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The potential of quinoline derivatives for the treatment of *Toxoplasma gondii* infection

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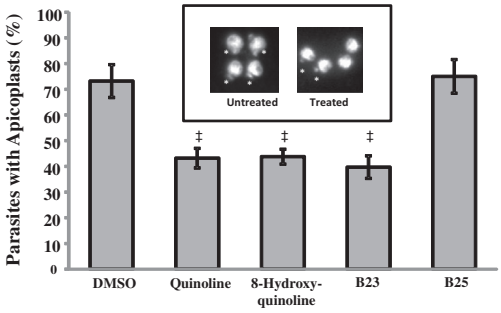
HIGHLIGHTS

- Quinoline derivatives are effective at inhibiting the growth of *Toxoplasma*.
- Some quinolones cause the disappearance of the apicoplast.
- 8-Hydroxyquinoline is very effective to inhibit the growth of *Toxoplasma*.

GRAPHICAL ABSTRACT



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ABSTRACT

Here we reported our investigation, as part of our drug repositioning effort, on anti-*Toxoplasma* properties of newly synthesized quinoline compounds. A collection of 4-aminoquinoline and 4-piperazinylquinoline analogs have recently been synthesized for use in cancer chemotherapy. Some analogs were able to outperform chloroquine, a quinoline derivative drug which is commonly used in the treatment of malaria and other parasitic infections. Herein 58 compounds containing one or two quinoline rings were examined for their effectiveness as potential anti-*Toxoplasma* compounds. Of these 58 compounds, 32 were efficient at inhibiting *Toxoplasma* growth (IC_{50} < 100 μM). Five compounds with single and simple quinoline rings exhibited similar cLogP values of ~2 and IC_{50} values between 5 and 6 μM, with one exception of 8-hydroxyquinoline whose IC_{50} value was 213 nM. The addition of one hydroxyl group at position 8 caused a 40-fold increase in the inhibitory effect of quinoline. A significant improvement in anti-*Toxoplasma* effect among quinoline derivatives was detected in **B11**, **B12**, **B23**, and **B24**, whose structures carry two quinoline rings, and their resultant cLogP values are ⩾7. Among these compounds, **B23** was the most effective compound with IC_{50} value of 425 ± 35 nM, and TI value of 4.9. It was also noted that compounds with at least one quinoline ring, displaying anti-*Toxoplasma* effects were capable of causing the disappearance of the apicoplast, a plastid-like organelle. When treated with quinoline, 8-hydroxyquinoline or **B23**, 40–45% of the parasites lost their apicoplasts. Our findings recapitulate the properties of quinoline derivatives in diminishing apicoplast. This could aid further investigations of anti-parasitic treatments specific to Apicomplexan. More importantly, **B12** and **B23** which harbor superior anti-cancer properties than chloroquine, have effective anti-*Toxoplasma* activity. These compounds therefore have significant potential for future development of chemotherapeutic agents for patients suffering from breast cancers and parasitic infection.

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1. Introduction

Quinoline alkaloids, heterocyclic aromatic organic (C_9H_7N) natural compounds, are known to harbor various medicinal properties and are present in a number of national pharmacopoeias. Their synthetic quinoline counterparts also carry important biological activities. 8-Hydroxyquinoline has growth inhibitory effects against gram positive bacteria and some lower level eukaryotes (Shen et al., 1999), and has been used in the veterinary sciences as an antimicrobial agent for topical treatment (Gershon et al., 1985). The mode of action of 8-hydroxyquinoline is a result of its chemical property as a metal chelator and thought to sequester metals necessary for important enzymes in micro-organisms (Shen et al., 1999). 8-Nitroquinoline has been successfully exploited in the treatment of topical pathogenic parasitic *Leishmania* infection (Paloque et al., 2012). As early as the 17th century, quinine [(*R*)-(6-Methoxyquinolin-4-yl)((2*S*,4*S*,8*R*)-8-vinylquinuclidin-2-yl)methanol], an active constituent from the barks of cinchona tree, was used both as a muscle relaxant to halt shivering due to the infection caused by *Plasmodium* spp. and as an anti-malaria agent. Malaria was and remains a serious and sometimes fatal disease. Due to an overwhelming demand for quinine and limited supplies of cinchona barks, the need for readily available and alternative sources of quinine-equivalent compounds gave rise to the discovery of the first synthetic acridine derivative, quinacrine [(*RS*)-*N*′-(6-chloro-2-methoxy-acridin-9-yl)-*N*,*N*-diethyl-pentane-1,4-diamine], around the time of the First World War (Butler et al., 2010). Soon after the discovery of quinacrine, a more potent quinoline derivative, chloroquine [(*RS*)-*N*′-(7-chloroquinolin-4-yl)-*N*,*N*-diethyl-pentane-1,4-diamine], was synthesized (Butler et al., 2010; Solomon and Lee, 2009). Quinine, quinacrine, and chloroquine are still currently used as anti-malaria agents (Butler et al., 2010). It was hypothesize that these compounds prevent the formation of hemozoin by *Plasmodium* parasites (Foley and Tilley, 1998). Hemozoin detoxifies free heme intracellularly, as free heme and heme-drug complexes destroy the parasite by creating oxidative stress (Slater, 1992). More detailed structural and functional studies showed that 7-halo substituted compounds are the most active compound. Chloroquine, a 4-aminoquinoline carrying a chlorine moiety at position 7, is an effective anti-malaria agent currently used in the treatment and prevention of malaria (Butler et al., 2010), and as a reference compound to evaluate the ability of chemicals to inhibit hemozoin formation (Foley and Tilley, 1998). Despite their effectiveness, emerging resistance of *Plasmodium* spp. has stimulated further drug discovery and development (Butler et al., 2010).

A simple backbone quinoline and 2-methyl-3-carbethoxyquinoline have also been shown to inhibit the growth of *Toxoplasma gondii*, a *Plasmodium*-related parasite. When treated with either simple quinoline or 2-methyl-3-carbethoxyquinoline, 52–57% of *Toxoplasma* lost their unique organelle called apicoplast (Smith et al., 2007). Similar activity was previously reported with ciprofloxacin, a fluoroquinolone derivative (Fichera and Roos, 1997). It was hypothesized that the crucial pharmacophore conferring this biological activity is the quinoline ring structure (Smith et al., 2007). Interestingly, the presence of an apicoplast is generally used in grouping *Plasmodium* and *Toxoplasma* parasites as pathogenic members of the Apicomplexa phylum. The apicoplast is a non-photosynthetic plastid organelle. In *Plasmodium* and *Toxoplasma* parasites, the apicoplast is crucial for three essential metabolic functions: the synthesis of heme, type II fatty acids and isoprenoid precursors (Sheiner et al., 2013), and thus has long been considered an ideal target for treating infection caused by Apicomplexan (Fichera and Roos, 1997). Pathogenic Apicomplexan, besides *Plasmodium* (malaria in humans) and *Toxoplasma* (toxoplasmosis in humans and animals), are the following. *Cryptosporidium* can cause cryptosporidiosis whose major symptom in humans is diarrhea (Tzipori and Ward, 2002). *Eimeria* is the major cause of coccidiosis in agriculturally important animals, including poultry, sheep, and cattle (Shirley and Harvey, 2000). *Babesia* parasites can cause a hemolytic disease known as babesiosis in humans and occasionally in domestic animals (Vannier and Krause, 2009). *Neospora* is an important protozoan pathogen in cattle and dogs, while *Theileria* are important cattle parasites (Katzer et al., 2011).

Drug repositioning can be described as the investigation of new medicinal uses and purposes for previously known and tested drugs (Solomon et al., 2010a, 2010b). Such practices could help overcome high costs and time often associated with the development of new drugs because the known and tested drugs have previously or generally been evaluated or recognized for their pharmacokinetics properties (Chong and Sullivan, 2007). The repositioning studies of chloroquine derivatives (Solomon et al., 2010a, 2010b) have been very valuable in generating a large collection of quinoline derivatives. By combining two important pharmacophores, 4-piperazinylquinolones and isatin structure, novel compounds were created with versatile biological effects. For example, **B12** and **B23** with their hybrid pharmacophore exhibit cancer-specific and are more effective than chloroquine (Solomon et al., 2010b). Due to the presence of multiple structural determinants of the hybrid pharmacophores, these newly generated compounds harbor great potentials to target different sites or molecules, which might result in the possibility to disrupt multiple cellular mechanisms simultaneously and/or to overcome drug resistance (Meunier, 2007).

Here we reported our evaluation for anti-*Toxoplasma* activity of a wide array of quinoline derivatives, ranging from simple molecules with null or one substitution to multiple substitutions (Tables 1–3). *Toxoplasma* was chosen as our model organism because (1) it can be easily cultivated in vitro using human fibroblast monolayers (HFF); (2) its doubling time is relatively short (~7 h) (Seeber and Boothroyd, 1996); (3) the availability of its 2F strain to express beta-galactosidase reporter system offers an accurate and effective growth evaluation (Seeber and Boothroyd, 1996), and (4) protocols for growth inhibition and toxicity assays are well documented (Seeber and Boothroyd, 1996; Jones-Brando et al., 2003). In general, *Toxoplasma* infection is quite common, affecting a quarter of the world's population, although not all infections lead to lethal toxoplasmosis. *Toxoplasma* infection can cause very mild or no symptom in healthy individuals, but becomes severe in immunocompromised individuals (Montoya and Rosso, 2005). The infection occurs via ingestion of contaminated food or water. If the infection takes place during pregnancy, the parasite could be transmitted to a fetus and could cause blindness and mental retardation. Current treatments for *Toxoplasma* infection include pyrimethamine in combination with sulfadiazine and/or clindamycin (Montoya and Rosso, 2005). Pyrimethamine and sulfadiazine inhibit the enzyme dihydrofolate reductase of the tetrahydrofolic acid synthesis, whereas clindamycin inhibits bacterial protein synthesis by inhibiting ribosomal translocation. These current treatments could have severe side effects such as, the inhibition of bone marrow formation and the teratogenic nature of pyrimethamine as a folate inhibitor, precluding it from being used in pregnant women during their first trimester. Sulfadiazine could cause allergic reactions in immunocompromised individuals (Montoya and Rosso, 2005; Peters et al., 2007). Because of these limitations associated with current treatments, it is of great importance to evaluate and find alternative safe treatments. Evaluation of the biological properties of quinoline compounds for their ability to inhibit the growth of *Toxoplasma* could reap benefits for the ongoing search for effective treatments against the devastating effects of apicomplexan infection.