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One-pot, multicomponent synthesis of 2,3-disubstituted quinazolinones with potent and selective activity against *Toxoplasma gondii*

Carla E. Brown [a], Tiffany Kong [a], Claudia Bordón [b], Robert Yolken [b], Lorraine Jones-Brando [b], James McNulty [a,*]

[a] Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario L8S 4M1, Canada
[b] Stanley Division of Developmental Neurovirology, Department of Pediatrics, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA

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ABSTRACT

The discovery of two quinazolinones with selective, single-digit micromolar activity (IC_{50} = 6–7 μM) against the tachyzoites of the apicomplexan parasite *Toxoplasma gondii* is reported. These potent and selective third generation derivatives contain a benzyloxybenzyl substituent at C2 and a bulky aliphatic moiety at N3. Here we show that these quinazolinones inhibit *T. gondii* tachyzoite replication in an established infection, but do not significantly affect host cell invasion by the tachyzoites.

Toxoplasmosis is a life-long infectious disease caused by the apicomplexan parasite *Toxoplasma gondii*. In intermediate hosts, e.g., humans, this obligate intracellular parasite can be present in both its rapidly replicating stage, tachyzoites, as well as the slowly replicating/non-replicating stage, bradyzoites. During an initial, acute infection tachyzoites invade and then replicate within a parasitophorous vacuole (PV) inside host cells and then gradually convert to bradyzoites, thereby forming tissue cysts. These encysted bradyzoites become permanent residents of the host's skeletal muscle, cardiac muscle and central nervous system and thus constitute a persistent (latent or chronic) infection (for a detailed review see Ref. [1a]). In contrast to in vivo infections, the tachyzoite stage is the predominant and most easily manipulated form of the parasite in vitro and thus is the primary target of our initial drug development experiments. Uninhibited, tachyzoites infect host cells, multiply until they egress from and lyse the cells, invade nearby susceptible cells, and then begin the process again until the entire cell monolayer is lysed. This is known as the lytic cycle.[1a]

Toxoplasma infection is very common with about 50% of the world's human population harboring a persistent, ordinarily asymptomatic infection. However, in developed countries, toxoplasmosis is the second leading cause of death due to food-borne illness.[1b] Further, acute toxoplasmosis as well as reactivation of persistent *T. gondii* infections can be life-threatening during fetal development and in immunocompromised individuals.[1b,2,3] In low-income countries, chronic *T. gondii* infection is highly prevalent among HIV-positive patients, and reactivation of these persistent infections is a major cause of mortality in this population.[4] Persistent *T. gondii* infection has also been associated with neurological illnesses such as schizophrenia[5] and bipolar disorder,[6] and several known anti-psychotic medications are moderately effective against in vitro *T. gondii* tachyzoite growth.[7] Current prescribed anti-parasitic chemotherapies for the treatment of *T. gondii* infection are effective against the parasite tachyzoite, the agent of acute disease, but are not effective in eliminating the encysted parasite bradyzoites, the agents of the persistent infection. In addition, resistance to these drugs is rapidly developing.[8] Consequently, there is an urgent need to develop new anti-Toxoplasma agents.

Quinazolinones contain a heterocyclic core that is found within both natural products and synthetic pharmaceuticals. Variously substituted quinazolinones have demonstrated antiparasitic,[9,10] antiviral,[11–13] antifungal,[14] antibiotic,[15] anticancer,[16–18] and analgesic activities,[19] illustrating the importance and value of this molecular scaffold in drug design. Given the known antiparasitic quinazolinones such as febrifugine and tryptanthrin, and structurally related heterocyclic scaffolds that display antiparasitic activity,[20–22] we hypothesized that synthetic access to 2,3-disubstituted quinazolinones might allow the discovery of novel compounds with activity against *T. gondii*. Here we describe the synthesis and structural optimization of quinazolinones and

* Corresponding author.
E-mail address: jmcnult@mcmaster.ca (J. McNulty).

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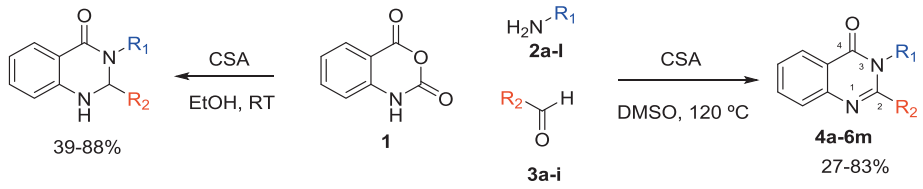
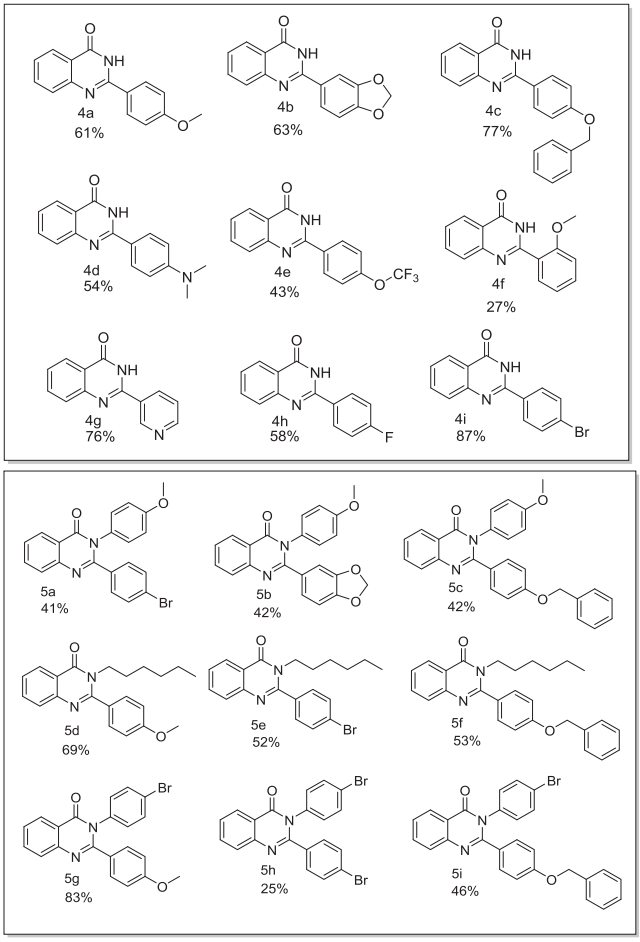


Scheme 1. Synthesis of 2- and 3-substituted quinazolinone analogs using CSA and DMSO.

discovery of potent and selective in vitro anti-tachyzoite agents through an iterative structure–activity study.

The condensation of *ortho*-amino benzamides with carbonyls produces quinazolinones in a process catalyzed by protic acids,[23,24] Lewis acids,[25] and other catalysts.[26,27] Aldehydes and ketones are common substrates for this chemistry, but diketones,[28] β-ketoesters,[23] and benzyl alcohols[29] can also be used. To generate quinazolinones from dihydroquinazolinone intermediates, additional oxidants are sometimes employed.[25,30,31] We previously reported a mild, one-pot multicomponent cyclization for the preparation of 2-substituted and 2,3-disubstituted quinazolinones (Scheme 1) and development of potent antiviral (HSV-1) agents through iterative structure–activity optimization studies.[32] We discovered that the condensation reaction conducted thermally in DMSO as solvent led exclusively to the aromatic quinazolinones in good yield (Scheme 1). On the basis of the precedents described (febrifugine and tryptanthrin), the three-component coupling strategy was used to prepare an initial compound array of nine quinazolinones (**4a**-**4i**; first generation) for initial screening against in vitro *T. gondii* (Scheme 2). Reaction of isatoic anhydride with various aldehydes and ammonium acetate under the conditions described, led to the assembly of this collection of N3 unsubstituted quinazolinones.



Scheme 2. Structure of the quinazolinones prepared in the first (**4a**-**4i**) generation, unsubstituted at N-3, and second generation (**5a**-**5i**) containing aryl or hexyl substituents at N-3.

Table 1
Anti-Toxoplasma activity of quinazolinones depicted in Scheme 2.

CPD	IC_{50} (μM)	IC_{90} (μM)	TD_{50} (μM)	TI
4a	20	121	125	6
4b	50	443	≥320	6
4c	153	4989	≥320	2
4d	14	281	≥320	23
4e	27	447	≥320	12
4f	56	266	305	5
4g	94	225	≥320	3
4h	29	260	≥320	11
4i	92	414	≥320	3
5a	32	81	≥320	10
5b	40	91	≥320	8
5c	4	14	4	1
5d	10	48	42	4
5e	60	193	88	1
5f	10	45	178	18
5g	31	83	196	6
5h	44	126	≥320	7
5i	3	12	5	2
ATV	0.2	0.6	21	111

On the basis of initial results (*vide infra*), a second generation of quinazolinones (**5a**-**5i**) with substituted aryl and alkyl substituents at N3 was generated. The structure (Scheme 2) and antiparasitic activity relationships (Table 1) of the pooled first and second generation quinazolinones are discussed together below.

We routinely perform three different assays to screen experimental compounds for in vitro activity against *T. gondii* tachyzoites.[7,33,34] These assays determine each compound's ability to 1) inhibit establishment of infection and continued growth of tachyzoites over 5 days, i.e. multiple rounds of the lytic cycle, 2) affect replication of intracellular tachyzoites in an established infection over a 24 h period, or 3) affect extracellular tachyzoites by inhibition of attachment to and penetration of tachyzoites through host cell membranes. Thus these three assays interrogate in vitro efficacy against the major steps of the lytic cycle of *T. gondii* tachyzoites, i.e., invasion of host cell, replication within the host cell, lysis of host cell and subsequent spread of infection to surrounding cells. All of the compounds (Scheme 2) were thus subjected to each of these assays using previously published protocols.[7,33,34] Data reported are compiled from three independent experiments unless otherwise noted.

Stock solutions (10 mM) of compounds were made in DMSO and stored at −25 °C. All further dilutions for use in assays discussed below were made with cell growth medium [Dulbecco's Modified Eagle Medium (D-MEM; Thermo-Fisher), 10% fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 units of penicillin G and 100 μg streptomycin sulfate per ml].

A detailed, published colourimetric assay protocol was employed for assessment of 5-day growth inhibition of both