parasite IC\textsubscript{50} values in vitro [53]. The efficacy of these three compounds in chronically infected mice remains to be described.

4.4. Drug Targets Involved in Mitochondrial Electron Transport Pathway

Mitochondrial Cytochrome bc1 Complex

The cytochrome bc1 complex (bc1), present in the mitochondrial electron transport chain, is a drug target for several apicomplexan parasitic infections, including toxoplasmosis, malaria and babesiosis. Mitochondrial bc1 inhibitors bind to the hydroquinone oxidation (Qo) or quinone reduction (Qi) site of this complex, hindering cell respiration by inhibition of the electron transport pathway [27]. Atovaquone, which is clinically available for alternate treatment and prophylaxis of toxoplasmosis, as well as malaria and babesiosis, is a well-described Qo-site inhibitor. However, due to mutations in the target binding site, the development of resistance limited its use in toxoplasmosis [88].

Endochin-like quinolones (ELQ), which are 4-(1H)-quinolone derivatives, target the Qi site of bc1. The most promising compounds, ELQ-271 and ELQ-316, effectively exhibited low IC\textsubscript{50} values for parasite growth inhibition, such as 0.100 and 0.007 nM, respectively. ELQ-271 inhibited human bc1 at 800 nM and ELQ-316 did not show human bc1 inhibi-
tion and was not toxic to HFF or human hepatocarcinoma cells (HepG2) at the highest concentration tested (10 µM). A reduction in the number of brain cysts by 88% in mice treated five weeks after infection with an ME49 T. gondii strain (type II strain) was also verified [54–56,63]. Due to the broad anti-apicomplexan activity of ELQ-316, combination with atovaquone was suggested to overcome the resistance issues associated with the latter [27]. Recently, McConnell et al. identified the compound ELQ-400 (also known as MMV671636, from Neospora caninum assays) as a promising candidate, capable of inhibiting both Qo and Qi sites of bc1 due to its structural flexibility and favorable substitution pattern. ELQ-400 decreased acute infection with a lethal type I strain of T. gondii in mice. In this assay, all mice survived and presented no signs of infection. ELQ-400 and ELQ-316 were simultaneously evaluated in vivo. Results indicate that both compounds are remarkably effective in decreasing infection in mice, however, they differ in tissue distribution and ability to prevent T. gondii from accessing the brain tissue, due to their distinct blood-brain barrier penetration capacity and half-life. In this regard, ELQ-400 was more effective in preventing the parasite from reaching the brain. Therefore, this compound is thought to effectively act on both acute and chronic phases of T. gondii infection [56,64].

5. Drug Repurposing Approach

Drug repurposing is a strategy for identifying new clinical uses for existing drugs with specific therapeutic indications [89]. This process is becoming an interesting strategy for drug discovery, as it involves potentially lower financial costs in drug development as well as shorter timelines [90].

The Medicines for Malaria Venture (MMV) foundation aims to reduce the burden of malaria by developing and facilitating access to new drug candidates. Thus, an open-access compound library called Malaria Box was made available for academics worldwide, with the purpose of identifying novel bioactive compounds against various pathogens. This library contained 400 blood-stage active anti-Plasmodium compounds. The screening led to the identification of seven potent anti-T. gondii compounds. Among these, the most potent and selective was the piperazine acetamide MMV007791 (compounds provided by the MMV foundation are generally designated by their MMV identifier codes) [91]. In 2015, MMV launched a novel open-access library, modeled after the Malaria Box, known as the Pathogen Box. It consists of 400 drug-like small molecules, contained in 96-well plates, with confirmed bioactivity against at least one of the following pathogens: Plasmodium, Mycobacterium, Kinetoplastids (such as Trypanosoma), Schistosoma, Cryptosporidium, helminths, and dengue virus. Eventually, since T. gondii is an apicomplexan parasite and morphologically similar to some of the parasites contained in this list, many compounds were found to have activity against this protozoan and even shed new insights into its biological pathways [64,65,92,93]. Spalenka et al. screened all 400 compounds for anti-T. gondii activity. Fifteen compounds demonstrated desired efficacy, of which eight presented selectivity and favorable in vitro effects on tachyzoite proliferation. The most active compound, MMV675968, is a diaminoquinazoline with known activity against the enzyme DHFR of Cryptosporidium, potentially targeting the same enzyme in T. gondii. This hypothesis was later assessed, and the compound exhibited an IC₅₀ of 0.02 µM and high selectivity towards parasite DHFR enzyme. Buparvaquone (MMV689480), a well-known hydroxynaphthoquinone with described activity against N. caninum, by inhibiting several enzymes involved in the mitochondrial electron transport pathway, was also identified as having good in vitro anti-T. gondii activity, with an IC₅₀ of 0.10 µM [65].

Recently, Murata et al. screened a chemical compound library, provided by the Drug Discovery Initiative from the University of Tokyo, and two promising compounds with anti-T. gondii activity were identified: tanshinone IIA, a compound with potential cancer cell growth inhibition; and hydroxyzine, a well-known first-generation antihistamine drug. T. gondii targets and mode of action are currently not known for these compounds, however, they were identified as effective in vitro inhibitors of tachyzoite growth, with reduced host cell toxicity. Moreover, tanshinone IIA and hydroxyzine showed inhibitory effects on the
growth of bradyzoites. Thus, data indicate that these compounds may represent novel lead compounds to treat acute toxoplasmosis as well as preventing reactivation of latent infection, particularly in immunocompromised individuals [66]. However, in vivo efficacy remains to be reported.

6. Concluding Remarks

Toxoplasmosis is widely distributed worldwide, and the current chemotherapy lacks efficacy and safety. Clinically available options are associated with a relevant spectrum of adverse side effects and generally induce poor compliance within patients. Drug discovery has developed enormously in the last decade, with several drug candidates showing promising results both in vitro and in vivo. This review underlines several promising compounds, drug targets and strategies for anti-\(T. gondii\) drug development. Molecular modifications play an important role in this regard, with the aim of improving pharmacokinetic characteristics, including blood–brain barrier access, bioavailability, and half-life. The use of liposomal nanoparticles may also be applied in drugs with promising in vitro results that lack the necessary pharmacokinetic profile. Epigenetics and modulation of gene expression offer vast possibilities in drug discovery, as \(T. gondii\) provides a unique HAT/HDAC system that allows various explorative strategies. Drug repurposing has been used for several decades in many different diseases and should be continuously explored.

It is of great importance to invest in the study of novel potent drug candidates. Compounds exhibiting fewer and milder side effects, being overall better tolerated in pregnant women and newborns, specifically, in comparison to current chemotherapy, should be prioritized. Ideally, candidates should be capable of acting on both acute replicating tachyzoite and latent bradyzoite stages, preventing acute disease and reactivation, and even allowing resolution of chronic infection. In addition, the drugs should be bioavailable, capable of reaching therapeutic concentration in all target tissues, such as the brain and eye, as well as concentrate appropriately in the placenta and fetal compartment, while avoiding the generation of drug-resistant strains. Finally, drug costs should be affordable, providing treatment in all world regions. The development of such drugs would revolutionize current \(T. gondii\) chemotherapy.

Author Contributions: M.d.S. and M.B. wrote the initial manuscript. M.d.S., M.B., C.T. and P.G. contributed to the article and approved the submitted version. All authors agreed to the published version of the manuscript.

Funding: This research was funded by Fundação para a Ciência e Tecnologia (FCT), Portugal, through projects UIDB/50006/2020, and PTDC/BTM-SAL/29786/2017. This work is financed by national funds from FCT-Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences-UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy-i4HB.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Lindsay, D.S.; Dubey, J.P. Chapter 6—Toxoplasmosis in wild and domestic animals. In Toxoplasma Gondii, 3rd ed.; Weiss, L.M., Kim, K., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 293–320.
2. Montazeri, M.; Mikaeili Galeh, T.; Moosazadeh, M.; Sarvi, S.; Dodangeh, S.; Javidnia, J.; Sharif, M.; Daryani, A. The global serological prevalence of Toxoplasma gondii in felids during the last five decades (1967–2017): A systematic review and meta-analysis. Parasit. Vectors 2020, 13, 82. [CrossRef] [PubMed]
3. Molan, A.; Nosaka, K.; Wang, W.; Hunter, M. Global status of Toxoplasma gondii infection: Systematic review and prevalence snapshots. Trop. Biomed. 2019, 36, 898–925. [PubMed]
4. Pittman, K.J.; Knoll, L.J. Long-Term Relationships: The Complicated Interplay between the Host and the Developmental Stages of Toxoplasma gondii during Acute and Chronic Infections. *Microbiol. Mol. Biol. Rev.* 2015, 79, 387–401. [CrossRef] [PubMed]

5. Saeij, J.P.; Boyle, J.P.; Boothroyd, J.C. Differences among the three major strains of Toxoplasma gondii and their specific interactions with the infected host. *Trends Parasitol.* 2005, 21, 476–481. [CrossRef]

6. Xiao, J.; Yolken, R.H. Strain hypothesis of Toxoplasma gondii infection on the outcome of human diseases. *Acta Physiol.* 2015, 213, 828–845. [CrossRef]

7. Kojchanowski, J.A.; Koshy, A.A. Toxoplasma gondii. *Curr. Biol.* 2018, 28, R770–R771. [CrossRef]

8. Paris, L. 106—Toxoplasmosis. In *Hunter’s Tropical Medicine and Emerging Infectious Diseases*, 10th ed.; Ryan, E.T., Hill, D.R., Solomon, T., Aronson, N.E., Endy, T.P., Eds.; Content Repository Only: London, UK, 2020; pp. 803–813.

9. Flegr, J.; Escudero, D.Q. Impaired health status and increased incidence of diseases in Toxoplasma-seropositive subjects—An explorative cross-sectional study. *Parasitology 2016*, 143, 1974–1989. [CrossRef]

10. Wang, Z.-D.; Liu, H.-H.; Ma, Z.-X.; Ma, H.-Y.; Li, Z.-Y.; Yang, Z.-B.; Zhu, X.-Q.; Xu, B.; Wei, F.; Liu, Q. Toxoplasma gondii Infection in Immunocompromised Patients: A Systematic Review and Meta-Analysis. *Front. Microbiol.* 2017, 8, 389. [CrossRef]

11. McAuley, J.B. Congenital Toxoplasmosis. *J. Pediatric Infect. Dis. Soc.* 2017, 4, 3 (Suppl. S1), S30–S35. [CrossRef]

12. Foulon, W.; Villena, I.; Stray-Pedersen, B.; Decoster, A.; Lappalainen, M.; Pinon, J.M.; Jenum, P.A.; Hedman, K.; Naessens, A. Treatment of toxoplasmosis during pregnancy: A multicenter study of impact on fetal transmission and children’s sequelae at age 1 year. *Am. J. Obs. Gynaecol.* 1999, 180, 410–415. [CrossRef]

13. Kieffer, F.; Wallon, M. Congenital toxoplasmosis. *Handb. Clin. Neurol.* 2013, 112, 1099–1101. [CrossRef]

14. Robbins, J.R.; Zeldovich, V.B.; Poukhanzki, A.; Boothroyd, J.C.; Bakardjiev, A.I. Tissue barriers of the human placenta to infection with Toxoplasma gondii. *Infect. Immun.* 2012, 80, 418–428. [CrossRef]

15. Ahmed, M.; Sood, A.; Gupta, J. Toxoplasmosis in pregnancy. *Eur J. Obs. Gynecol. Reprod. Biol.* 2020, 255, 44–50. [CrossRef] [PubMed]

16. Borges, M.; Magalhaes Silva, T.; Brito, C.; Teixeira, N.; Roberts, C.W. How does toxoplasmosis affect the maternal-foetal immune interface and pregnancy? *Parasite Immunol.* 2019, 41, e12606. [CrossRef] [PubMed]

17. Garweg, J.G.; Stanford, M.R. Therapy for ocular toxoplasmosis—The future. *Ocul. Immunol. Inflamm.* 2013, 21, 300–305. [CrossRef] [PubMed]

18. Khan, K.; Khan, W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. *Parasitol. Int.* 2018, 67, 715–721. [CrossRef]

19. Burgdorf, K.S.; Trabjerg, B.B.; Pedersen, M.G.; Nissen, J.; Banasik, K.; Pedersen, O.B.; Sørensen, E.; Nielsen, K.R.; Larsen, M.H.; Erikstrup, C.; et al. Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. *Brain Behav. Immun.* 2019, 79, 152–158. [CrossRef]

20. Fond, G.; Capdevielle, D.; Macgregor, A.; Attal, J.; Larue, A.; Brittner, M.; Ducasse, D.; Boulenger, J.P. Toxoplasma gondii: A potential role in the genesis of psychiatric disorders. *L’Encéphale* 2013, 39, 38–43. [CrossRef]

21. Yalin Sapmaz, Ş.; Şen, S.; Ozkan, Y.; Kandemir, H. Relationship between Toxoplasma gondii seropositivity and depression in children and adolescents. *Psychiatry Res.* 2019, 278, 263–267. [CrossRef] [PubMed]

22. Fuglewicz, A.J.; Piotrowski, P.; Stodolak, A. Relationship between toxoplasmosis and schizophrenia: A review. *Adv. Clin. Exp. Med.* 2017, 26, 1031–1036. [CrossRef]

23. Khademvatan, S.; Saki, J.; Khajeddin, N.; Izadi-Mazidi, M.; Beladi, R.; Shafiee, B.; Salehi, Z. Toxoplasma gondii Exposure and the Risk of Schizophrenia. *Jundishapur J. Microbiol.* 2014, 7, e12776. [CrossRef] [PubMed]

24. Sutterland, A.L.; Fond, G.; Kuin, A.; Koeter, M.W.J.; Lutter, R.; van Gool, T.; Yolken, R.; Szoke, A.; Leboyer, M.; de Haan, L. Beyond the association. Toxoplasma gondii in schizophrenia, bipolar disorder, and addiction: Systematic review and meta-analysis. *Acta Psychiatr. Scand.* 2015, 132, 161–179. [CrossRef]

25. Dunay, I.R.; Gajurel, K.; Dhakal, R.; Liesenfeld, O.; Montoya, J.G. Treatment of Toxoplasmosis: Historical Perspective, Animal Models, and Current Clinical Practice. *Clin. Microbiol. Rev.* 2018, 31. [CrossRef] [PubMed]

26. Montoya, J.G.; Remington, J.S. Management of Toxoplasma gondii infection during pregnancy. *Clin. Infect. Dis.* 2008, 47, 554–566. [CrossRef] [PubMed]

27. Falcón, V.C.; Turró, I.; Pons, J.; et al. Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. *Brain Behav. Immun.* 2019, 79, 152–158. [CrossRef]

28. Burgdorf, K.S.; Trabjerg, B.B.; Pedersen, M.G.; Nissen, J.; Banasik, K.; Pedersen, O.B.; Sørensen, E.; Nielsen, K.R.; Larsen, M.H.; Erikstrup, C.; et al. Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. *Brain Behav. Immun.* 2019, 79, 152–158. [CrossRef]

29. Ardabili, S.; Kohl, J.; Gül, G.; Hodel, M. What obstetricians should be aware of: Serious side effects of antibiotic toxoplasmosis treatment in pregnancy. *BMJ Case Rep.* 2021, 14, e240809. [CrossRef] [PubMed]

30. Ben-Harari, R.R.; Goodwin, E.; Casoy, J. Adverse Event Profile of Pyrimethamine-Based Therapy in Toxoplasmosis: A Systematic Review. *Drugs R D* 2017, 17, 523–544. [CrossRef]

31. Borkowski, P.K.; Brydak-Godowska, J.; Basiak, W.; Olszyńska-Krowicka, M.; Rabczenko, D. Adverse Reactions in Antifolate-Treated Toxoplasmic Retinochoroiditis. *Adv. Exp. Med. Biol.* 2018, 1108, 37–48. [CrossRef]

32. Guaraldo, L.; Villar, B.P.F.; Durão, N.M.G.; Louro, V.C.; Quintana, M.S.B.; Curi, A.L.L.; Neves, E.S. Ocular toxoplasmosis: Adverse reactions to treatment in a Brazilian cohort. *Trans. R Soc. Trop. Med. Hyg.* 2018, 112, 188–192. [CrossRef]

33. Iaccheri, B.; Fiore, T.; Papadaki, T.; Androudi, S.; Foster, C. Adverse Reactions with Antitoxoplasma Therapy. *Investig. Ophthalmol. Vis. Sci.* 2003, 44, 1415.
34. Shammaa, A.M.; Powell, T.G.; Benmerzoug, I. Adverse outcomes associated with the treatment of Toxoplasma infections. Sci. Rep. 2021, 11, 1035. [CrossRef] [PubMed]

35. Rajapakse, S.; Chiranan Shivanthan, M.; Samaranayake, N.; Rodrigo, C.; Deepika Fernando, S. Antibiotics for human toxoplasmosis: A systematic review of randomized trials. Pathog. Glob. Health 2013, 107, 162–169. [CrossRef]

36. Paquet, C.; Yudin, M.H. No. 285-Toxoplasmosis in Pregnancy: Prevention, Screening, and Treatment. J. Obs. Gynaecol. Can. 2018, 40, e687–e693. [CrossRef] [PubMed]

37. Dexamethasone for Cerebral Toxoplasmosis-ClinicalTrials.gov Identifier: NCT04341155. Available online: https://ClinicalTrials.gov/show/NCT04341155 (accessed on 6 September 2021).

38. Konstantinovic, N.; Guegan, H.; Stajner, T.; Belaz, S.; Robert-Gangneux, F. Treatment of toxoplasmosis: Current options and future perspectives. Food Waterborne Parasit. 2019, 15, e00306. [CrossRef]

39. Doliwa, C.; Escotte-Binet, S.; Aubert, D.; Velard, F.; Schmid, A.; Geers, R.; Villena, I. Induction of sulfadiazine resistance in vitro in Toxoplasma gondii. Exp. Parasitol. 2013, 133, 131–136. [CrossRef]

40. Meneceur, P.; Boudouyre, M.A.; Aubert, D.; Villena, I.; Menotti, J.; Sauvage, V.; Garin, J.F.; Derouin, F. In vitro susceptibility of various genotypic strains of Toxoplasma gondii to pyrimethamine, sulfadiazine, and atovaquone. Antimicrob. Agents Chemother. 2008, 52, 1269–1277. [CrossRef]

41. Silva, L.A.; Reis-Cunha, J.L.; Bartholomeu, D.C.; Vitor, R.W.A. Genetic Polymorphisms and Phenotypic Profiles of Sulfadiazine-Resistant and Sensitive Toxoplasma gondii Isolates Obtained from Newborns with Congenital Toxoplasmosis in Minas Gerais, Brazil. PLoS ONE 2017, 12, e0170689. [CrossRef]

42. Barros, M.; Teixeira, D.; Vilanova, M.; Correia, A.; Teixeira, N.; Borges, M. Vaccines in Congenital Toxoplasmosis: Advances and Perspectives. Front. Immunol. 2021, 11, 3900. [CrossRef]

43. Doggett, J.S.; Ojo, K.K.; Fan, E.; Maly, D.J.; van Voorhis, W.C. Bumped Kinase Inhibitor 1294 Treats Established Toxoplasma gondii. Antimicrob. Agents Chemother. 2014, 58, 3547–3549. [CrossRef]

44. Vidadala, R.S.; Rivas, K.L.; Ojo, K.K.; Hulverson, M.A.; Zambriski, J.A.; Bruzual, I.; Schultz, T.L.; Huang, W.; Zhang, Z.; Scheele, S.; et al. Development of an Orally Available and Central Nervous System (CNS) Penetrant Toxoplasma gondii Calcium-Dependent Protein Kinase 1 (TgCDPK1) Inhibitor with Minimal Human Ether-a-go-go-Related Gene (hERG) Activity for the Treatment of Toxoplasmosis. J. Med. Chem. 2016, 59, 6531–6546. [CrossRef]

45. Rutaganira, F.U.; Barks, J.; Dhabson, M.S.; Wang, Q.; Lopez, M.S.; Long, S.; Radke, J.B.; Jones, N.G.; Maddirala, A.R.; Janetka, J.W.; et al. Inhibition of Calcium Dependent Protein Kinase 1 (CDPK1) by Pyrazolopyrimidine Analogs Decreases Establishment and Reoccurrence of Central Nervous System Disease by Toxoplasma gondii. J. Med. Chem. 2017, 60, 9976–9989. [CrossRef]

46. McLeod, R.; Muench, S.P.; Rafferty, J.B.; Kyle, D.E.; Mui, E.J.; Kirisits, M.J.; Mack, D.G.; Roberts, C.W.; Samuel, B.U.; Lyons, R.E.; et al. Triclosan inhibits the growth of Plasmodium falciparum and Toxoplasma gondii by inhibition of apicomplexan Fab I. Int. J. Parasitol. 2001, 31, 109–113. [CrossRef]

47. El-Zawawy, L.A.; El-Said, D.; Mossallam, S.F.; Ramadan, H.S.; Younis, S.S. Triclosan and triclosan-loaded liposomal nanoparticles in the treatment of acute experimental toxoplasmosis. Exp. Parasitol. 2015, 149, 54–64. [CrossRef] [PubMed]

48. Jackowski, S.; Murphy, C.M.; Cronan, J.E., Jr.; Rock, C.O. Acetoacetyl-acyl carrier protein synthase. A target for the antibiotic thiolactomycin. J. Biol. Chem. 1989, 264, 7624–7629. [CrossRef]

49. Mageed, S.N.; Cunningham, F.; Hung, A.W.; Silvestre, H.L.; Wen, S.; Blundell, T.L.; Abell, C.; McConkey, G.A. Pantothenic Acid Biosynthesis in the Parasite Toxoplasma gondii: A Target for Chemotherapy. Antimicrob. Agents Chemother. 2014, 58, 6345–6353. [CrossRef] [PubMed]

50. Andrews, K.T.; Haque, A.; Jones, M.K. HDAC inhibitors in parasitic diseases. Immuno. Cell Biol. 2012, 90, 66–77. [CrossRef]

51. Vanagas, L.; Jeffers, V.; Bogado, S.S.; Dalmasso, M.C.; Sullivan, W.J., Jr; Angel, S.O. Toxoplasma histone acetylation remodelers as novel drug targets. Expert Rev. Anti-Infect. 2012, 10, 1189–1201. [CrossRef]

52. Bougdour, A.; Maubon, D.; Baldacci, P.; Ortet, P.; Bastien, O.; Bouillon, A.; Barale, J.-C.; Pelloux, H.; Ménard, R.; Hakimi, M.-A. Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. J. Exp. Med. 2009, 206, 953–966. [CrossRef]

53. Maubon, D.; Bougdour, A.; Wong, Y.-S.; Brenier-Pinchart, M.-P.; Curt, A.; Hakimi, M.-A.; Pelloux, H. Activity of the Histone Deacetylase Inhibitor FR235222 on Toxoplasma gondii: Inhibition of Stage Conversion of the Parasite Cyst Form and Study of New Derivative Compounds. Antimicrob. Agents Chemother. 2010, 54, 4843–4850. [CrossRef]

54. Alday, P.H., Jr; Bruzal, I.; Pou, S.; Nilsen, A.; Riscoe, M.K.; Doggett, J. Mechanism of Action of ELQ-316 Against Toxoplasma gondii: Evidence for Qi Site Inhibition of Cytochrome bc1. Open Forum Infect. Dis. 2016, 3. [CrossRef]

55. Alday, P.H.; Bruzual, I.; Nilsen, A.; Pou, S.; Winter, R.; Ben Mamoun, C.; Riscoe, M.K.; Doggett, J.S. Genetic Evidence for Cytochrome bc Qi Site Inhibition by 4(1H)-Quinolone-3-Diarylethers and Antimycin in Toxoplasma gondii. Antimicrob. Agents Chemother. 2017, 61, e01866-16. [CrossRef]

56. McConnell, E.V.; Bruzual, I.; Pou, S.; Winter, R.; Dodean, R.A.; Smilstein, M.J.; Krollenbrock, A.; Nilsen, A.; Zakharov, L.N.; Riscoe, M.K.; et al. Targeted Structure-Activity Analysis of Endochin-like Quinolones Reveals Potent Qi and Qo Site Inhibitors of Toxoplasma gondii and Plasmodium falciparum Cytochrome bc(1) and Identifies ELQ-400 as a Remarkably Effective Compound against Acute Experimental Toxoplasmosis. ACS Infect. Dis. 2018, 4, 1574–1584. [CrossRef]
57. Müller, J.; Aguado-Martínez, A.; Ortega-Mora, L.-M.; Moreno-Gonzalo, J.; Ferre, I.; Hulverson, M.A.; Choi, R.; McCloskey, M.C.; Barrett, L.K.; Maly, D.J.; et al. Development of a murine vertical transmission model for Toxoplasma gondii oocyst infection and studies on the efficacy of bumped kinase inhibitor (BKI)-1294 and the napthoquinone buparvaquone against congenital toxoplasmosis. *J. Antimicrob. Chemother.* 2017, 72, 2334–2341. [CrossRef]

58. Schaefer, D.A.; Betzer, D.P.; Smith, K.D.; Millman, Z.G.; Michalski, H.C.; Menchaca, S.E.; Zambriski, J.A.; Ojo, K.K.; Hulverson, M.A.; Arnold, S.L.M.; et al. Novel Bumped Kinase Inhibitors Are Safe and Effective Therapeutics in the Calf Clinical Model for Cryptosporidiosis. *J. Infect. Dis.* 2016, 214, 1856–1864. [CrossRef] [PubMed]

59. Sánchez-Sánchez, R.; Ferre, I.; Re, M.; Ramos, J.I.; Regidor-Cerrillo, J.; Pizarro Díaz, M.; González-Huecas, M.; Tabanera, E.; Benavides, J.; Hemphill, A.; et al. Treatment with Bumped Kinase Inhibitor 1294 Is Safe and Leads to Significant Protection against Abortion and Vertical Transmission in Sheep Experimentally Infected with *Toxoplasma gondii* during Pregnancy. *Antimicrob. Agents Chemother.* 2019, 63, e02527-18. [CrossRef] [PubMed]

60. El-Zawawy, L.A.; El-Said, D.; Mossallam, S.F.; Ramadan, H.S.; Younis, S.S. Preventive prospective of triclosan and triclosan-liposomal nanoparticles against experimental infection with a cystogenic ME49 strain of Toxoplasma gondii. *Acta Trop.* 2015, 141, 103–111. [CrossRef]

61. Stec, J.; Fomovska, A.; Afanador, G.A.; Muensch, S.P.; Zhou, Y.; Lai, B.-S.; El Bissati, K.; Hickman, M.R.; Lee, P.J.; Leed, S.E.; et al. Modification of triclosan scaffold in search of improved inhibitors for enoyl-acyl carrier protein (ACP) reductase in Toxoplasma gondii. *ChemMedChem* 2013, 8, 1138–1160. [CrossRef]

62. Martins-Duarte, E.S.; Jones, S.M.; Gilbert, I.H.; Atella, G.C.; de Souza, W.; Vommaro, R.C. Thiolactomycin analogues as potential anti-Toxoplasma gondii agents. *Parasitol. Int.* 2009, 58, 411–415. [CrossRef]

63. Doggett, J.S.; Nilsen, A.; Forquer, I.; Wegmann, K.W.; Jones-Brando, L.; Yolken, R.H.; Bordón, C.; Charman, S.A.; Katneni, K.; Schultz, T.; et al. Endochin-like quinolones are highly efficacious against acute and latent experimental toxoplasmosis. *Proc. Natl. Acad. Sci. USA* 2012, 109, 15936–15941. [CrossRef] [PubMed]

64. Secrèrieu, A.; Costa, I.C.C.; O'Neill, P.M.; Cristiano, M.L.S. Antimalarial Agents as Therapeutic Tools Against Toxoplasmosis-A Short Bridge between Two Distant Illnesses. *Molecules* 2020, 25, 1574. [CrossRef]

65. Spalenga, J.; Escotte-Binet, S.; Bakiri, A.; Hubert, J.; Renault, J.-H.; Velard, F.; Duchateau, S.; Aubert, D.; Huguenin, A.; Villena, I. Discovery of New Inhibitors of Toxoplasma gondii via the Pathogen Box. *Antimicrob. Agents Chemother.* 2018, 62, e01640-17. [CrossRef]

66. Murata, Y.; Sugi, T.; Weiss, L.M.; Kato, K. Identification of compounds that suppress Toxoplasma gondii tachyzoites and bradyzoites. *PLoS ONE* 2012, 7, e0178203. [CrossRef]

67. Seebor, F.; Steinfelder, S. Recent advances in understanding apicomplexan parasites. *F1000Research* 2016, 5, 1369. [CrossRef] [PubMed]

68. Dubremetz, J.F.; Garcia-Réguet, N.; Conseil, V.; Fourmaux, M.N. Apical organelles and host-cell invasion by Apicomplexa. *Int. J. Parasitol.* 1998, 28, 1007–1013. [CrossRef]

69. Lüder, C.G.; Stanway, R.R.; Chaussepied, M.; Langsley, G.; Heussler, V.T. Intracellular survival of apicomplexan parasites and host cell modification. *Int. J. Parasitol.* 2009, 39, 163–173. [CrossRef]

70. Portes, J.; Barrias, E.; Travisso, R.; Attias, M.; de Souza, W. Toxoplasma gondii Mechanisms of Entry Into Host Cells. *Front. Cell Infect. Microbiol.* 2020, 10, 294. [CrossRef]

71. Portes, J.A.; de Souza, W. Development of an in vitro system to study the developmental stages of Toxoplasma gondii using a genetically modified strain expressing markers for tachyzoites and bradyzoites. *Parasitol. Res.* 2019, 118, 3479–3489. [CrossRef] [PubMed]

72. Plattner, F.; Soldati-Favre, D. Hijacking of host cellular functions by the Apicomplexa. *Annu. Rev. Microbiol.* 2008, 62, 471–487. [CrossRef] [PubMed]

73. Mayor, S.; Parton, R.G.; Donaldson, J.G. Clathrin-independent pathways of endocytosis. *Cold Spring Harb. Perspect. Biol.* 2014, 6, a016758. [CrossRef]

74. Cardew, E.M.; Verlinde, C.L.M.J.; Pohl, E. The calcium-dependent protein kinase 1 from Toxoplasma gondii as target for structure-based drug design. *Parasitology* 2018, 145, 210–218. [CrossRef]

75. Ojo, K.K.; Larson, E.T.; Keyloun, K.R.; Castaneda, L.J.; DeRoche, A.E.; Inamudi, K.K.; Kim, J.E.; Arakaki, T.L.; Murphy, R.C.; Zhang, L.; et al. Toxoplasma gondii calcium-dependent protein kinase 1 is a target for selective kinase inhibitors. *Nat. Struct. Mol. Biol.* 2010, 17, 602–607. [CrossRef]

76. Lourido, S.; Zhang, C.; Lopez, M.S.; Tang, K.; Barks, J.; Wang, Q.; Wildman, S.A.; Shokat, K.M.; Sibley, L.D. Optimizing small molecule inhibitors of calcium-dependent protein kinase 1 to prevent infection by Toxoplasma gondii. *J. Med. Chem.* 2013, 56, 3068–3077. [CrossRef] [PubMed]

77. Murphy, R.C.; Ojo, K.K.; Larson, E.T.; Castellanos-Gonzalez, A.; Perera, B.G.K.; Keyloun, K.R.; Kim, J.E.; Bhandari, J.G.; Muller, N.R.; Verlinde, C.L.M.J.; et al. Discovery of Potent and Selective Inhibitors of CDPK1 from C. parvum and T. gondii. *ACS Med. Chem. Lett.* 2010, 1, 331–335. [CrossRef] [PubMed]

78. Winzer, P.; Müller, J.; Aguado-Martínez, A.; Rahman, M.; Balmer, V.; Manser, V.; Ortega-Mora, L.M.; Ojo, K.K.; Fan, E.; Maly, D.J.; et al. In Vitro and In Vivo Effects of the Bumped Kinase Inhibitor 1294 in the Related Cyst-Forming Apicomplexans *Toxoplasma gondii* and *Neospora caninum*. *Antimicrob. Agents Chemother.* 2015, 59, 6361–6374. [CrossRef] [PubMed]
79. Vandenberg, J.I.; Perry, M.D.; Perrin, M.J.; Mann, S.A.; Ke, Y.; Hill, A.P. hERG K(+) channels: Structure, function, and clinical significance. *Physiol. Rev.* 2012, 92, 1393–1478. [CrossRef]

80. Mazumdar, J.; Wilson, E.H.; Masek, K.; Hunter, C.A.; Striepen, B. Apicoplast fatty acid synthesis is essential for organelle biogenesis and parasite survival in *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. USA* 2006, 103, 13192–13197. [CrossRef]

81. Mazumdar, J.; Wilson, E.H.; Masek, K.; Hunter, C.A.; Striepen, B. Apicoplast fatty acid synthesis is essential for organelle biogenesis and parasite survival in *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. USA* 2006, 103, 13192–13197. [CrossRef]

82. Ramakrishnan, S.; Docampo, M.D.; MacRae, J.I.; Ralton, J.E.; Rupasinghe, T.; McConville, M.J.; Striepen, B. The intracellular parasite *Toxoplasma gondii* depends on the synthesis of long-chain and very long-chain unsaturated fatty acids not supplied by the host cell. *Mol. Microbiol.* 2015, 97, 64–76. [CrossRef]

83. Dubois, D.; Fernandes, S.; Amiar, S.; Dass, S.; Katris, N.J.; Botté, C.Y.; Yamaryo-Botté, Y. *Toxoplasma gondii* acetyl-CoA synthetase is involved in fatty acid elongation (of long fatty acid chains) during tachyzoite life stages. *J. Lipid Res.* 2018, 59, 994–1004. [CrossRef]

84. Ramakrishnan, S.; Docampo, M.D.; MacRae, J.I.; Pujol, F.M.; Brooks, C.F.; van Dooren, G.G.; Hiltunen, J.K.; Kastaniotis, A.J.; McConville, M.J.; Striepen, B. Apicoplast and Endoplasmic Reticulum Cooperate in Fatty Acid Biosynthesis in Apicomplexan Parasite *Toxoplasma gondii*. *J. Biol. Chem.* 2012, 287, 4957–4971. [CrossRef]

85. Sullivan, W.J., Jr.; Hakimi, M.A. Histone mediated gene activation in *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* 2006, 148, 109–116. [CrossRef] [PubMed]

86. Sullivan, W.J., Jr.; Jeffers, V. Mechanisms of *Toxoplasma gondii* persistence and latency. *FEMS Microbiol. Rev.* 2012, 36, 717–733. [CrossRef] [PubMed]

87. Angel, S.O.; Vanagas, L.; Ruiz, D.M.; Cristaldi, C.; Saldarriaga Cartagena, A.M.; Sullivan, W.J. Emerging Therapeutic Targets Against *Toxoplasma gondii*: Update on DNA Repair Response Inhibitors and Genotoxic Drugs. *Front. Cell. Infect. Microbiol.* 2020, 10, 289. [CrossRef]

88. McFadden, D.C.; Tomavo, S.; Berry, E.A.; Boothroyd, J.C. Characterization of cytochrome b from *Toxoplasma gondii* and Qo domain mutations as a mechanism of atovaquone-resistance. *Mol. Biochem. Parasitol.* 2000, 108, 1–12. [CrossRef] [PubMed]

89. Ashburn, T.T.; Thor, K.B. Drug repositioning: Identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.* 2004, 3, 673–683. [CrossRef] [PubMed]

90. Pushpakom, S.; Iorio, F.; Eyers, P.A.; Escott, K.J.; Hopper, S.; Wells, A.; Doig, A.; Guilliams, T.; Latimer, J.; McNamee, C.; et al. Drug repurposing: Progress, challenges and recommendations. *Nat. Rev. Drug Discov.* 2019, 18, 41–58. [CrossRef]

91. Boyom, F.F.; Fokou, P.V.T.; Tchokouaha, L.R.Y.; Spangenberg, T.; Mfopa, A.N.; Kouipou, R.M.T.; Mbouna, C.J.; Donfack, V.F.D.; Zollo, P.H.A. Repurposing the Open Access Malaria Box To Discover Potent Inhibitors of *Toxoplasma gondii* and *Entamoeba histolytica*. *Antimicrob. Agents Chemother.* 2014, 58, 5848–5854. [CrossRef]

92. Patra, A.T.; Hingmire, T.B.; Belekar, M.; Xiong, A.; Subramanian, G.; Bozdech, Z.; Preiser, P.; Shanmugam, D.; Chandramohanadas, R. Whole Cell Phenotypic Screening Of MMV Pathogen Box identifies Specific Inhibitors of *Plasmodium falciparum* merozoite maturation and egress. *bioRxiv* 2019, 772434. [CrossRef]

93. Radke, J.B.; Burrows, J.N.; Goldberg, D.E.; Sibley, L.D. Evaluation of Current and Emerging Antimalarial Medicines for Inhibition of *Toxoplasma gondii* Growth in Vitro. *ACS Infect. Dis.* 2018, 4, 1264–1274. [CrossRef]