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In vitro and *in vivo* activity of 3-alkoxy-1,2-dioxolanes against *Schistosoma mansoni*

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Objectives: Compounds characterized by a peroxidic skeleton are an interesting starting point for antischistosomal drug discovery. Previously a series of 3-alkoxy-1,2-dioxolanes, which are chemically stable cyclic peroxides, demonstrated significant *in vitro* activity against *Plasmodium falciparum*. We aimed to evaluate the potential of these compounds against *Schistosoma mansoni* and elucidate the roles of iron and peroxidic groups in activity.

Methods: Drugs were tested against juvenile and adult stages of *S. mansoni in vitro* and *in vivo*. Selected structures were assessed *in vitro* against schistosomes in the presence of additional iron sources. In addition, drugs were tested *in vitro* and *in vivo* against *Echinostoma caproni*, a non-blood-feeding intestinal fluke. Finally, the activity of non-peroxidic analogues was evaluated.

Results: Three dioxolanes displayed IC_{50}s $\leq$20.1 μM against adult schistosomes and values as low as 4.2 μM against newly transformed schistosomula. Nonetheless, only moderate, non-significant worm burden reductions were observed after treatment of mice harbouring adult infections. Drugs lacked activity against juvenile schistosomes *in vivo*. Two selected dioxolanes showed *in vitro* activity against *E. caproni* down to concentrations of 5 mg/L, but none of the compounds revealed *in vivo* activity. All tested non-peroxidic analogues lacked activity *in vitro* against both parasites.

Conclusions: Selected dioxolanes presented interesting *in vitro* activity, but low *in vivo* activities have to be overcome to identify a lead candidate. Although the inactivity of non-peroxidic analogues underlines the necessity of a peroxide functional group, incubation of adult schistosomes with additional iron sources did not alter activity, supporting an iron-independent mode of activation.

Keywords: chemotherapy, peroxides, non-peroxidic analogues, schistosomiasis

Introduction

Schistosomiasis is a neglected tropical disease caused by trematode flatworms of the genus *Schistosoma*. Five species of schistosomes infect humans, with *Schistosoma haematobium*, *Schistosoma japonicum* and *Schistosoma mansoni* being responsible for the main burden of schistosomiasis.[1] Approximately 200 million people are affected by schistosomiasis, mainly in sub-Saharan Africa.[2] Praziquantel is currently the gold standard for the treatment of schistosome infections. Because of the threat of drug resistance and the limitations in the activity profile of praziquantel (the drug lacks activity against the juvenile schistosome stages), identification of potential drug candidates has a high priority.

The antischistosomal potential of the antimalarials artemisinin and its semisynthetic derivatives artesunate and artemether has been studied thoroughly in the past three decades, in *in vitro* and *in vivo* studies, and in clinical trials.[3–5] Given the promising activity profile of the artemisinins, with particularly high activities observed against juvenile schistosomes, different groups of fully synthetic peroxides have been studied *in vivo* and *in vitro* in recent years.[6,7] For example, the synthetic trioxolane OZ418 was recently introduced as a promising drug candidate showing high worm burden reductions (WBRs) of 80% and 86% against *S. mansoni* and *S. haematobium*, respectively.[8]

The high antimalarial activity of 1,2,4-trioxolanes triggered investigations with 1,2-dioxolanes, which are structurally

analogous five-membered ring peroxides offering enhanced chemical stability. However, the dioxolanes proved much less active than the corresponding 1,2,4-trioxolanes against *Plasmodium falciparum in vitro* and *Plasmodium berghei in vivo*.[9] This reduction in activity has been attributed to a decreased tendency for scission of the alkoxy radicals derived from Fe(II) activation of the 1,2-dioxolane peroxide;[10] β-scission to generate carbon radicals is considered important for the activity of peroxide antimalarials.[11] 3-Alkoxy-1,2-dioxolanes, which undergo activation by Fe(II) to generate oxygen-substituted alkoxy radicals closely related to the intermediates derived from trioxolanes, generate products indicative of efficient β-scission and have been shown to have promising antimalarial efficacy.[10]

To our knowledge the antischistosomal activity of the 3-alkoxy-1,2-dioxolanes has not been studied to date. In the present work 18 selected 3-alkoxy-1,2-dioxolanes were tested on *S. mansoni in vitro* and active candidates were followed up *in vivo*. To investigate whether the peroxide pharmacophore is an essential requirement for antischistosomal activity, non-peroxidic analogues of active compounds were synthesized and their antischistosomal potential was determined. In addition, the relationship between the parasite's haemoglobin consumption and the antischistosomal activity of compounds was studied *in vitro* by testing active compounds on the non-blood-feeding foodborne trematode *Echinostoma caproni* and evaluating the *in vitro* activity of active compounds under different incubation conditions, in media containing haemin, haemoglobin or red blood cells.

Materials and methods

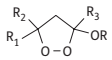
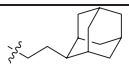
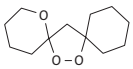
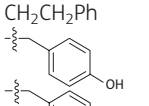
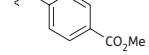
Drugs

The 18 3-alkoxy-1,2-dioxolane substrates illustrated in Table 1 were prepared based upon methods described by Schiaffo *et al*.[10] Two non-peroxidic analogues were prepared as illustrated in Figure S1 (available as Supplementary data at *JAC* Online).

Animals and parasites

Animal studies were conducted following Swiss regulations on animal welfare at the Swiss Tropical and Public Health Institute (Basel, Switzerland, permission no. 2070). Three-week-old (weight ∼14 g) female NMRI mice were purchased from Charles River (Sulzfeld, Germany) or Harlan Laboratories (Horst, The Netherlands). Before starting experiments, animals were allowed to adapt for 1 week under controlled conditions (temperature ∼22°C; humidity ∼50%; 12 h light and 12 h dark cycle; free access to rodent diet and water). Infection of mice with *S. mansoni* (Liberian strain) was performed subcutaneously by injection

Table 1. Chemical structures of investigated 3-alkoxy-1,2-dioxolanes and determined IC_{50} values (mean of three experiments) against newly transformed schistosomula (NTS) and adult *S. mansoni* 72 h post-compound exposure; *r* represents goodness of fit (conformity with $r \geq 0.85$)

					IC_{50} (μM) for *S. mansoni*			
Compound	R_1	R_2	R_3	R	NTS	*r*	adult	*r*
1	Me	Me	Me		31.1	0.9	11.9	1.0
2	Me	Bu	Me	CH_2CH_2Ph	7.5	1.0	12.4	1.0
3	-$(CH_2)_5$-		CH_2CH_2Ph	CH_2CH_2Ph	4.2	0.9	20.1	0.8
4	Me	Me	CH_2Ph	Me	101.0	0.7	49.2	1.0
5					17.0	0.7	76.4	1.0
6	Me	Me	Me	5,6-epoxyhexyl	35.9	0.9	101.5	1.0
7	Me	Me	Me	CH_2CH_2CN	89.2	1.0	137.0	1.0
8	-$(CH_2)_5$-		Me	$CH(OH)CH_2OH$	90.9	0.9	138.4	1.0
9	Me	Me	Me	$(CH_2)_9CO_2Me$	89.7	0.9	169.3	0.9
10	Me	Me	Me	$CH_2CH_2CO_2Me$	76.0	1.0	206.2	1.0
11	Me	Me	Me	CH_2CH_2OMe	69.2	0.8	207.4	0.9
12	Me	Me	Me	CH_2CH_2 CH_2OH	42.6	0.8	235.8	0.9
13	Me	Me	Me	$(CH_2)_4CO_2Me$	59.8	1.0	283.3	0.6
14	Me	Me	Me	$CH(OH)CH_2OH$	102.5	0.9	>436	
15	Me	Me	Me	CH_2CH_2Ph	2.7	0.9	58.4	0.9
16	-$(CH_2)_5$-		Me	CH_2CH_2Ph	2.8	0.9	49.4	0.9
17	-$(CH_2)_5$-		Me		4.1	0.9	71.8	1.0
18	Me	Me	Me		4.8	0.9	109.3	0.9
Praziquantel					2.2	0.9	0.08[a]	
Artesunate					5.0	0.9	41.2	0.8

[a]As described by Keiser *et al*.[26]

of 80–100 cercariae. Cercariae were harvested from infected intermediate host snails (*Biomphalaria glabrata*) by exposure to light for 3 h, following the standard procedures of our laboratory. For *in vitro* and *in vivo* studies with *E. caproni*, mice were infected intragastrically with 30 metacercariae harvested from infected *B. glabrata* snails.

In vitro screening

Preparation of NTS and adult schistosomes

NTS were obtained by mechanical transformation of *S. mansoni* cercariae.[12,13] Cercariae were collected as described above. The schistosomula suspension was adjusted to a concentration of 100 NTS per 50 μL with Medium 199 (Invitrogen, Carlsbad, CA, USA) supplemented with 5% heat-inactivated fetal calf serum (iFCS), 100 U/mL penicillin and 100 mg/L streptomycin (Invitrogen). To ensure completed conversion from cercariae to NTS, suspensions were incubated at 37°C in an atmosphere of 5% CO_2 in ambient air for a minimum of 12–24 h until use.[14]

Adult flukes were harvested from hepatic portal veins and mesenteric veins of infected NMRI mice (7–8 weeks post-infection) following standard procedures.[6] Schistosomes were placed in RPMI 1640 culture medium supplemented with 5% iFCS, 100 U/mL penicillin and 100 mg/L streptomycin at 37°C in an atmosphere of 5% CO_2 until use.

Preparation of adult E. caproni

Infected mice were killed 2 weeks post-infection. Trematodes were harvested from the excised small intestine and placed in RPMI medium supplemented with 100 U/mL penicillin, 100 mg/L streptomycin and 1% α-D-glucose (Sigma Aldrich, St Louis, MO, USA). Flukes were maintained at 37°C in an atmosphere of 5% CO_2 in ambient air until use.

Drug susceptibility assays with NTS

Drug dilution series with concentrations ranging from 0.37 to 90 mg/L (0.37, 1.1, 3.3, 10, 30, 90 mg/L) were prepared in 96-well flat-bottom plates (BD Falcon, USA) using supplemented medium (with iFCS and antibiotics). Prepared NTS suspension containing 100 NTS per 50 μL was added to each well to yield a total volume of 250 μL per well. The highest DMSO concentration (1.1%) used, diluted in Medium 199, served as control. Plates were incubated at 37°C in an atmosphere of 5% CO_2. NTS were evaluated by microscopic readout (Carl Zeiss, Germany, magnification ×80) with regard to death, changes in motility, viability and morphological alterations 24, 48 and 72 h post-drug exposure. As described previously, drug effects were evaluated using a viability scale.[12,13] Parasite fitness, morphology and motility were classified with scores ranging from 3 (normal activity, no morphological changes) to 0 (all worms dead). Duplicate examinations were performed for each concentration and experiments were repeated at least three times. IC_{50} values of the investigated drugs based on motility scale values obtained at the 72 h timepoint were determined using CompuSyn software (Version 3.0.1, 2007; ComboSyn, Inc.).

Drug susceptibility assay with adult schistosomes

In vitro screening on adult flukes was performed in 24-well flat-bottom plates (BD Falcon, USA). Supplemented RPMI 1640 medium (with iFCS and antibiotics) and drug stock solutions (10 mg/mL) were used to obtain final test concentrations of 1–90 mg/mL (1.1, 3.3, 10, 30, 90 mg/L) in wells with a final volume of 1.4 mL. Finally, three schistosomes of both sexes were added to each well. The highest concentration of DMSO (0.3%) in medium served as control. Twenty-four, 48 and 72 h post-drug exposure, phenotypes were monitored using the motility scale described by Ramirez *et al.*[15] and an inverse microscope (Carl Zeiss, Germany, magnification ×80). Experiments were performed three times. IC_{50} values were calculated with CompuSyn software as described above for NTS (Version 3.0.1, 2007; ComboSyn, Inc.).

Drug susceptibility assay of adult S. mansoni in the presence of haemin, haemoglobin or red blood cells

Lead candidates were incubated as described above, but using different RPMI culture medium conditions. A haemin solution (1.5 mM) was prepared as follows: 50 mg haemin chloride (Fluka Analytical, The Netherlands) was dissolved in 10 mL of 0.1 M NaOH, 0.5 mL of 1 M HCl and 39.5 mL of PBS (pH = 7.4). The haemoglobin solution (0.23 mM) was prepared using 750 mg haemoglobin from bovine blood (Sigma Aldrich, USA) dissolved in the same amounts of NaOH, HCl and PBS as used above. Finally, supplemented RPMI media were prepared by adding 8% haemin solution, 10% haemoglobin solution or 2% red blood cells from red blood cell concentrate (blood group A Rhesus positive) to final concentrations in well plates of 120 μM for haemin, 23 μM for haemoglobin or 2% for red blood cells. At timepoints 24, 48 and 72 h post-exposure, phenotypes were monitored using the motility scale as described above using an inverse microscope (Carl Zeiss, Germany, magnification ×80) and data were compared between various incubation conditions.[15]

Isothermal microcalorimetry (IMC) drug assay with adult S. mansoni

Two non-peroxidic analogues (compounds 19, 20) and one selected dioxolane (compound 16) were further characterized using IMC as described by Manneck *et al.*[16] Briefly, heat production and motility (derived from noise amplitudes) of schistosomes were measured using a 48-channel microcalorimeter (model TAM 48; TA Instruments, New Castle, DE, USA) over a period of 5 days. Samples were prepared in glass ampoules with 2900 μL of medium (supplemented RPMI 1640) containing four adult worms. Pre-warmed (37°C) ampoules were placed in channels and equilibration was performed for 12 h until a stable signal was observed. Drug suspensions (concentration of 900 mg/L) in supplemented medium (volume 100 μL) were injected, using 1 mL syringes (BD Plastipak, Becton Dickinson S.A., Madrid, Spain), to reach the final concentration of 30 mg/L per ampoule. Ampoules with dead worms served as the negative control and ampoules with worms treated with the highest concentration of DMSO (0.3%) served as the positive control. Heat flow was recorded as 1 data point per 1 min over at least 120 h. Tests at each concentration were performed three times.

Drug susceptibility assay with adult E. caproni

Assays were prepared in 24-well flat-bottom plates (Costar). Drug dilutions were prepared with drug stock solutions and supplemented RPMI medium (with antibiotics and glucose) to obtain final drug concentrations of 5–100 mg/L (5, 10, 50, 100 mg/L) in a total volume of 2 mL per well. Six to nine trematodes were used (one or two worms per well) for each experimental group. The highest concentration of DMSO (1%) served as control. Plates were incubated at 37°C in an atmosphere of 5% CO_2. Twenty-four, 48 and 72 h post-drug exposure, phenotypes and mortality of worms were monitored as described elsewhere.[12]

In vivo screening

Studies with S. mansoni

Groups of four infected NMRI mice characterized by a patent schistosome infection (49 days post-infection) or a juvenile *Schistosoma* infection

(21 days post-infection) were treated orally with the test drug using single oral doses of 400 mg compound per kg body weight. Seven to nine untreated mice served as controls. Animals harbouring an adult *Schistosoma* infection were killed by the CO_2 method 14 days post-treatment, and mice treated at the juvenile infection stage were sacrificed 4 weeks post-treatment. Mice were then dissected and worms sexed and counted.[6] Worm burdens of treated mice were compared with those of untreated animals and reductions of worm burden calculated.

Studies with E. caproni

Four NMRI mice were treated intragastrically with 400 mg/kg of test compounds 2 weeks post-infection with *E. caproni*. Four mice were left untreated and served as controls. One week post-treatment, mice were euthanized with CO_2. At necropsy, the intestines were removed from the pylorus to the ileocaecal valve, placed in a Petri dish and opened longitudinally. All *E. caproni* worms were removed and counted.

Statistics

Parasite motility of treated and untreated NTS and adult trematodes was calculated as mean (±SD) using Microsoft Excel software. Motility data obtained from experiments with various media containing iron sources were compared using the Mann–Whitney test (considered significant at $P \leq 0.05$). IC_{50} values were determined using the CompuSyn software (Version 3.0.1, 2007; ComboSyn, Inc.). For the comparison of IC_{50} values we used the Kruskal–Wallis test (considered significant at $P \leq 0.05$). The Kruskal–Wallis test was also used for *in vivo* studies, comparing the medians of the responses between the treatment and control groups. A difference in median was considered to be significant at a significance level of 5% (StatsDirect statistical software, version 2.7.2.; StatsDirect Ltd, UK). Noise amplitudes and heat flows observed in calorimetric *in vitro* assays with adult *S. mansoni* were analysed using R software and Microsoft Excel. As described by Manneck *et al.*,[17] noise amplitude values follow an exponential decay. Endpoints of worm motility were determined by the intersection of the sample amplitude curve with the background signal noise of dead worms.

Results

In vitro screening against S. mansoni

In a first step, 14 3-alkoxy-1,2-dioxolanes were studied against adult *S. mansoni in vitro*. Three of the compounds (1, 2 and 3) demonstrated good antischistosomal activity with IC_{50}s of 11.9, 12.4 and 20.1 μM, respectively, against adult flukes. The remaining 11 substances revealed only minor activity (IC_{50}s between 49.2 and 283.3 μM) or lacked activity (compound 14). Good activities were observed against the juvenile schistosome stage (NTS) for compounds 2 (IC_{50} 7.5 μM) and 3 (IC_{50} 4.2 μM). Moderate activity was detected for compounds 1 (IC_{50} 31.1 μM), 5 (IC_{50} 17.0 μM) and 6 (IC_{50} 35.9 μM) against NTS. Finally, only minor activity was recorded for the remaining nine compounds against NTS (IC_{50} 42.6–102.5 μM). *In vitro* findings are summarized in Table 1. For comparison, activities of standard antischistosomal drugs, praziquantel and artesunate, are also shown in Table 1.

Influence of haemin, haemoglobin or red blood cells on in vitro antischistosomal activity

Incubation of adult schistosomes with various concentrations (1.1, 3.3, 10, 30 mg/L) of the three active alkoxydioxolanes (1–3) in supplemented media containing haemin, haemoglobin or red blood cells showed no significant differences in activity. The motilities recorded 72 h post-exposure of *S. mansoni* with compound 1 at different concentrations did not vary among the different media tested (Figure 1). Slightly higher, though not significant ($P > 0.05$) activities were detected for compounds 2 and 3 when incubated in the presence of haemin or haemoglobin. In the presence of these media all worms had died 72 h post-exposure to the tested compounds at a concentration of 10 mg/L and 30 mg/L.

In vivo activity of selected alkoxydioxolanes against S. mansoni

The three lead structures 1–3 identified by prior *in vitro* screening, were tested in a juvenile as well as an adult *S. mansoni* infection mouse model (Table 2). All studied alkoxydioxolanes lacked *in vivo* activity against juvenile *S. mansoni* (Table 2; WBRs 0%–4%). However, moderate, non-significant activities were observed with compounds 1 and 2 against adult *S. mansoni* with WBRs of 42.5% and 37.0%, respectively. Compound 3 showed only low activity against adult *S. mansoni in vivo* with a WBR of 15.1%. Dead worms were detected in mouse livers after treatment of adult infections with compounds 1, 2 and 3. The presence of a patent schistosoma infection in treated animals was confirmed by observing granulous tissue and *Schistosoma* eggs within all livers.

In vitro and in vivo activity of modified lead dioxolanes against S. mansoni

Given the low *in vivo* activity of the three test drugs, we were motivated to investigate four additional chemically related alkoxydioxolanes *in vitro* against NTS and adult schistosomes, followed by *in vivo* studies on a patent *S. mansoni* infection. All four compounds (15–18) showed very high activities against NTS, represented by low IC_{50} values (2.7–4.8 μM) (summarized in Table 1). However, the four drugs revealed only low activities against adult schistosomes *in vitro*, with IC_{50}s between 49.4 and 109.3 μM. Moderate, but non-significant, *in vivo* WBRs of 32.0% and 21.3% were achieved with compounds 15 and 17, respectively. Low activities with WBRs of 12.0% and 16.4% were observed with compounds 16 and 18, respectively (summarized in Table 3).

In vitro and in vivo effect against E. caproni

Lead structures 1–3 as well as the four related structures (15–18) were tested *in vitro* on freshly harvested *E. caproni*. Data are summarized in Table 4. Six of seven compounds (1, 3, 15–18) showed 100% worm mortality 24 h post-drug exposure at a concentration of 50 mg/L. Two of the compounds (16 and 17) killed all worms 24 h post-incubation at a 5-fold lower concentration of 10 mg/L. Incubation of adult *E. caproni* with three spirocyclohexyl compounds (3, 16 and 17) at 5 mg/L for 72 h resulted in death of all worms. Compounds 15–17 were followed

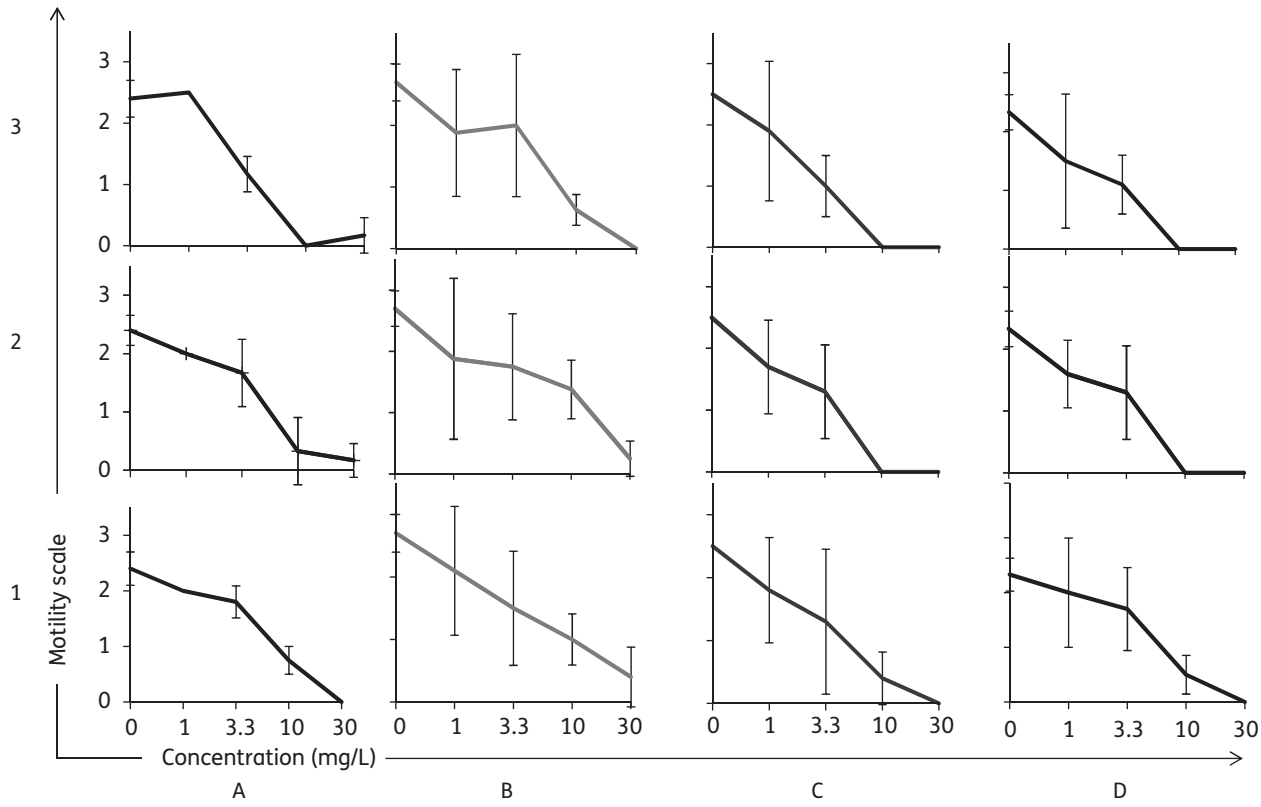


Figure 1. Motility of adult schistosomes 72 h post-treatment with various concentrations (1.1, 3.3, 10 and 30 mg/L) of compounds 1, 2 or 3 in four different incubation settings. (a) Standard incubation with supplemented RPMI. (b) Addition of 2% red blood cells from red blood cell concentrate. (c) Addition of 120 μM haemin. (d) Addition of 23 μM haemoglobin.

Table 2. *In vivo* activity following a single oral dose of 400 mg/kg of selected 3-alkoxy-1,2-dioxolanes in mice harbouring juvenile or adult *S. mansoni*

Group	No. of mice investigated	No. of mice that died	No. of mice cured	TWR (%)	*P* value	FWBR (%)	*P* value
Control	8	0	0	–	–	–	–
Juvenile infection							
1	4	0	0	0	0.73	0	0.86
2	4	0	0	4.1	0.67	5.9	0.38
3	4	0	0	0	0.87	0	0.44
Adult infection							
1	4	0	0	42.5	0.27	47.1	0.23
2	4	0	0	37.0	0.35	41.2	0.35
3	4	0	0	15.1	0.86	17.7	0.73

TWR, total worm burden reduction; FWBR, female worm burden reduction.

up *in vivo*. Treatment of *E. caproni*-infected mice with a single oral dose of 400 mg/kg of compound 15 resulted in a low WBR of 17.4%. Compounds 16 and 17 lacked *in vivo* activity (Table 5).

Table 3. *In vivo* activity following a single oral dose of 400 mg/kg of selected 3-alkoxy-1,2-dioxolanes in mice harbouring adult *S. mansoni*

Group	No. of mice investigated	No. of mice that died	No. of mice cured	TWR (%)	*P* value	FWBR (%)	*P* value
Control[a]	7	0	0	–	–	–	–
Control[b]	9	0	0	–	–	–	–
15[a]	4	1	0	32.0	0.1	37.8	0.1
16[a]	4	1	0	12.0	0.7	5.4	0.7
17[a]	4	1	0	21.3	0.4	35.1	0.3
18[b]	4	0	0	16.4	0.9	11.9	0.7

TWR, total worm burden reduction; FWBR, female worm burden reduction.
[a]Versus control.
[b]Versus control.

In vitro activity of non-peroxidic analogues

To elucidate the role and necessity of the peroxide core of the alkoxydioxolanes for trematocidal activity we tested the *in vitro* activity of two alkoxy-substituted tetrahydrofurans (19, 20) prepared as non-peroxidic analogues of the alkoxydioxolanes. Both derivatives lacked activity at concentrations of 30 and 90 mg/L

Table 4. *In vitro* mortality of *E. caproni* worms at timepoints 24, 48 and 72 h post-drug treatment with selected compounds

Drug	Drug concentration (mg/L)	No. of worms observed	Percentage of worms that died after indicated time			
			0 h	24 h	48 h	72 h
Control	–	20	0	0	0	0
1	100	8	0	100		
	50	8	0	100		
	10	7	0	43	100	
	5	8	0	25	75	88
2	100	6	0	100		
	50	6	0	0	100	
	10	6	0	17	67	100
	5	7	0	0	29	57
3	100	6	0	100		
	50	7	0	100		
	10	7	0	57	100	
	5	9	0	22	100	
15	100	6	0	100		
	50	6	0	100		
	10	7	0	86	100	
	5	8	0	0	38	75
16	100	6	0	100		
	50	6	0	100		
	10	7	0	100		
	5	6	0	50	67	100
17	100	6	0	100		
	50	7	0	100		
	10	6	0	100		
	5	7	0	0	100	
18	100	6	0	100		
	50	7	0	100		
	10	6	0	67	83	100
	5	6	0	0	17	33

Table 5. *In vivo* activity following a single oral dose of 400 mg/kg of selected 3-alkoxy-1,2-dioxolanes against *E. caproni* in mice

Group	No. of mice investigated	No. of mice cured	Worms	TWR (%)	*P* value
Control	4	0	23.0 (0)	–	–
15	4	0	19.0 (7.2)	17.4	0.2
16	4	0	27.8 (4.5)	0	0.2
17	4	0	23.0 (7.8)	0	0.5

TWR, total worm burden reduction.

against *S. mansoni*. Also, *E. caproni* was not affected at 50 and 100 mg/L. No effect against adult *S. mansoni* could be observed by microscopic examination 72 h post-treatment as well as by IMC 6 days after treatment. According to IMC, no loss of motility was detected for either non-peroxidic compound over an incubation period of 6 days (concentration 30 mg/L). For comparison, treatment of adult *S. mansoni* with 30 mg/L of the peroxidic analogue of compound 19 (compound 16) revealed a reduction in heat flow and complete loss of motility was detected 90 h post-exposure (Figure 2).

Discussion

The antischistosomal activity of semisynthetic artemisinins, frequently used in malaria treatment, synthetic trioxolanes and hybrid molecules of quinines and trioxanes has been well described.[7,8] Compounds characterized by a peroxidic skeleton are therefore an interesting starting point for antischistosomal drug discovery. Hence, we were interested in elucidating the antischistosomal potential of recently introduced alkoxydioxolanes as well as their structural needs for activity against schistosomes.[10]

Three of 14 compounds tested (compounds 1–3) showed promising *in vitro* activity against adult *S. mansoni* flukes. Two of these compounds (2 and 3) also revealed very good efficacy against the juvenile stage (NTS) *in vitro*. The antischistosomal *in vitro* activity seems to be enhanced by the presence of a bulky substituent at C3, since the three lead compounds all feature a large alkoxide side chain at C3. The remaining non-active 11 compounds do not display this feature. This finding is in accordance with observations reported by Schiaffo *et al.*[10] on the antimalarial structure–activity relationship of similar compounds. These studies revealed that antimalarial activity is enhanced by a spirocyclohexyl group at C5/C5′ and by the presence of a steric bulk at C3. However, for antischistosomal activity it does not seem to matter whether substitution at C5/C5′ is a dimethyl (compound 2) or spirocyclohexyl (compound 3) group. On the other hand, it is interesting to note that, with regard to *E. caproni*, the three most active compounds (3, 16 and 17) all display spirocyclohexyl units.

In our *in vivo* studies we observed only moderate, non-significant activities of alkoxydioxolanes in *S. mansoni*-infected NMRI mice. Compound 1 showed the highest *in vivo* activity against adult *S. mansoni*, achieving a total WBR of 42.5% and a female WBR of 47.1%. In contrast to results reported for artemisinins, surprisingly low WBRs were observed against juvenile *S. mansoni* harboured in mice.[18] A recent study conducted with a library of dioxolanes revealed that the majority of the compounds tested against rat and human microsomes were metabolized rapidly. It was concluded that significant optimization of the groups attached to the dioxolane core is needed before a viable candidate for drug development could be identified.[19] Hence, it is likely that the alkoxydioxolanes tested in this study also exhibit bioavailability problems which can likely play a role in limiting *in vivo* activities.

In the present work we also studied the activity of alkoxydioxolanes in the presence of various additional iron sources, since haemoglobin metabolism, the potential target of these drugs, is a common feature of both *Schistosoma* and *Plasmodium*

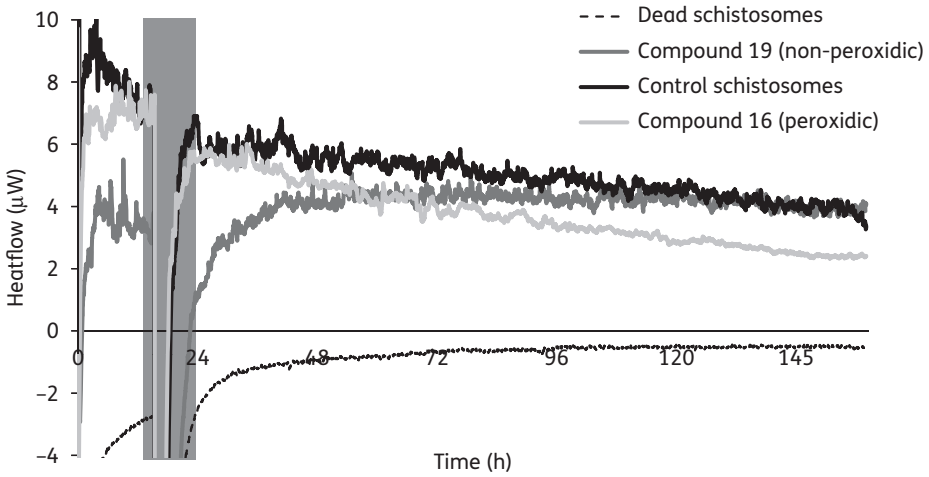


Figure 2. Course of heat flow (μW) over time (h) after treatment of schistosomes with compound 19 (non-peroxidic) or compound 16 (peroxidic). Control schistosomes correspond to schistosomes treated with DMSO at the same concentration as used for drugs (0.3%). Amplitudes of curves represent the motility of schistosomes.

spp.[20] Earlier studies investigated the relationship between Fe(II) reactivity, the efficiency of haem alkylation and antimalarial activity for various peroxides *in vitro*. The authors stated that Fe(II) reactivity for the tested peroxide heterocycles is a necessary, but insufficient, property of antimalarial peroxides,[21] and the alkoxydioxolanes have been shown to undergo cleavage to alkoxy radicals in the presence of iron(II).[10] Interestingly, we did not observe significant differences in susceptibility of the tested agents in the different iron-source-containing media. This finding is in contrast to the haemin-dependent antischistosomal *in vitro* effect of the trioxolane OZ78 on *S. mansoni* and *S. japonicum*, whereas similar haemin-independent activity was described for OZ209.[6,22] Similarly, it was recently shown that the antimalarial arylmethanol mefloquine, a drug class also described to interfere with haemoglobin degradation in *Plasmodium*, revealed a 57-fold lower IC_{50} in the presence of haemoglobin against adult *S. mansoni in vitro*.[23] Our results suggest that the alkoxydioxolanes possess an iron-independent mechanism of action on schistosomes *in vitro*. This discovery is supported by the observed high *in vitro* activities of three compounds (3, 16 and 17) at concentrations as low as 5 mg/L against the non-haematophagous intestinal fluke *E. caproni*.

Nonetheless, the results with the isosteric compounds that lack a peroxide functional group underline the necessity of the peroxidic core for trematocidal activity. Both compounds lacked *in vitro* activity against adult *S. mansoni* and *E. caproni*. Similar results were recently demonstrated in studies with the liver fluke *Fasciola hepatica*. While OZ78 has excellent *in vitro* and *in vivo* activity against *F. hepatica*, its non-peroxidic analogue failed to show an effect against the fluke.[24] The peroxidic feature seems therefore to play a role in the iron-independent mode of action. The basis for the iron-independent activity of the alkoxydioxolanes is unclear. These molecules, like all peroxides, are oxidants, and in principle capable of reaction with strongly nucleophilic or reducing agents. Alternatively, it is possible that the alkoxydioxolanes undergo activation via acid-catalysed ring opening of the peroxyacetal core to generate a more reactive 3-hydroperoxyketone; a similar model has been proposed to account for the antimalarial activity of artemisinin.[25]

In conclusion, we have demonstrated that a number of alkoxydioxolanes are characterized by good *in vitro* antischistosomal activity and non-significant *in vivo* effects on *S. mansoni*, with compound 1 being the most promising candidate. Similarities, but also differences, exist between antimalarial and antischistosomal activity of alkoxydioxolanes. The peroxidic bond is essential to antischistosomal activity, but activation of the molecules seems to be independent of iron. The low *in vivo* activity of this drug class, which may result from limited bioavailability, represents a challenge that would need to be overcome in order to identify an antischistosomal lead candidate.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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