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Antileishmanial and Antitrypanosomal Activities of the 8-Aminoquinoline Tafenoquine▽

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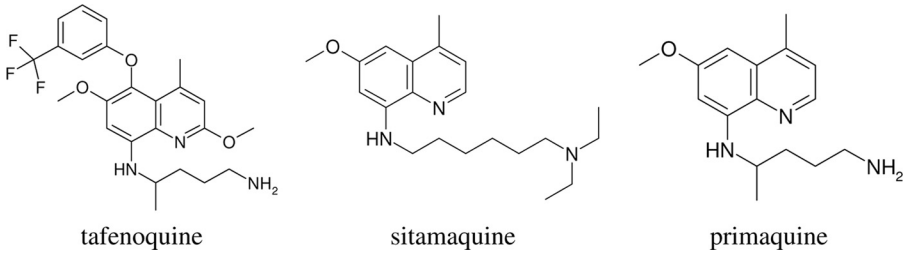
The 8-aminoquinoline tafenoquine showed significant *in vitro* activity against *Leishmania* species, including *L. donovani* amastigotes in macrophages, with 50% inhibitory concentrations (IC_{50}s) between 0.1 and 4.0 μM for both pentavalent antimony (SbV)-sensitive and SbV-resistant strains and by oral administration in BALB/c mice, with 50% effective dose (ED_{50}) values of 1.2 to 3.5 mg/kg for 5 days. Tafenoquine was less active against intracellular *Trypanosoma cruzi* amastigotes, with an IC_{50} of 21.9 μM.

The neglected tropical diseases leishmaniasis, Chagas' disease, and human African trypanosomiasis (HAT), caused by trypanosomatid parasites, have a limited number of drugs for treatment and control, all with limitations of toxicity, variable efficacy, long dosing regimens, and/or parenteral administration. Recent reviews have outlined the advances made in the chemotherapy of these diseases over the past decade for visceral leishmaniasis (VL) (1), cutaneous leishmaniasis (CL) (18), Chagas' disease (22), and human African trypanosomiasis (2).

The search for new treatments for these diseases has adopted various strategies, including rational design of drugs (7, 15), screening libraries of synthetic and natural products (11), and therapeutic switching. The more rapid development of a new treatment by the latter approach has been recently demonstrated for Chagas' disease with ergosterol biosynthesis inhibitors (22) and for leishmaniasis with miltefosine and paromomycin (8, 20). The 8-aminoquinolines (Fig. 1) have a long history as antiprotozoal drugs, in particular as antimalarials. Since the 1950s, several have also been reported as being active against *Leishmania* and *Trypanosoma* parasites (13, 21). Interest in the activity of this class of compounds for these diseases has been kept in focus by the clinical trials of sitamaquine (WR6026) for VL (12, 23). Sitamaquine also has anti-*Trypanosoma cruzi* activity (6). Research on another 8-aminoquinoline, NPC1161, has identified an enantiomer with significant antileishmanial activity and a lower toxicity profile (17). Tafenoquine (TFQ) (WR238605), developed, like many agents of this class, by the Walter Reed Army Institute of Research (WRAIR), is now in clinical trials for the radical cure of *Plasmodium vivax* by GlaxoSmithKline (GSK) and the Medicines for Malaria Venture (MMV) (16). We present here the results of studies of the *in vitro* and *in vivo* activities of TFQ against *Leishmania donovani* and *Trypanosoma cruzi*. Studies on the mechanism of action of TFQ against *Leishmania* and activity against *Trypanosoma brucei* subsp. will be reported elsewhere.

Early tests of TFQ against the promastigotes of different *Leishmania* species demonstrated 50% inhibitory concentrations (IC_{50}s) below 3 μM (data not shown). Of more clinical relevance, TFQ (GSK, United Kingdom) activity was evaluated, *in vitro*, against intracellular amastigotes of *L. donovani* MHOM/ET/67/HU3 (from East Africa), *L. donovani* MHOM/IN/82/DD8 (from India), and *L. donovani* BHU1 and BHU3 (antimony-resistant strains from India generously donated by Shyam Sundar). Infected murine peritoneal macrophages were exposed to the drug as previously described (24). The percent infection was calculated, and the IC_{50}s were derived (Prism). Subsequently, TFQ was further evaluated in the BALB/c mouse-*L. donovani* model of infection (9). Eight-



tafenoquine sitamaquine primaquine

FIG. 1. Structures of tafenoquine, sitamaquine, and primaquine.

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TABLE 1. Activity of TFQ enantiomers against *L. donovani* SbV[a]-sensitive strains by the amastigote-peritoneal exudate macrophage model

Drug (unit of activity)	IC_{50} (±95% confidence interval) for strain[b]:				Dose achieving cytotoxicity in KB cells
	HU3	DD8	BHU3	BHU11	
TFQ (μM)					
Racemate	1.75 ± 1.3	1.52 ± 1.02	2.26 ± 1.4	3.69 ± 0.9	6.6
Positive enantiomer	2.26 ± 1.8	3.28 ± 0.45	0.18 ± 3.3	ND[c]	7.4
Negative enantiomer	4.04 ± 2.2	2.56 ± 0.56	0.10 ± 0.85	ND	7.0
Sitamaquine (μM)	1.03 ± 1.02	1.86 ± 0.2	1.36 ± 0.56	2.35 ± 0.6	506
SbV (μg/ml)	4.49 ± 1.83	13.79 ± 10.17	>50	>50	>300
Miltefosine (μM)	2.01 ± 0.84	4.85 ± 3.89	1.07 ± 0.2	1.77 ± 0.2	31
Amphotericin B (μM)	0.03 ± 0.01	0.04 ± 0.05	0.02 ± 0.01	0.07 ± 0.2	0.7

[a] SbV, pentavalent antimony.
[b] Results for HU3 represent an average of four tests, and those for DD8 represent an average of two tests.
[c] ND, not done.

week-old, female mice (Charles River, United Kingdom) were infected with amastigotes harvested from a donor animal. After 7 days, the mice were treated with TFQ formulated in 10% Tween 80–ethanol (EtOH) 70:30 double-distilled water (ddH_2O), at 5 mg/kg, by the oral route, for 5 consecutive days. On day 14, the mice were euthanized and liver impression smears were made at necropsy. The amastigote burden was calculated (Leishman-Donovan units [LDUs]) (4), the percent inhibition was derived, and 50% effective dose (ED_{50}) values were calculated. TFQ hydrochloride (racemate batch R146390, positive enantiomer batch R206420, and negative enantiomer batch R206422) and sitamaquine tosylate (batch SLV3L004) were donated by GSK. Miltefosine was donated by Astra Zeneca, United Kingdom, and amphotericin B deoxycholate (Fungizone) was purchased from a commercial supplier. All *in vivo* experiments were carried out under license at the London School of Hygiene & Tropical Medicine (LSHTM) according to UK Home Office regulations.

The efficacy of TFQ against *T. cruzi* (Tulahuen-LacZ strain) (5) was tested against amastigotes *in vitro*. Peritoneal macrophages were infected with *T. cruzi* harvested from feeder cell layers and exposed to TFQ. β-Galactosidase activity was measured by the addition of Nonidet P-40 (detergent) and chlorophenol red β-D-thiogalactopyranoside (CPRG; developer). Ninety-six-well assay plates were read at 570 λ, and IC_{50}s were calculated. Benznidazole (Roche, Switzerland) was used as a positive control.

Both the racemate and positive and negative enantiomers of TFQ were active against intracellular amastigotes of all of the *L. donovani* strains tested (see Table 1 for IC_{50}s) and compared favorably with the standard drugs tested alongside. In the BALB/c mouse model, TFQ was equally active against both antimony-sensitive and antimony-resistant strains (BHU1 and BHU3), with no difference seen between the racemate and enantiomers. At 5 mg/kg, TFQ achieved 99% inhibition against all *L. donovani* species, with the enantiomers performing similarly. In a subsequent dose-response experiment, the ED_{50} values ranged from 1.01 to 3.5 mg/kg (Table 2).

We have shown that TFQ, an 8-aminoquinoline in development for the treatment of malaria (21) has, like other drugs of the same class, potential as an oral antileishmanial agent. In both *in vitro* and rodent models of *Leishmania* infection, TFQ had similar potency to sitamaquine, the drug currently in clinical development for VL, and NPC111B, which is in preclinical development (21). The limitation of this class has been toxicity, which is of special concern for glucose-6-phosphate dehydrogenase (G6PD)-deficient patients. The extensive antimalarial safety data for TFQ, along with clinical data on sitamaquine

TABLE 2. *In vivo* activities of TFQ, sitamaquine, and SbV[a] in *L. donovani*-BALB/c mouse models

Drug (dose)	In vivo activity (% inhibition [±95% confidence interval]) for strain:			ED_{50} (mg/kg) for strain DD8[b]
	HU3	BHU1	BHU3	
TFQ (5 mg/kg)[c]				
Racemate	99.32 ± 0.31	99.49 ± 0.66	100	1.47 ± 3.9
Positive enantiomer	99.12 ± 0.45	ND[d]		1.01 ± 9.7
Negative enantiomer	99.03 ± 0.52	ND		3.5 ± 4.8
Sitamaquine (5 mg/kg)	94.48 ± 0.29	ND	98.7 ± 0.6	2.2 ± 7.2
SbV (15 mg/kg)	70.93 ± 10.7	Inactive at 100 mg/kg	Inactive at 100 mg/kg	60% inhibition

[a] SbV, pentavalent antimony.
[b] Note that amphotericin B (AmBisome) given intravenously at 1 mg/kg for 3 days gives 93% inhibition.
[c] TFQ results represent oral administration for 5 days.
[d] ND, not done.

TABLE 3. *In vitro* activity of TFQ versus *T. cruzi*

T. cruzi strain	IC_{50}, μM (95% confidence interval)				
	TFQ			Sitamaquine	Benznidazole
	Racemate	Positive enantiomer	Negative enantiomer		
Tulahuen-LacZ	21.9 (3.6–40.3)	17.5 (5.5–29.5)	15 (4.6–48.2)	1.46 (0.93–2.31)	6.6 (1.56–27.6)

for VL, could support the design of appropriate treatment regimes for VL with TFQ. TFQ might also be an oral partner of interest in combination therapies for the treatment of VL (10, 19).

The activities of several series of 8-aminoquinolines against the causative pathogen of Chagas' disease have been published (13). Some have undergone preclinical development (23): for example, moxipraquine for Chagas' disease (3). Sitamaquine showed potential for prevention of *T. cruzi* transmission through blood transfusion, with activity against trypomastigotes at 4°C (6). We did not find TFQ to be as active *in vitro* against *T. cruzi* as other 8-aminoquinolines (Table 3), as others have previously reported (3, 13, 14).

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