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Organometallic Derivatives of Decoquinatate targeted towards *Toxoplasma gondii*

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ABSTRACT: Toxoplasmosis is an infection contracted by exposure to *Toxoplasma gondii* and can have deleterious health effects on pregnant women and their children. Current treatments for the infection are complex and have considerable undesired side effects, raising the need for new treatments. Herein we report the synthesis, characterisation, and biological testing of some organometallic derivatives of the commercially available, broad spectrum antiparasitic, decoquinatate (DCQ). The cyclic secondary amine of decoquinatate was functionalised with a range of groups (i.e., ferrocene, ruthenocene and phenyl) with either methyl or vinyl bridges. Through measurement of half maximal inhibitory concentrations (IC₅₀) and *T. gondii* proliferation assays, it was found that ferrocenylvinyl-DCQ and an oxygen-alkylated phenylvinyl-DCQ side product reduced proliferation of the parasite by 84% at 1 μM, which approached that of the parent drug (96%). These data provide a possible roadmap for future investigations on the derivatisation of DCQ to yield better treatments for toxoplasmosis, particularly the functionalisation of the cyclic ketone.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasitic one-celled eukaryote (specifically an apicomplexan) that causes the infectious disease toxoplasmosis. This parasite is capable of

infecting any warm-blooded mammal.^{1,2} In humans, toxoplasmosis can arise from contact with the parasite via raw or undercooked meat, unwashed vegetables and fruits or consumption of contaminated water. Some patients show no symptoms when infected while others develop flu-like symptoms including fever, aching muscles, fatigue, and swollen glands. Infected pregnancy cases can lead to miscarriage and serious complications, while patients with weak immune systems can have their eyes or brain affected, leading to issues with balance and coordination, seizures, lung problems and blurry vision.

First-line treatment of toxoplasmosis involves combination therapy with pyrimethamine and sulfadiazine or clindamycin. Pyrimethamine has high toxic side effects on bone marrow, which is mitigated by co-administration of leucovorin (folinic acid). Pyrimethamine is also teratogenic, and so affected patients in their first trimester of pregnancy are given spiramycin instead which has moderate effectiveness for reducing congenital transmission. Due to the toxic side effects of current treatments, complicated administration regimes, and decreased effectiveness of alternative treatments, there is a need to develop new effective *T. gondii* antiparasitic drugs with reduced side effects and simpler dose profiles.²⁻⁴

Decoquinate (DCQ) is a broad-spectrum anti-parasitic drug. It has known activity against *coccidia*, *toxoplasma*, *cryptosporidia* and *neospora*. It is nontoxic, and it is commercially available (Deccox®) in formulations for long-term oral administration as a feed additive in suspension, powder or capsule form. DCQ works effectively as a preventive treatment for coccidiosis in ruminants and poultry. After absorption, DCQ is rapidly eliminated via bile and faeces and to a lesser extent also in the urine. Its target tissues are skin and fat, and metabolic equilibrium in these tissues is reached after 3 days.⁵

The mechanistic activity of DCQ in the *Toxoplasma* parasite has been studied⁶ as well as with *Plasmodium*.^{7,8} Available reports screening DCQ with *Plasmodium* showed that DCQ interrupts the parasite life cycle via inhibition of electron transport in mitochondria, by docking within the ubiquinol-binding pocket in cytochrome B.^{9,10} What is more interesting is that docking can still occur within the resistant form of the protein given that the resistance-conferring mutation at the residues of Ala122 and Tyr126 are not involved in binding DCQ. Rather, the residues binding to DCQ are conserved. Reports exploring biological activity of organic derivatives of DCQ by Beteck *et al.* also showed that the long C10 alkyl chain was key to selectivity (**Figure 1**).^{11,12}

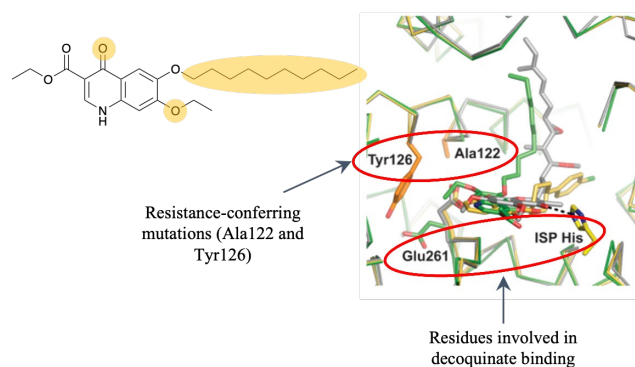


Figure 1. Superposition of DCQ (green) and atovoquone (yellow) with cytochrome *b* subunit of bovine cytochrome *bc*₁ complex co-crystallised with stigmatellin A (grey, PDB ID = 1SQX). Figure adapted from reference¹⁰ with permission from ACS. Components of DCQ thought to be important in selectivity are highlighted on the structure in yellow.

The rationale behind synthesising ferrocenyl analogues of DCQ stems from previously published studies by Jaouen, Biot and co-workers, which showed that ferrocenyl analogues of tamoxifen and chloroquine had improved bioactivities compared to the original parent compounds.¹³⁻¹⁶ Further studies showed that the ferrocenyl component made a difference by producing reactive oxygen species, increasing lipophilicity of the molecule, and provided additional mechanisms of action to that of the original drug.¹⁷⁻²² At our end, we have, over the past years, used this strategy to unveil novel lead compounds against different parasitic diseases such as schistosomiasis or fungal agents.^{13,16,23,24}

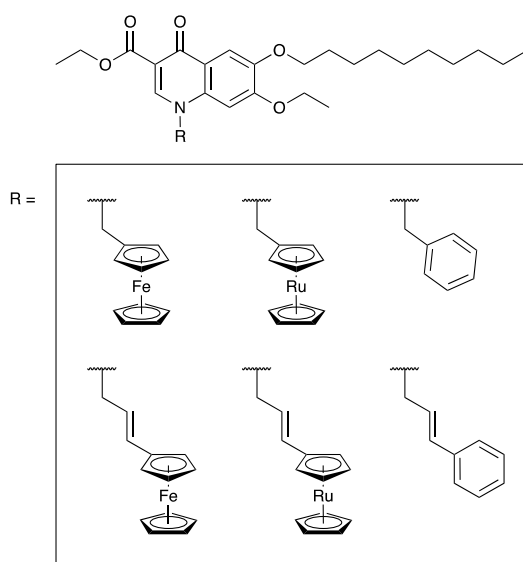


Figure 2. Structures of synthesised DCQ derivatives. Ferrocenylmethyl (FcMDCQ) and vinyl (FcVDCQ); ruthenocenylmethyl (RcMDCQ) and vinyl (RcVDCQ); phenylmethyl (PhMDCQ) and vinyl (PhVDCQ). For underivatized

With this in mind, we have synthesised and characterised analogues of DCQ, containing ferrocenyl moieties. In addition to this, ruthenocenyl and benzyl derivatives were also synthesised. The rationale for their inclusion in the series was to help elucidate possible mechanisms of action. Ruthenocene lacks the redox activity of ferrocene while the phenyl derivatives lack a metal centre but is aromatic. We hypothesise having the ferrocenyl moiety would improve activity compared to the parent compound (**Figure 2**). An additional *O*-alkylated derivative was isolated and subjected to biological testing (**Figure 3**).

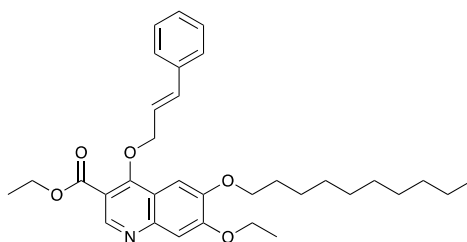


Figure 3. Additional *O*-alkylated derivative isolated from synthesis of phenylvinyl-DCQ adduct (O-PhVDCQ).

RESULTS AND DISCUSSION

Synthesis of DCQ derivatives. Compounds were prepared by direct treatment of DCQ with (ferrocenylmethyl) trimethylammonium iodide, benzyltrimethylammonium iodide, (ruthenocenylmethyl)-trimethylammonium iodide, (2-ferrocenylvinyl)methyl trimethylammonium iodide, (2-phenylvinyl)methyl trimethylammonium iodide, and (2-ruthenocenylvinyl)methyl trimethylammonium, respectively, with K_2CO_3 in acetonitrile. All newly synthesized compounds were unambiguously characterized by 1H , ^{13}C NMR spectroscopy, IR spectroscopy and ESI mass spectrometry (**Figures S4-S10**). The purity of individual compounds was confirmed by microanalysis.

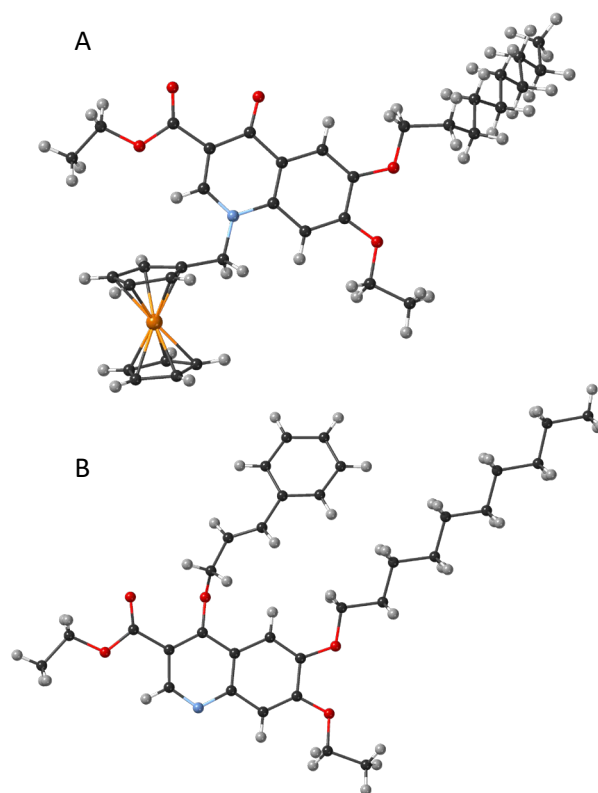


Figure 4. X-ray crystallography structures of (A) FcMDCQ, and (B) *O*-PhVDCQ.

Solid-state crystal structures of some DCQ derivatives, and unreported starting material were collected, further validating the desired structures (**Figure 4** and **S3**). The unexpected product, *O*-PhVDCQ, was identified through crystallisation and X-ray analysis and ^1H NMR.

X-ray crystallography. The crystal structures of two DCQ derivatives were determined by single crystal X-ray diffraction studies: the ferrocenylmethyl-DCQ (FcMDCQ) and the *O*-(2-phenylvinyl)methyl-DCQ (*O*-PhVDCQ) compounds (**Figure 4**). In the structure of FcMDCQ, the DCQ molecule is coordinated to the ferrocenylmethyl moiety through the nitrogen atom. The nitrogen atom exhibits a trigonal-planar geometry with three N—C bond distances of 1.346(5) and 1.399(5) Å in the pyridine ring and 1.489(6) Å in coordination with the metal fragment. In *para*-position, the C—O bond distance of 1.246(5) Å indicates the presence of a double C=O bond and confirms the quinolone form of the DCQ (two double bonds in the pyridine ring). In the structure of the *O*-alkylated DCQ derivative *O*-PhVDCQ, the nitrogen is unsubstituted, the N—C bond distances within the pyridine ring are significantly shorter than those in FcMDCQ (1.315(4) vs. 1.346(5) and 1.370(3) vs. 1.399(5) Å, respectively) and in

para-position the C—O bond distance of 1.364(3) Å indicates the presence of a single C—O bond, due to the coordination of the phenylvinyl group on the oxygen atom. As expected, the O-alkylated DCQ derivative exhibits an aromatic heterocyclic ring with three delocalised double bonds. It is worth to note that, except the phenylvinyl group which is oriented perpendicular to the central rings as shown by the C4-C5-O3-C13 torsion angle of 82.9(1)°, the entire molecule of O-PhVDCQ is almost planar. Indeed, even the long and flexible decyl group in its regular all-trans-torsional arrangement is almost coplanar with the quinoline rings, the dihedral angle between the mean planes of the corresponding atoms being 15.3(1)°. To the opposite, in FcMDCQ the decyl group is not as linearly arranged as in O-PhVDCQ and is oriented nearly perpendicular to the central rings (the dihedral angle between the mean planes of the corresponding atoms is 70.2(2)°). Nevertheless, the relative orientation of this long alkyl chain is only a consequence of the geometrical arrangement of the molecules at close contact in the crystals. Thereby, several types of intermolecular interactions are evidenced in the structures: a) π – π stackings involving the pyridine units, C–H \cdots π interactions between the decyl ligand and the cyclopentadienyl ring, and weak O–H \cdots O and C–H \cdots O hydrogen bonds involving the isolated water molecules in FcMDCQ, b) π – π stackings involving the central rings, and weak C–H \cdots O hydrogen bonds involving the phenylvinyl group and one of the carboxylic oxygen atom in O-PhVDCQ.

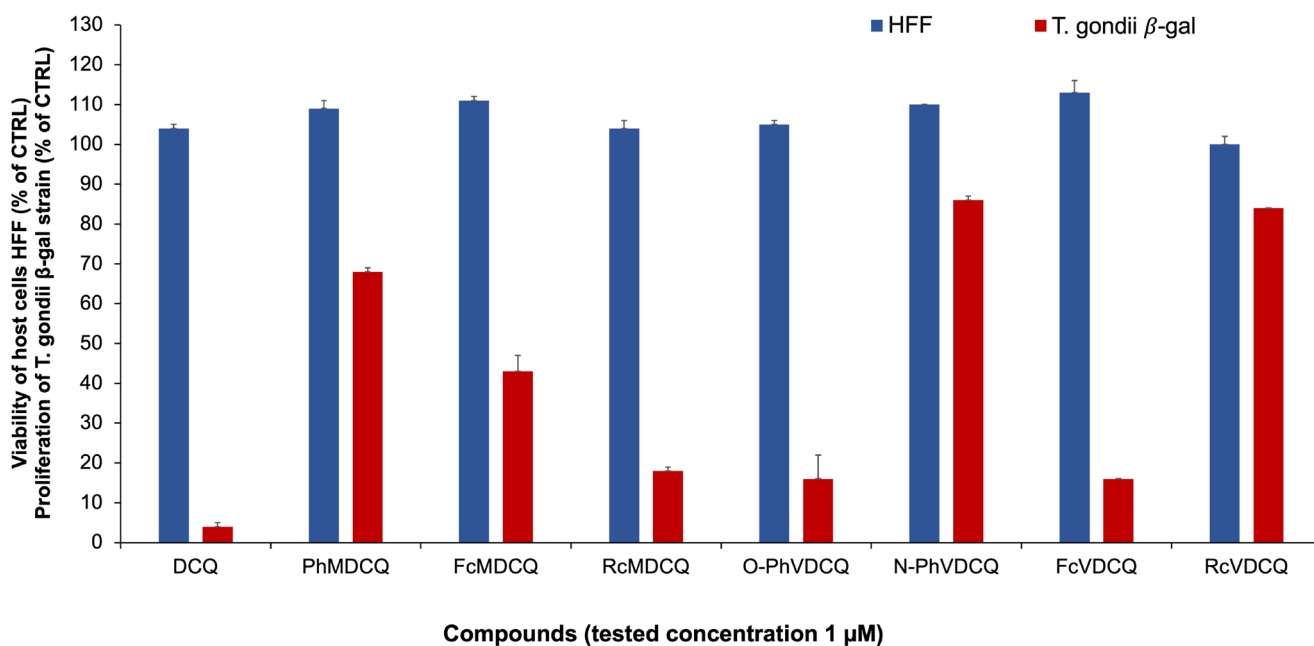


Figure 5. Clustered column chart showing *in vitro* activities at 1 μM of DCQ and derivatives on HFF viability (red) and *T. gondii* β-gal proliferation (blue). Control HFF cells and *T. gondii* β-gal tachyzoites treated with 0.1% DMSO were attributed 100% viability, and proliferation, respectively. For each assay, standard deviations were calculated from triplicates and are displayed on the plot.

In vitro bioassays. The 7 compounds together with decoquinatone were screened for biological activity *in vitro* against *T. gondii* β -gal, a transgenic strain that constitutively expresses β -galactosidase, which is grown in human foreskin fibroblasts (HFF) monolayers. The effects on uninfected HFF host cells were assessed as well. For the primary screening, cell cultures were exposed during 3 days to 1 and 0.1 μ M of each compound, including the reference compound decoquinatone. The viability of HFF cultures following drug treatments was measured by Alamar Blue assay, and the proliferation of *T. gondii* was quantified by measuring β -galactosidase activity. The results of this primary screening are presented as percentage in relation to untreated control cultures in **Table S1**. The results obtained at concentration of 1 μ M of tested compound for *T. gondii* and HFF are presented in **Figure 5**, in relation to controls (CTR), namely HFF treated with 0.1 % DMSO exhibiting 100 % viability, and *T. gondii* β -gal tachyzoites treated with 0.1 % DMSO showing 100 % proliferation. DCQ is active at both concentrations against *T. gondii* β -gal proliferation, and, like all other compounds tested, does not show toxicity to the host cells, as well as all the other compounds tested. In terms of antiparasitic activities, the DCQ derivatives did not have a notable activity against *T. gondii* *in vitro* at a concentration of 0.1 μ M (**Figure S2**). However, at 1 μ M, three compounds had significant activity, namely, RcMDCQ, O-PhVDCQ and FcVDCQ, which reduced the parasite proliferation by 82%, 86%, and 86%, respectively, approaching the efficacy of the parent decoquinatone (96%). N-PhVDCQ and RcVDCQ showed minimal inhibitory activity, reducing proliferation by ~15% while PhMDCQ and FcMDCQ, with the shorter CH₂ linkage showed moderate activity with parasite reduction of ~30% and 60%, respectively.

IC₅₀ values were determined for all compounds, including undervatised DCQ (**Table 1**). Values were not obtained for PhMDCQ, FcMDCQ and RcVDCQ as the dose-response curves did not plateau at zero viability before reaching 2.5 μ M. IC₅₀ values for RcMDCQ and the N- and O-PhVDCQ derivatives ranged from 0.6 to 0.7 μ M, while that of FcVDCQ was two-fold higher (1.4 μ M). No derivatives reached the IC₅₀ value of underivatized DCQ, which was determined to be 0.0024 μ M. Increased efficacy of O-alkylated DCQ derivatives relative to DCQ alone has been studied previously, with O-carbamate derivatives reaching nanomolar IC₅₀ values.²⁴ Such organometallic derivatives could be of interest in the future.

Table 1: Inhibitory concentration (IC₅₀) value (μM) on *T. gondii* β-gal and effect at 2.5 μM on HFFs viability of DCQ. (a) values at 95% confidence interval; upper limit (LS), lower limit (LI).

Compound	IC ₅₀ (μM)	[LS; LI] (a)	HFF viability at 2.5 μM
DCQ	0.0024	[0.003; 0.0004]	No compounds affected viability at 2.5 μM
PhMDCQ	>2.5	-	
FcMDCQ	>2.5	-	
RcMDCQ	0.7	[0.5; 1.0]	
N-PhVDCQ	0.6	[0.3; 0.9]	
O-PhVDCQ	0.6	[0.4; 0.8]	
FcVDCQ	1.4	[1.1; 1.7]	
RcVDCQ	>2.5	-	

CONCLUSIONS

Functionalisation of the commercially available, broad spectrum anti-parasitic drug, decoquinate was successful, as confirmed by exhaustive characterisation. However, biological evaluation of these derivatives revealed that substitution of the cyclic secondary amine by organometallic or aromatic groups reduced the drug's efficacy against *T. gondii*. Despite this, some promising effects arose from substitution with ferrocenyl vinyl, and ruthenocenyl methyl groups, with 86%, and 82% reduction in proliferation of the parasite, respectively, when treated at 1 μM. Of interest was the significant efficacy of the *O*-alkylated *O*-PhVDCQ derivative, potentially opening a new avenue of investigation in the pursuit of an effective organometallic derivative of DCQ. Substitution of groups onto the cyclic ketone may yield improved efficacy, as has been reported elsewhere.²⁴

EXPERIMENTAL SECTION

Ferrocenylmethyl-DCQ (FcMDCQ). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K₂CO₃ (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). A solution of (ferrocenylmethyl) trimethylammonium iodide (110 mg, 0.287 mmol) in acetonitrile (10 mL)

was added, dropwise *via* a cannula, to the suspension and heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). FcMDCQ was isolated as an orange solid (59 mg, 40%). ¹H NMR (400 MHz, CDCl₃): δ 8.51 (s, 1H), 7.87 (s, 1H), 6.83 (s, 1H), 5.05 (s, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 4.22-4.18 (m, 9H), 4.15-4.08 (m, 4H), 1.88-1.80 (m, 4H), 1.48 (t, *J* = 7.0 Hz, 3H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.26-1.23 (m, 12H), 0.89-0.85 (t, *J* = 7.2 Hz, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): 173.48, 166.2, 153.0, 147.67, 147.3, 134.2, 123.3, 110.0, 108.4, 99.3, 81.5, 69.1, 68.9, 68.4, 65.0, 60.7, 53.6, 31.9, 29.6, 29.5, 29.4, 29.3, 28.9, 26.0, 22.7, 14.6, 14.4, 14.1 ppm. Molecular wt: 615.59 g.mol⁻¹. +ESI 199.2 [FcCH₂]⁺, 616.8 [M+H]⁺, 638.8 [M+Na]⁺, 654.8 [M+K]⁺. Elemental analysis: C₃₃H₄₅FeNO₅ + 1.5 H₂O Calculated C 64.61, H 7.33, N 2.15; Found C 64.46, H 7.23, N 2.03.

Ruthenocenylmethyl-DCQ (RcMDCQ). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K₂CO₃ (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). A solution of (ruthenocenylmethyl) trimethylammonium iodide (123 mg, 0.287 mmol) in acetonitrile (10 mL) was added, dropwise *via* a cannula, to the suspension and heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). (2-Ruthenocenylvinyl)methyl-DCQ was isolated as a pale brown solid (42.5 mg, 27%). ¹H NMR (400 MHz, CDCl₃): δ 8.45 (s, 1H), 7.89 (s, 1H), 6.84 (s, 1H), 4.90 (s, 2H), 4.55-4.53 (m, 7H), 4.51-4.50 (m, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.17-4.10 (m, 4H), 1.89-1.82 (m, 2H), 1.50 (t, *J* = 6.8 Hz, 3H), 1.41 (t, *J* = 7.0 Hz, 3H) 1.35-1.27 (m, 12H), 0.87 (t, *J* = 7.2 Hz, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 173.6, 166.3, 153.2, 148.0, 147.8, 134.4, 123.4, 110.1, 108.6, 99.3, 86.6, 71.5, 70.6, 70.3, 69.3, 65.2, 60.9, 52.9, 32.0, 29.7, 29.7, 29.5, 29.4, 29.0, 26.1, 22.8, 14.7, 14.6, 14.2 ppm. Molecular wt 660.82 g.mol⁻¹. HRMS (ESI) *m/z*: [M+H]⁺ Calcd for C₃₅H₄₅NO₅RuH 662.2414. Found 662.2427; (Error: 2.0 ppm). Elemental analysis: C₃₅H₄₅NO₅Ru + 1 H₂O Calculated C 61.93, H 6.98, N 2.06; Found C 61.23, H 7.01, N 2.03.

Phenylmethyl-DCQ (PhMDCQ). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K₂CO₃ (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). A solution of benzyl trimethylammonium iodide (80 mg, 0.287 mmol) in acetonitrile (10 mL) was added, dropwise *via* a cannula, to the suspension and heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). PhMDCQ was isolated as a white solid (69.1 mg, 57%). ¹H NMR (400 MHz, CDCl₃): δ 8.54 (s, 1H), 7.89 (s, 1H), 7.41-7.28 (m, 3H), 7.22-7.09 (m, 2H), 6.62 (s, 1H), 5.34 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 4.10 (t, *J* = 6.8 Hz, 3H), 3.91 (q, *J* = 7.0 Hz, 2H), 1.83 (p, *J* = 6.9 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.38-1.23 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 173.7, 166.5, 153.0, 148.4, 147.8, 134.7, 134.3, 129.6, 128.8, 126.2, 123.7, 110.7, 108.5, 99.9, 69.3, 64.8, 61.0, 58.1, 32.0, 29.9, 29.7, 29.7, 29.5, 29.5, 29.0, 26.1, 22.8, 14.6, 14.4, 14.3, 1.2 ppm.

Molecular wt: 507.67 g.mol⁻¹. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₃₁H₄₁NO₅H 508.3057. Found 508.3063; (Error: 1.1 ppm). Elemental analysis: C₃₁H₄₁NO₅.H₂O Calculated C 70.83, H 8.25, N 2.66; Found C 71.20, H 7.97, N 2.59.

(2-Ferrocenylvinyl)methyl-DCQ (*FcVDCQ*). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K₂CO₃ (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). A solution of (ferrocenylvinyl)methyl trimethylammonium iodide (118 mg, 0.287 mmol) in acetonitrile (10 mL) was added, dropwise *via* a cannula, to the suspension and the mixture heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). *FcVDCQ* was isolated as an orange solid (63 mg, 41%). ¹H NMR (400 MHz, CDCl₃): δ8.43 (s, 1H), 7.91 (s, 1H), 6.82 (s, 1H), 6.31 (d, J = 15.8 Hz, 1H), 5.87 (dt, J = 15.7, 5.6 Hz, 1H), 4.73 (dd, J = 5.6, 1.6 Hz, 2H), 4.39 (q, J = 7.2 Hz, 2H), 4.29 (t, J = 1.9 Hz, 2H), 4.23 (t, J = 1.8 Hz, 2H), 4.17 (q, J = 7.0 Hz, 2H), 4.11 (t, J = 6.8 Hz, 2H), 4.03 (s, 5H), 1.84 (p, J = 6.9 Hz, 2H), 1.50 (t, J = 7.0 Hz, 3H), 1.41 (t, J = 7.1 Hz, 3H), 1.37 – 1.12 (m, 14H), 0.87 (t, J = 7.1, 6.7 Hz, 2H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ173.7, 166.5, 153.2, 147.9, 147.4, 134.4, 133.3, 123.7, 118.2, 110.6, 108.7, 99.6, 81.0, 69.5, 69.3, 67.1, 65.2, 60.0, 55.9, 32.0, 29.7, 29.7, 29.5, 29.5, 29.0, 26.1, 22.8, 14.8, 14.6, 14.3 ppm. Molecular wt: 641.63 g.mol⁻¹. HRMS (ESI) m/z: [M]⁺ Calcd for C₃₇H₄₇FeNO₅H 642.2876. Found 642.2875; (Error: -0.2 ppm). Elemental analysis: C₃₇H₄₇FeNO₅ + 0.5 H₂O Calculated C 67.47, H 7.35, N 2.13; Found C 67.75, H 7.66, N 2.22.

(2-Ruthenocenylvinyl)methyl-DCQ (*RcVDCQ*). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K₂CO₃ (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). To the suspension was added, dropwise *via* a cannula, a solution of (2-ruthenocenylvinyl)methyl trimethylammonium iodide (131 mg, 0.287 mmol) in acetonitrile (10 mL) and the mixture heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). *RcVDCQ* was isolated as a pale brown solid (27 mg, 16%). ¹H NMR (400 MHz, CDCl₃): δ8.38 (s, 1H), 7.90 (s, 1H), 6.77 (s, 1H), 6.18 (d, J = 15.8 Hz, 1H), 5.82 (dt, J = 15.7, 5.6 Hz, 1H), 4.68 (t, J = 1.5 Hz, 3H), 4.56 (t, J = 1.7 Hz, 2H), 4.42 (s, 5H), 4.39 (q, J = 7.2 Hz, 2H), 4.21-4.02 (m, 4H), 1.85 (p, J = 6.9 Hz, 2H), 1.52 (t, J = 7.0 Hz, 3H), 1.41 (t, J = 7.1 Hz, 3H), 1.38 – 1.17 (m, 14H), 0.87 (t, J = 6.7 Hz, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ173.7, 166.5, 153.2, 147.9, 147.4, 134.4, 132.4, 123.6, 118.0, 110.6, 108.6, 99.6, 85.0, 71.3, 71.2, 65.2, 61.0, 55.8, 32.0, 29.8, 29.7, 29.7, 29.5, 29.5, 29.0, 26.1, 22.8, 14.8, 14.6, 14.3 ppm. Molecular wt: 686.86 g.mol⁻¹. HRMS (ESI) m/z: [M]⁺ Calcd for C₃₇H₄₇NO₅RuH 688.2570. Found 688.2588; (Error: 2.5 ppm). Elemental analysis: C₃₇H₄₇NO₅Ru Calculated C 64.70 H 6.90 N 2.04; Found C 64.77, H 7.27, N 2.22.

N-(2-Phenylvinyl)methyl-DCQ (*N*-PhVDCQ). Under an inert atmosphere, DCQ (100 mg, 0.239 mmol) and K_2CO_3 (99 mg, 0.718 mmol) were stirred in dry acetonitrile (60 mL). To the suspension was added, dropwise *via* a cannula, a solution of (2-phenylvinyl)methyl bromide (57 mg, 0.287 mmol) in acetonitrile (10 mL) and the mixture heated at reflux overnight. Volatiles were removed and the residue purified by flash column (3:1 ethyl acetate/hexane to neat ethyl acetate). *N*-PhVDCQ was isolated as a white solid (55 mg, 28%). 1H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 7.89 (s, 1H), 7.32-7.28 (m, 5H), 6.84 (s, 1H), 6.56-6.52 (d, J = 16.0 Hz, 1H), 6.30 (dt, J = 16.0, 5.5 Hz, 1H), 4.91 (dd, J = 5.6, 1.7 Hz, 2H), 4.37 (q, J = 7.1 Hz, 2H), 4.12 (m, 4H), 1.88-1.81 (m, 2H), 1.45 (t, J = 7.1 Hz, 3H), 1.40 (t, J = 7.1 Hz, 3H), 1.32-1.25 (m, 14H), 0.87 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.7, 172.8, 166.3, 153.3, 147.9, 147.6, 135.3, 134.5, 134.4, 128.9, 128.8, 126.7, 123.5, 121.8, 110.6, 108.5, 99.4, 69.3, 65.1, 60.9, 56.0, 32.0, 29.7, 29.6, 29.5, 29.4, 29.0, 26.1, 22.8, 22.7, 14.6, 14.5, 14.2 ppm. Molecular wt: 533.71 g.mol⁻¹. HRMS (ESI) m/z : [M]⁺ Calcd for $C_{33}H_{43}NO_5H$ 534.3214. Found 534.3212; (Error: -0.4 ppm). Elemental analysis: $C_{33}H_{43}NO_5 + 1 H_2O$ Calculated C 71.84, H 8.22, N 2.54; Found C 71.09, H 8.43, N 2.23.

O-(2-Phenylvinyl)methyl-DCQ (*O*-PhVDCQ). Under an inert atmosphere, DCQ (150 mg, 0.36 mmol) and K_2CO_3 (149 mg, 1.08 mmol) were stirred in dry acetonitrile (150 mL). To the suspension, cinnamyl bromide (106 mg, 0.54 mmol) was added and the reaction mixture was heated at reflux overnight. Upon cooling to RT, the mixture was filtered and volatiles removed *in vacuo* and the residue purified by column chromatography (hexane ethyl acetate). *O*-PhVDCQ was obtained as a white solid (8 mg, 4%). 1H NMR (400 MHz, $CDCl_3$): δ 9.08 (s, 1H), 7.49 (s, 1H), 7.42-7.37 (m, 3H), 7.36-7.30 (m, 2H), 7.29-7.25 (m, 1H), 6.76 (d, J = 15.9 Hz, 1H), 6.51 (dt, J = 15.9, 6.4 Hz, 1H), 4.90 (dd, J = 6.4, 1.3 Hz, 2H), 4.64 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.0 Hz, 2H), 4.05 (t, J = 6.8 Hz, 2H), 1.54 (t, J = 7.0 Hz, 3H), 1.44 (t, J = 7.2 Hz, 3H), 1.41-1.20 (m, 16H), 0.88 (t, J = 6.85 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.4, 162.8, 154.1, 150.4, 150.0, 149.0, 136.3, 134.3, 128.8, 128.4, 126.8, 124.3, 119.0, 113.6, 108.9, 102.3, 69.4, 64.7, 61.4, 32.1, 29.71, 29.67, 29.5, 29.0, 14.6, 14.5, 14.3 ppm. Molecular wt: 533.71 g mol⁻¹. HRMS (ESI) m/z : [M]⁺ Calcd for $C_{33}H_{43}NO_5H$ 534.3210. Found 534.3214; (Error: -0.7 ppm). Elemental analysis: $C_{33}H_{45}NO_5$ Calculated C 73.91, H 8.47, N 2.61; Found C 74.30, H 8.28, N 2.63.

X-ray crystallography. All single crystal X-ray diffraction studies were performed at 160(1) K on a Rigaku Oxford Diffraction Synergy-Pilatus diffractometer using the copper X-ray radiation (λ = 1.54184 Å) from a dual wavelength X-ray source and an Oxford Instruments Cryojet XL cooler. The provided single crystals were covered with a polybutene oil, selected and mounted on a loop fixed on a goniometer head. Pre-experiments, data collections, data reductions and analytical absorption corrections²⁵ were performed with the program suite *CrysAlisPro*.²⁶ Using

Olex2,²⁷ the structures were solved with the SHELXT²⁸ small molecule structure solution program and refined with the SHELXL program package²⁹ by full-matrix least-squares minimization on F². PLATON³⁰ was used to check the result of the X-ray analyses. The crystal data collections and structure refinement parameters are shown in Tables S2 – S4 . CCDC 2035352 (for FcMDCQ), 2159223 (for O-PhVDCQ) and 2159224 (for (2-ruthenocenylnyl)methyl trimethylammonium iodide) contain the supplementary crystallographic data for these compounds, and can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. In the crystal structure of FcMDCQ, the terminal CH₂CH₃ group was disordered over two sets of positions refined with site-occupancy factors of 0.157(8) and 0.843(8).

In vitro culture of parasites and host cells. If not otherwise stated, tissue culture media were purchased from Gibco-BRL and biochemical reagents from Sigma-Aldrich. Human foreskin fibroblasts (HFF) were maintained in DMEM cell culture media containing 10% fetal calf serum (FCS; Gibco-BRL) and antibiotics as described.³¹ *T. gondii* β-gal strain (transgenic *T. gondii* RH expressing the β-galactosidase gene from *E. coli*)³² were maintained in HFF cells and isolated from their host cells as previously described.³¹

In vitro assessment of drug efficacy against *T. gondii* and host cells. To study the effects of compounds against *T. gondii* tachyzoites *in vitro*, 1 mM stock solutions of compounds were prepared in DMSO, and stored at –20 °C. For assessment of drug efficacy against *T. gondii* tachyzoites, parasites were isolated, and assays were performed using HFF as host cells as previously described.³¹ 5×10³ HFF cells were grown to confluence in 96-well plates in phenol-red free culture medium at 37 °C with 5 % CO₂. Cultures were infected with freshly isolated *T. gondii* β-gal tachyzoites (1×10³ per well), and drugs were added concomitantly with infection. Initial assessments of drug efficacy were done by exposing parasite cultures to 0.1 and 1 μM of each compound for a period of three days, or 0.1 % DMSO was added as a control. For IC₅₀ determination, decoquinatone was added at 7 concentrations: 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1, and 1 μM. After three days of incubation at 37 °C, 5 % CO₂, medium was removed, and cell cultures were permeabilized by using 90 μL PBS containing 0.05 % Triton-X-100. After addition of 10 μL of 5 mM chlorophenol red-β-d-galactopyranoside (CPRG; Roche Diagnostics) dissolved in PBS, the absorption shift was measured at 570 nm wavelength at various time points on a VersaMax multiplate reader (Bucher Biotec, Basel, Switzerland). For the initial screening at 0.1 and 1 μM, the activity, measured as the release of chlorophenol red over time, was calculated as percentage from DMSO control, which represented 100 % of *T. gondii* β-gal growth. For the IC₅₀ assays, the activity measured as the release of chlorophenol red over time was proportional to the number of live parasites down to 50% per well. IC₅₀ values were calculated after the logit-log-transformation of relative growth

and subsequent regression analysis. All calculations were performed using the corresponding software tool contained in the Excel software package (Microsoft). Cytotoxicity assays on uninfected confluent HFF were performed also in 96 well plates by exposing HFF to a concentration range of 0.1, 1, and for decoquinatone also 2.5 μ M and assessment of the viability by Alamar Blue assay.³³

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Additional data, crystallographic structures and tables (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interests. Use of copyrighted material from Figure 3C of *ACS Chem. Biol.*, **2011**, 6, 11, 1214-1222 (<https://pubs.acs.org/doi/10.1021/cb200105d>) adapted for Figure 1 of this publication was approved by ACS. Further permissions related to the material excerpted should be directed to the ACS.

ABBREVIATIONS

DCQ, decoquinatate; FcMDCQ, ferrocenylmethyl decoquinatate; RcMDCQ, ruthenocenylmethyl decoquinatate; PhMDCQ, phenylmethyl decoquinatate; FcVDCQ, ferrocenylvinylmethyl decoquinatate; RcVDCQ, ruthenocenylvinylmethyl decoquinatate; N-PhVDCQ, N-alkylated phenylvinylmethyl decoquinatate; O-PhVDCQ, O-alkylated phenylvinylmethyl decoquinatate; HFF, human foreskin fibroblasts; CTR, control; half maximal inhibitory concentration, IC₅₀.

REFERENCES

- (1) Tenter, A. M.; Heckerth, A. R.; Weiss, L. M. *Toxoplasma Gondii: From Animals to Humans*. *Int. J. Parasitol.* **2000**, 30, 1217–1258.
- (2) Schwartzman, J. D.; Maguire, J. H. *Toxoplasmosis*. In *Tropical Infectious Diseases: Principles, Pathogens and Practice*; W. B. Saunders: Edinburgh, U.K., 2011; pp 722–728.
- (3) Montoya, J. G.; Liesenfeld, O. *Toxoplasmosis*. *Lancet* **2004**, 363, 1965–1976.
- (4) Montoya, J. G.; Remington, J. S. Management of *Toxoplasma Gondii* Infection during Pregnancy. *Clin. Infect. Dis.* **2008**, 47, 554–566.
- (5) Duszynski, D. W.; Kvicerova, J.; Seville, R. S. Treatment and Drug Therapies of Coccidiosis in Carnivora. In *The Biology and Identification of the Coccidia (Apicomplexa) of Carnivores of the World*; Academic Press, 2018; pp 445–463.
- (6) Pfeifferkorn, E. R.; Borotz, S. E.; Nothnagel, R. F. Mutants of *Toxoplasma Gondii* Resistant to Atovaquone (566C80) or Decoquinatate. *J. Parasitol.* **1993**, 74 (4), 559–564.
- (7) Wang, H.; Li, Q.; Zhang, J.; Zeng, Q.; Zhang, P.; Xie, L.; Lee, P. J.; Roncal, N.; Melendez, V.; Hickman, M.; Kozar, M. P. Nanoparticle Formulations of Decoquinatate Increase Antimalarial Efficacy against Liver Stage Plasmodium Infections in Mice. *Nanomed. J.* **2014**, 10 (1), 57–65.
- (8) Kumar, S.; Kumari, R.; Pandey, R. *New Insight-Guided Approaches to Detect, Cure, Prevent and Eliminate Malaria*; 2015; Vol. 252.
- (9) Da Cruz, F. P.; Martin, C.; Buchholz, K.; Lafuente-Monasterio, M. J.; Rodrigues, T.; Sönnichsen, B.; Moriera, R.; Gamo, F. J.; Marti, M.; Mota, M. M. Drug Screen Targeted at Plasmodium Liver Stages Identifies a Potent Multistage Antimalarial Drug. *J. Infect. Dis.* **2012**, 205, 1278–1286.
- (10) Nam, T. G.; McNamara, C. W.; Bopp, S.; Dharia, N. V.; Meister, S.; Bonamy, G. M.; Plouffe, D. M.; Kato, N.; McCormack, S.; Bursulaya, B. A Chemical Genomic Analysis of Decoquinatate, a Plasmodium Falciparum Cytochrome b Inhibitor. *ACS Chem. Biol.* **2011**, 6, 1214–1222.
- (11) Beteck, R. M.; Seldon, R.; Coertzen, D.; van der Watt, M. E.; Reader, J.; Mackenzie, J. S.; Lamprecht, D. A.; Abraham, M.; Eribez, K.; Müller, J. Accessible and Distinct Decoquinatate Derivatives Active against Mycobacterium Tuberculosis and Apicomplexan Parasites. *Commun. Chem.* **2018**, 1, 62.
- (12) Beteck, R. M.; Coertzen, D.; Smit, F. J.; Birkholtz, L. M.; Haynes, R. K.; N'Da, D. D. Straightforward Conversion of Decoquinatate into Inexpensive Tractable New Derivatives with Significant Antimalarial Activities. *Bioorganic Med. Chem.* **2016**, 26, 3006–3009.

- (13) Patra, M.; Gasser, G. The Medicinal Chemistry of Ferrocene and Its Derivatives. *Nat. Rev. Chem.* **2017**, *1*, 0066.
- (14) Navarro, M.; Biot, C. Ligand Design in Medicinal Inorganic Chemistry. In *Metal-based Antimalarial Agents*; 2014.
- (15) Jaouen, G.; Vessières, A.; Top, S. Ferrocifen Type Anti Cancer Drugs. *Chem. Soc. Rev.* **2015**, *44* (24), 8802–8817.
- (16) Ong, Y. C.; Roy, S.; Andrews, P. C.; Gasser, G. Metal Compounds against Neglected Tropical Diseases. *Chem. Rev.* **2019**, *112* (2), 730–796.
- (17) Dubar, F.; Egan, T. J.; Pradines, B.; Kuter, D.; Ncokazi, K. K.; Forge, D.; Paul, J. F.; Pierrot, C.; Kalamou, H.; Khalife, J. The Antimalarial Ferroquine: Role of the Metal and Intramolecular Hydrogen Bond in Activity and Resistance. *ACS Chem. Biol.* **2011**, *6*, 275–287.
- (18) Dubar, F.; Biot, C. On the Molecular Mechanisms of the Antimalarial Action of Ferroquine. In *Bioorganometallic Chemistry: Applications in Drug Discovery, Biocatalysis, and Imaging*; Jaouen, G., Salmain, M., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA, 2015; pp 144–164. <https://doi.org/10.1073/pnas.78.8.4858>.
- (19) Chavain, N.; Vezin, H.; Dive, D.; Touati, N.; Paul, J. F.; Buisine, E.; Biot, C. Investigation of the Redox Behavior of Ferroquine, A New Antimalarial. *Mol. Pharm.* **2008**, *5*, 710–716.
- (20) Biot, C.; Taramelli, D.; Forfar-Bares, I.; Maciejewski, L. A.; Boyce, M.; Nowogrocki, G.; Brocard, J. S.; Basilico, N.; Olliaro, P.; Egan, T. J. Insights into the Mechanism of Action of Ferroquine, Relationship between Physicochemical Properties and Antiplasmodial. *Mol. Pharm.* **2005**, *2*, 185–193.
- (21) Hagen, H.; Marzenell, P.; Jentsch, E.; Wenz, F.; Veldwijk, M. R.; Mokhir, A. Aminoferrocene-Based Prodrugs Activated by Reactive Oxygen Species. *J. Med. Chem.* **2012**, *55* (2), 924–934.
- (22) Ocasio, C. A.; Sansook, S.; Jones, R.; Roberts, J. M.; Scott, T. G.; Tsoureas, N.; Coxhead, P.; Guille, M.; Tizzard, G. J.; Coles, S. J.; Hochegger, H.; Bradner, J. E.; Spencer, J. Pojamide: An HDAC3-Selective Ferrocene Analogue with Remarkably Enhanced Redox-Triggered Ferrocenium Activity in Cells. *Organometallics* **2017**, *36* (17), 3276–3283.
- (23) Gasser, G.; Metzler-Nolte, N. The Potential of Organometallic Complexes in Medicinal Chemistry. *Curr. Opin. Chem. Biol.* **2012**, *16* (1–2), 84–91. <https://doi.org/10.1016/j.cbpa.2012.01.013>.
- (24) Ramseier, J.; Imhof, D.; Anghel, N.; Hänggeli, K.; Beteck, R. M.; Balmer, V.; Ortega-Mora, L.-M.; Sanchez-Sanchez, R.; Ferre, I.; Haynes, R. K.; Hemphill, A. Assessment of the Activity of Decoquinone and Its Quinoline-O-Carbamate Derivatives against *Toxoplasma Gondii* In Vitro and in Pregnant Mice Infected with *T. Gondii* Oocysts. *Molecules* **2021**, *26* (21), 6393. <https://doi.org/10.3390/molecules26216393>.
- (25) Clark, R. C.; Reid, J. S. The Analytical Calculation of Absorption in Multifaceted Crystals. *Acta Cryst.* **1995**, *51* (887).
- (26) *CrysAlisPro*; Rigaku Oxford Diffraction Ltd: Yarnton, Oxfordshire, England, 2019.
- (27) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. A Complete Structure Solution, Refinement and Analysis Program. *J. Appl. Cryst.* **2009**, *49* (339).
- (28) Sheldrick, G. M. SHELXT - Integrated Space-Group and Crystal-Structure Determination. *Acta Cryst. A* **2015**, *71* (3).
- (29) Sheldrick, G. M. Crystal Structure Refinement with SHELXL. *Acta Cryst. C* **2015**, *71* (3).
- (30) Spek, A. L. Structure Validation in Chemical Crystallography. *Acta Cryst. D* **2009**, *65* (148).
- (31) Barna, F.; Debache, K.; Vock, C. A.; Küster, T.; Hemphill, A. In Vitro Effects of Novel Ruthenium Complexes in *Neospora Caninum* and *Toxoplasma Gondii* Tachyzoites. *Antimicrob. Agents Chemother.* **2013**, *57* (11), 5747–5754. <https://doi.org/10.1128/AAC.02446-12>.
- (32) McFadden, D. C.; Seeber, F.; Boothroyd, J. C. Use of *Toxoplasma Gondii* Expressing Beta-Galactosidase for Colorimetric Assessment of Drug Activity In Vitro. *Antimicrob. Agents Chemother.* **1997**, *41* (9), 1849–1853. <https://doi.org/10.1128/AAC.41.9.1849>.

- (33) Müller, J.; Aguado-Martinez, A.; Manser, V.; Balmer, V.; Winzer, P.; Ritler, D.; Hostettler, I.; Arranz-Solís, D.; Ortega-Mora, L.; Hemphill, A. Buparvaquone Is Active against *Neospora Caninum* in Vitro and in Experimentally Infected Mice. *Int. J. Parasitol. Drugs Drug Resist.* **2015**, 5 (1), 16–25. <https://doi.org/10.1016/j.ijpddr.2015.02.001>.

TABLE OF CONTENT

Organometallic functionalisation of the broad spectrum antiparasitic, decoquinone, has shed light on new possibilities for the improvement of the drug's efficacy against *Toxoplasma gondii*.

