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Design, synthesis and biological evaluation of praziquantel and endoperoxide conjugates as antischistosomal agents

Background: The widespread use of praziquantel for the treatment of schistosomiasis has led to concerns over the potential development of drug resistance. Therefore, the discovery of novel antischistosomal agents is imperative. In this study, a series of praziquantel and endoperoxide conjugates were synthesized and evaluated as potential antischistosomal agents. **Results:** Some compounds exhibited high efficacy against both adult and juvenile *Schistosoma*, in *in vitro* studies. Structure-activity relationship (SAR) analysis revealed that compounds with amide bond linker and cyclopentyl adjacent to the 1,2,4,5-tetraoxane pharmacophore displayed the highest efficacy. Overall, compounds showed consistent activity against *Schistosoma japonicum* and *Schistosoma mansoni*. *In vivo* study resulted in moderate but statistically significant activity. **Conclusion:** Important preliminary results were obtained from thorough activity evaluation of praziquantel-endoperoxide conjugates. Further pharmacokinetic property investigation is necessary to improve *in vivo* efficacy.

Schistosomiasis is listed by WHO as one of the 17th neglected tropical diseases (NTDs) [1]. The approximate global prevalence of schistosomiasis is 200 million and the annual death of this disease is about two hundred thousand. The disease is endemic in 76 countries worldwide, mainly in South America, southeast Asia and especially Africa, where infections are mediated through contact with cercariae released from infected snails in water. There are three main human pathogenic schistosome species, namely, *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium*.

Chemotherapy is the mainstay to fight against schistosome-acquired infections. After over 40 years of application, praziquantel (PZQ) (1, Figure 1) is still the only drug of choice for the treatment of schistosomiasis. This drug is highly effective against all three species of schistosomes at the adult stage, while barely efficient against the immature parasites [2,3]. Nonetheless, PZQ resistant or poorly sensitive strains have been identified in African countries [4,5] and resistant strain was also induced in the laboratory [6]. There

are concerns that the widespread use of PZQ could potentially lead to emergence of resistant parasites in the near future. Hence, there is an urgent need for new drugs with novel mechanism of action.

Recently, there is an increasing interest in the discovery of agents that can concomitantly address more than one biological target for NTD treatment. It was believed that hybridization of two or more known pharmacophores would endow the new entity with functions from both selected templates [7]. For example, a hybrid compound named Trioxaquantel (2, Figure 1) and its derivatives were expected to possess antischistosomal activity [8]. A series of compounds named Trioxaquinone (3, Figure 1) were designed and tested for their antimalarial and antischistosomal activity [9,10]. Conjugates bearing PZQ and synthetic endoperoxide pharmacophore were evaluated for antimalarial activity [11]. Organometallic derivatives of PZQ (4, Figure 1) were designed and evaluated as antischistosomal agents [12,13]. However, not all of these designed conjugates demonstrated expected biological activity.

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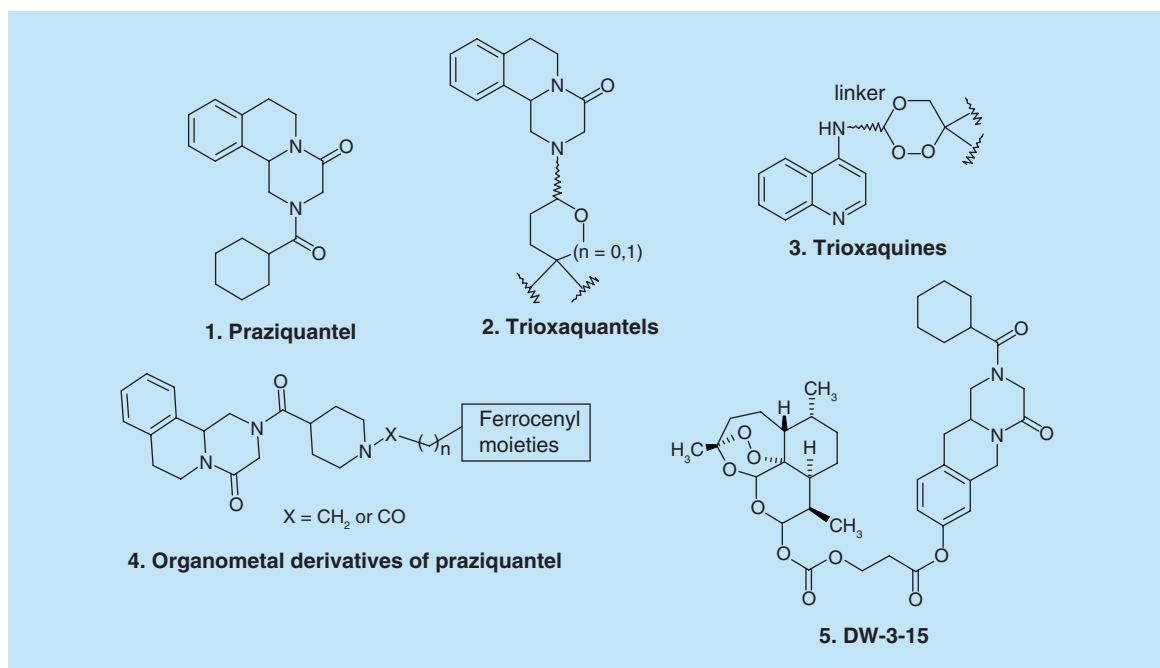


Figure 1. Structures of (1) Praziquantel, (2) Trioxaquantels, (3) Trioxaquinines, (4) Organometal derivatives of praziquantel and (5) DW-3-15.

This fact clearly emphasizes the need for a fundamentally different approach for the discovery of novel **PZQ derivatives** against schistosomiasis.

In the course of investigating PZQ derivatives as antischistosomal agents, we have reported DW-3-15 (5, Figure 1), an artesunate and PZQ hybrid, as a novel and promising prodrug with good efficacy against both adult and juvenile schistosomes [14,15]. However, DW-3-15 has a molecular weight >700 Dalton, which is much higher than the Lipinski defined MW500 for a drug-like molecule. Moreover, the artemisinin derivative artesunate has limited natural resources. Low molecular weight surrogates of the artesunate endoperoxide

pharmacophore obtainable by chemical preparation were extensively studied and reported. These structures include the 1,2,4,5-tetraoxane, 1,2,4-trioxolane and 1,2,4-trioxanes. Especially, as an effective antimalarial candidate, the synthetic compound OZ439 with 1,2,4-trioxolane pharmacophore offers new hope for a single-dose cure of uncomplicated malaria [16]. Inspired by these findings, we report here the design and synthesis of a series of endoperoxide and PZQ conjugates. We anticipated that they would have increased bioavailability, and thereby improved antischistosomal activity, by reducing the molecular weight. The antischistosomal activity of these compounds was evaluated both *in vitro*

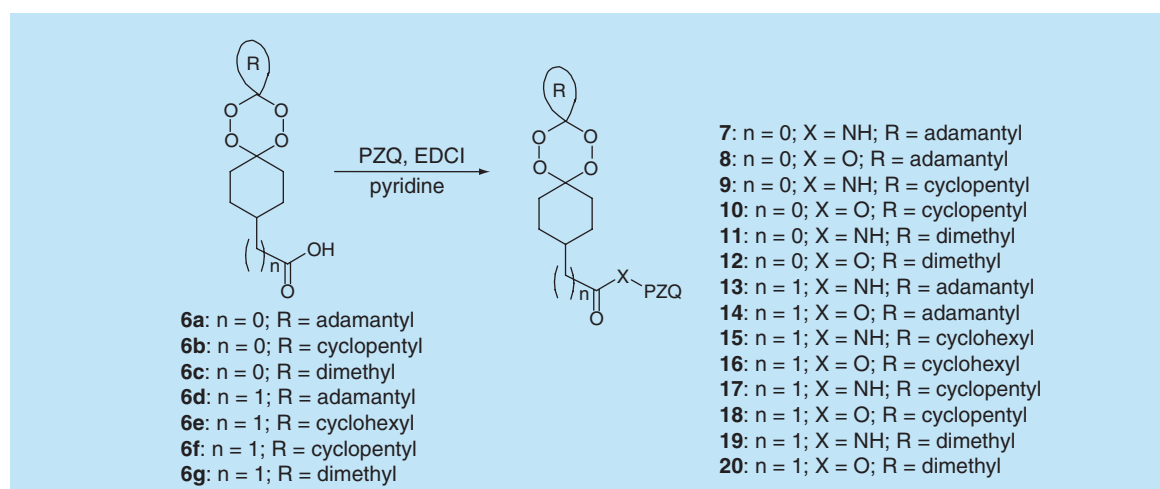


Figure 2. Chemical synthesis route for compounds 7-20.

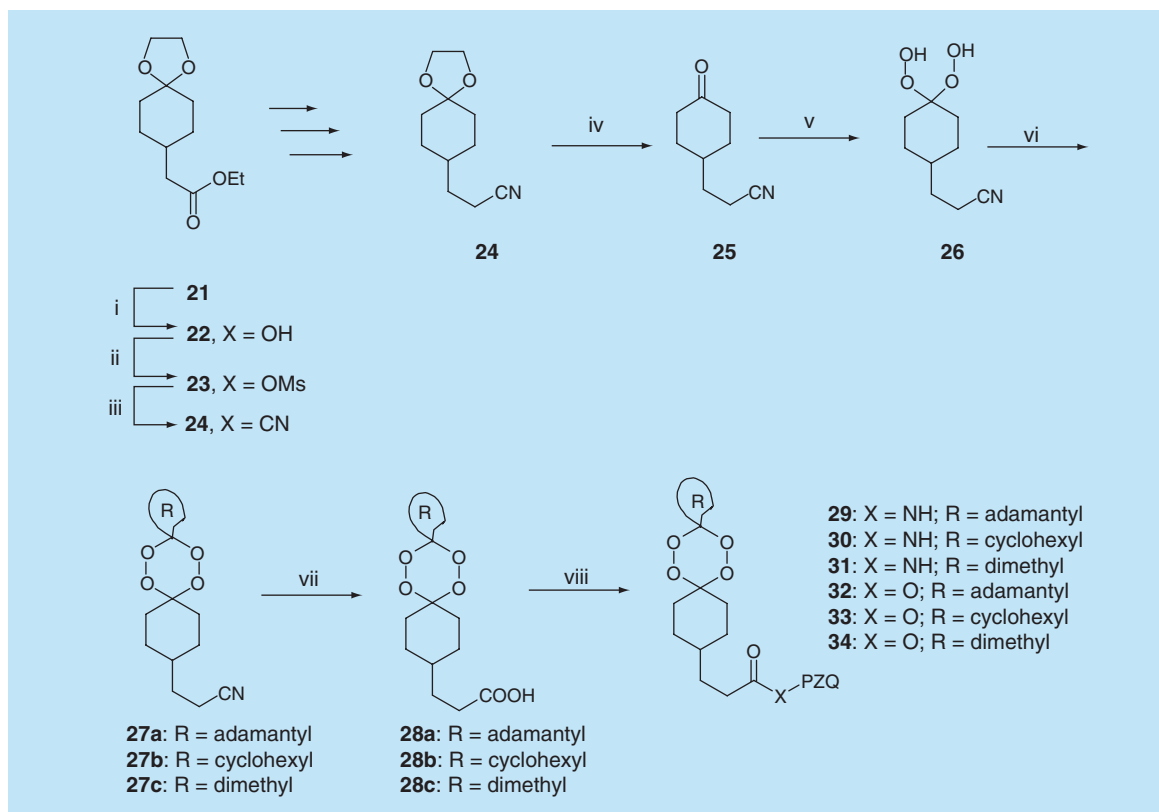


Figure 3. Chemical synthesis route for compounds 29–34.

and *in vivo*. A comparative study on *in vitro* activities in two parasite species, *Schistosomamansoni* and *Schistosoma japonicum*, was also conducted.

Results & discussion

Chemistry

Reaction intermediates **6a**, **6b** and **6c** were prepared according to our previously described procedure [17]. Intermediates **6d**, **6e**, **6f** and **6g** were prepared according to a method reported by Amewu [18]. Condensation of the above carboxylic acids with 10-OH PZQ or 10-NH₂ PZQ [14] in the presence of coupling reagent EDCI afforded target compounds 7–20 (Figure 2).

Carboxylic acids **28a–c** bearing the 1,2,4,5-tetraoxane moiety were synthesized in 7-step (Figure 2) starting from **21**. Lithium aluminum hydride (LAH) reduction of the ester group gave the terminal alcohol **22** (90%), which was followed by transformation of alcohol to mesylate (86%) and subsequent substitution by sodium cyanide afforded cyanide **24** (80%), that was treated with HCl to provide the ketone **25** (55%). The 1,1-dihydroperoxide derivative **26** was obtained by reaction of ketone with 30% H₂O₂ in 40% yield. Reaction of **26** with the corresponding ketone provided 1,2,4,5-tetraoxanes **27a–c**. Treatment of **27a–c** with NaOH converted the cyanide group to carbox-

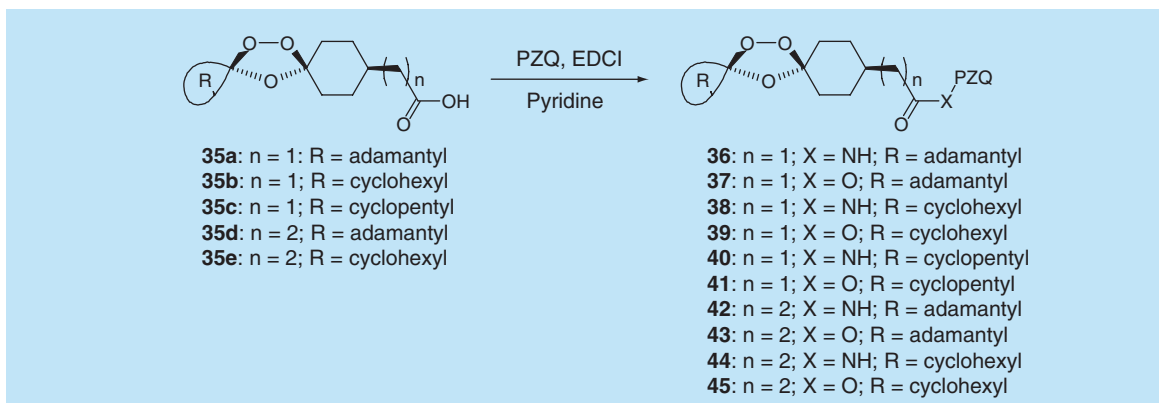


Figure 4. Chemical synthesis route for compounds 36–45.

Key terms:

Hybrid: Conjugate which is composed of and possesses functionality from two different pharmacophores.

Antischistosomal agent: Compound which can kill or shows efficacy against schistosoma.

Praziquantel derivatives: Compounds derived from praziquantel, or compounds containing the praziquantel moiety.

ylic acid to give **28a–c**. Finally, coupling of 10-NH₂ or 10-OH PZQ with the above carboxylic acids afforded the synthetic target compounds **29–34** (Figure 3).

Synthesis conditions and reagents: LAH, THF, 0°C; MsCl, TEA, THF; KCN, DMSO, 120°C; HCl, acetone; 30% H₂O₂, conc. HCl, DCM, CH₃CN; ketone, PMA, MgSO₄, DCM; NaOH, EtOH; PZQ, EDCl, pyridine.

Ozonide acids **35a–e** were prepared using the method reported by Jonathan [19]. Compounds **36–45** were prepared using the above mentioned EDCl coupling method (Figure 4).

In vitro evaluation of compound activity

Activity against *S. japonicum* in vitro

All prepared compounds were evaluated for their *in vitro* ability against both adult and juvenile *Schistosoma japonicum* according to a previously described method [20]. The results of repeated experiments against adult worm are shown in Table 1.

As shown in Table 1, most compounds displayed efficient worm killing ability. Specifically, compounds **9, 11, 12, 15, 17, 19, 31, 33** and **44** exhibited better potency than positive control PZQ: these compounds killed all worms within 72 h at concentrations lower than 25 μM, and several compounds displayed good efficacy at 5 μM. The activity of **7, 20, 34, 38, 40** and **45** was comparable to that of PZQ: at 25 μM, these compounds reduced the worm mobility score to <20%. The rest of the compounds displayed weaker worm killing capability. Overall, male worms were more vulnerable to these hybrid compounds.

In terms of structure activity relationship, several observations were concluded: compounds with amide bond linker displayed higher potency than compounds with ester linker (**9** vs **10, 15** vs **16, 17** vs **18, 19** vs **20, 31** vs **34, 38** vs **39, 40** vs **41**); the inhibitory activity of compounds with the CH₂CH₂ linker displayed low potency compared with those with shorter linkers (**15** vs **30, 16** vs **33, 19** vs **31, 20** vs **34, 36** vs **42** and **37** vs **43**); compounds bearing the cyclopentyl moiety exhibited higher inhibitory potency compared with those with cyclohexyl, adamantyl and isopropyl

moieties (**40** vs **36** and **38; 41** vs **37** and **39; 9** vs **11; 17** vs **19, 15** and **13; 18** vs **20, 16** and **14**); compounds with the 1,2,4,5-tetraoxane pharmacophore exhibited higher worm killing ability compared with compounds with 1,2,4-trioxane pharmacophore (**38** vs **15, 39** vs **16, 40** vs **17**).

Assessment of compound activity against juvenile worm in vitro

The inhibitory effect of these compounds against juvenile *Schistosoma japonicum* *in vitro* was also evaluated, and the result was displayed in Table 2.

Based on the above data, it was concluded that the compounds exhibited weak activity against juvenile worm compared with the adult worm, suggesting that the adult worms were more sensitive to these compounds *in vitro*. In general, compounds showed consistent activity against juvenile and the adult worm. Notably, compound **17** exhibited the most potent worm killing ability against the juvenile worms.

In order to infer the activity spectrum of these PZQ-endoperoxide conjugates, we have evaluated *in vitro* the activities of these compounds against adult *Schistosoma mansoni* as well (Table 3).

As shown in Table 3, the compounds can be classified into three categories: compounds killing all worms within 24 h (compounds **8, 9, 10, 11, 12, 17, 31, 34, 45**), compounds killing all worms within 72 h (compounds **19, 30, 33**) and poorly efficient compounds with worms remaining alive 72 h post treatment. Interestingly, among the nine most efficient compounds for the two parasite species, five of them (compounds **9, 11, 12, 17, 31**) are common.

In vivo efficacy evaluation in infected mice model

The effects of single 400 mg/kg oral dose of selected conjugates on worm burden reductions in 35-day-old adult and 21-day-old juvenile *S. japonicum* harbored in mice were evaluated, and the results are summarized in Table 4 and Table 5, respectively. Although conjugates **9, 16, 19** and **44** showed high efficacy against the adult worm *in vitro*, these compounds had no or low effect *in vivo*. Conjugates **11, 15, 17** and **31** showed >20%, but <35%, of worm killing activity against adult *S. japonicum*. Conjugates **17** and **19** had no statistically significant worm reduction effect against the juvenile worm. By comparison, conjugates **7, 9** and **11** showed significant activities on juvenile *S. japonicum*.

Overall, the *in vivo* activity of all conjugates was lower than that of the art-PZQ conjugate DW-3–15 [15]. The high *in vitro* activity was not transformed into high *in vivo* worm killing activity. This suggests that either these conjugates have a poor oral bioavailability, or different drug administration mode should be pursued

Table 1. Effects of the compounds on adult <i>Schistosoma japonicum</i> <i>in vitro</i> .								
Compound (No.)	Concentration (μM)	Worm [†]	24 h		48 h		72 h	
			Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
Control	50	♂	100	100	100	100	100	100
DMSO		♀	100	100	100	100	100	100
7	50[‡]	♂	100	33.3	75.0	33.3	0	0
		♀	100	66.7	25.0	8.3	0	0
	25	♂	100	50.0 \pm 10.0	40.0 \pm 20.0	13.3 \pm 13.3	0	0
		♀	100	66.7 \pm 23.0	100	41.7 \pm 12.3	25.0 \pm 25.0	8.3 \pm 8.3
8	50[‡]	♂	100	73.3	60.0	60.0	60.0	46.7
		♀	75.0	25.0	75.0	25.0	25.0	8.3
9	25[‡]	♂	25.0	8.3	0	0	0	0
		♀	0	0	0	0	0	0
	10[‡]	♂	100	50.0	0	0	0	0
		♀	100	33.3	0	0	0	0
	5	♂	100	66.7 \pm 13.3	0	0	0	0
		♀	100	33.3 \pm 33.3	0	0	0	0
10	50[‡]	♂	80.0	26.7	0	0	0	0
		♀	25.0	8.3	0	0	0	0
	25	♂	100	33.3 \pm 8.3	100	33.3 \pm 8.3	100	33.3 \pm 8.3
		♀	100	33.3 \pm 16.6	100	33.3 \pm 16.6	100	33.3 \pm 16.6
11	25	♂	100	50.0 \pm 25.0	0	0	0	0
		♀	100	33.3 \pm 33.3	0	0	0	0
	10	♂	100	58.3 \pm 15.3	20.0 \pm 10.0	6.7 \pm 3.3	0	0
		♀	100	60.0 \pm 20.0	50.0 \pm 10.0	16.7 \pm 3.3	0	0
12	25	♂	100	33.3 \pm 33.3	100	33.3 \pm 33.3	0	0
		♀	100	33.3 \pm 33.3	0	0	0	0
	10	♂	100	33.3 \pm 33.3	100	33.3 \pm 6.7	50	16.7 \pm 16.6
		♀	100	33.3 \pm 33.3	100	33.3 \pm 6.7	50	16.7 \pm 8.3
13	50[‡]	♂	100	91.7	100	91.7	100	66.7
		♀	100	66.7	100	66.7	100	33.3
14	50[‡]	♂	100	33.3	20.0	6.7	20.0	6.7
		♀	100	66.7	50.0	16.7	100	33.3
15	25	♂	100	33.3 \pm 8.3	0	0	0	0
		♀	100	33.3 \pm 8.3	0	0	0	0
	10	♂	100	66.7 \pm 16.7	0	0	0	0
		♀	100	33.3 \pm 6.7	0	0	0	0
16	50[‡]	♂	0	0	0	0	0	0

[†]For each group, 4–5 male or female worms were used.
[‡]Experiments were performed once. The others were done three times, the average \pm SD is given. The most efficient compounds are shown in bold font. See supporting information for percent mobile worm and mobility score definitions.
PZQ: Praziquantel.

Table 1. Effects of the compounds on adult <i>Schistosoma japonicum in vitro</i> (cont.).								
Compound (No.)	Concentration (μ M)	Worm [†]	24 h		48 h		72 h	
			Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
		♀	0	0	0	0	0	0
	25	♂	100	33.3 ± 6.7	100	33.3 ± 6.7	25.0 ± 25.0	8.3 ± 8.3
		♀	50	33.3 ± 6.7	100	33.3 ± 6.7	0	0
	10	♂	100	33.3 ± 33.3	75.0 ± 25.0	25.0 ± 5.0	75.0 ± 25.0	25 ± 22.1
		♀	100	16.7 ± 6.7	0	0	0	0
17	25	♂	25	8.3 ± 8.3	0	0	0	0
		♀	50	33.3 ± 33.3	50.0 ± 25.0	16.7 ± 16.7	0	0
	10	♂	75	25.0 ± 25.0	0	0	0	0
		♀	25	8.3 ± 8.3	0	0	0	0
	5	♂	100	44.4 ± 22.2	66.7 ± 33.3	22.2 ± 11.1	0	0
		♀	100	33.3 ± 6.7	50.0 ± 25.0	16.7 ± 16.7	25 ± 0.0	8.3 ± 8.3
18	50	♂	0 [‡]	0 [‡]	0 [‡]	0 [‡]	0 [‡]	0 [‡]
		♀	0 [‡]	0 [‡]	0 [‡]	0 [‡]	0 [‡]	0 [‡]
	25	♂	100	33.3 ± 16.7	100	33.3 ± 16.7	25 ± 0.0	25 ± 22.1
		♀	100	33.3 ± 16.7	100	33.3 ± 16.7	25 ± 0.0	25 ± 8.3
19	25	♂	100	33.3 ± 16.7	0	0	0	0
		♀	100	33.3 ± 16.7	33.3 ± 33.3	11.1 ± 6.7	0	0
	10	♂	50.0 ± 25.0	16.7 ± 16.7	0	0	0	0
		♀	0	0	0	0	0	0
	5	♂	100	66.7 ± 33.3	100	66.7 ± 33.3	100	41.6 ± 14.4
		♀	100	33.3 ± 33.3	50.0 ± 25.0	16.7 ± 16.7	25.0 ± 25.0	8.3 ± 8.3
20	50 [‡]	♂	0	0	0	0	0	0
		♀	0	0	0	0	0	0
	25	♂	100	33.3 ± 16.7	100	33.3 ± 16.7	50.0 ± 0.0	16.7 ± 22.1
		♀	100	33.3 ± 16.7	75.0 ± 25.0	25.0 ± 22.1	75.0 ± 25.0	25.0 ± 22.1
29	50 [‡]	♂	100	100	100	100	100	58.3
		♀	100	100	100	93.3	100	53.3
32	50 [‡]	♂	0	0	0	0	0	0
		♀	0	0	0	0	0	0
	25	♂	100	33.3 ± 33.3	100	33.3 ± 33.3	25 ± 25	8.3 ± 0
		♀	100	33.3 ± 33.3	50.0 ± 33.3	16.7 ± 16.7	0	0
	10	♂	100	33.3 ± 33.3	100	33.3 ± 33.3	100	33.3 ± 8.3
		♀	100	66.7 ± 8.3	100	66.7 ± 8.3	100	66.7 ± 8.3

[†]For each group, 4–5 male or female worms were used.

[‡]Experiments were performed once. The others were done three times, the average \pm SD is given. The most efficient compounds are shown in bold font. See supporting information for percent mobile worm and mobility score definitions.

PZQ: Praziquante.

Table 1. Effects of the compounds on adult <i>Schistosoma japonicum</i> <i>in vitro</i> (cont.).								
Compound (No.)	Concentration (μ M)	Worm [†]	24 h		48 h		72 h	
			Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
31	25	♂	100	41.7 ± 8.3	0	0	0	0
		♀	100	33.3 ± 6.7	0	0	0	0
	10	♂	100	50.0 ± 25.0	50.0 ± 25.0	25.0 ± 12.5	0	0
		♀	75.0 ± 25.0	25.0 ± 25.0	0	0	0	0
30	50 [‡]	♂	100	33.3	100	33.3	100	33.3
		♀	100	33.3	100	33.3	100	33.3
33	50 [‡]	♂	0	0	0	0	0	0
		♀	0	0	0	0	0	0
	25	♂	50.0 ± 25.0	16.7 ± 16.7	0	0	0	0
		♀	25.0 ± 25.0	8.3 ± 8.3	0	0	0	0
	10	♂	100	66.7 ± 33.3	100	55.6 ± 10.4	33.3 ± 33.3	22.2 ± 22.2
		♀	100	66.7 ± 33.3	40.0 ± 20.0	13.3 ± 13.3	40.0 ± 20.0	13.3 ± 13.3
34	50 [‡]	♂	100	33.3	100	33.3	25	8.3
		♀	100	33.3	0	0	0	0
	25	♂	100	33.3 ± 0.0	100	33.3 ± 0.0	75.0 ± 25.0	25.0 ± 22.1
		♀	100	33.3 ± 0.0	50.0 ± 25.0	16.7 ± 16.7	25.0 ± 0.0	8.3 ± 8.3
36	50 [‡]	♂	100	66.7	100	33.3	25.0	8.3
		♀	100	66.7	100	66.7	50.0	16.7
	25	♂	100	77.5 ± 2.5	83.3 ± 16.7	44.4 ± 11.1	66.7 ± 33.3	33.3 ± 8.3
		♀	100	66.7 ± 16.7	100	33.3 ± 8.3	100	33.3 ± 8.3
37	50 [‡]	♂	40.0	15.3	0	0	0	0
		♀	100	46.7	0	0	0	0
	25	♂	100	66.7 ± 33.3	100	50.0 ± 12.5	100	33.3 ± 8.3
		♀	100	33.3 ± 8.3	100	33.3 ± 8.3	100	33.3 ± 8.3
38	50 [‡]	♂	0	0	0	0	0	0
		♀	0	0	0	0	0	0
	25	♂	100	66.7 ± 33.3	66.7 ± 33.3	22.2 ± 11.1	33.3 ± 33.3	11.1 ± 11.1
		♀	100	50.0 ± 25.0	100	33.3 ± 33.3	50.0 ± 25.0	16.7 ± 22.1
39	50 [‡]	♂	0	0	0	0	0	0
		♀	25	8.3	0	0	0	0
	25	♂	100	83.3 ±	100	41.7 ±	100	33.3 ± 8.3
		♀	100	66.7 ±	100	66.7 ±	100	58.3 ± 22.1
40	50 [‡]	♂	75.0	58.3	0	0	0	0

[†]For each group, 4–5 male or female worms were used.
[‡]Experiments were performed once. The others were done three times, the average ±SD is given. The most efficient compounds are shown in bold font. See supporting information for percent mobile worm and mobility score definitions.
PZQ: Praziquantel.

Table 1. Effects of the compounds on adult <i>Schistosoma japonicum in vitro</i> (cont.).								
Compound (No.)	Concentration (μM)	Worm [†]	24 h		48 h		72 h	
			Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
25		♀	75.0	25.0	25.0	8.3	25.0	8.3
		♂	100	44.4 ±11.1	0	0	0	0
10		♀	100	66.7 ±33.3	100	33.3 ±33.3	50.0 ±25.0	16.7 ±16.7
		♂	100	75.0 ±25.0	75.0 ±25.0	41.7 ±8.3	75.0 ±25.0	33.3 ±8.3
41	50 [‡]	♀	100	83.3 ±16.7	100	50.0 ±25.0	100	50.0 ±25.0
		♂	100	8.3	0	0	0	0
25		♀	100	8.3	0	0	0	0
		♂	100	33.3 ±16.7	100	33.3 ±16.7	75.0 ±25.0	25.0 ±25.0
42	50 [‡]	♀	100	33.3 ±16.7	100	33.3 ±16.7	75.0 ±25.0	25.0 ±25.0
		♂	100	40.0	40.0	13.3	0	0
25		♀	100	40.0	0	0	0	0
		♂	100	66.7 ±33.3	80.0 ±10.0	46.7 ±23.3	60.0 ±20.0	20.0 ±6.7
43	50 [‡]	♀	100	66.7 ±33.3	100	41.7 ±16.7	100	33.3 ±8.3
		♂	100	40.0	80.0	20.0	25.0	13.3
25		♀	100	58.3	100	25.0	0	0
		♂	100	100	100	100	100	100
44	25 [‡]	♀	100	75.0 ±25.0	100	75.0 ±25.0	100	75.0 ±25.0
		♂	0	0	0	0	0	0
10		♀	0	0	0	0	0	0
		♂	100	60.0 ±10.0	20.0 ±10.0	16.7 ±16.7	0	0
45	50 [‡]	♀	75.0 ±25.0	25.0 ±25.0	0	0	0	0
		♂	25	8.3	0	0	0	0
25		♀	25	8.3	0	0	0	0
		♂	75.0 ±25.0	25.0 ±25.0	75.0 ±25.0	25.0 ±25.0	75.0 ±25.0	25.0 ±25.0
PZQ	50 [‡]	♀	75.0 ±25.0	25.0 ±25.0	75.0 ±25.0	25.0 ±25.0	50.0 ±25.0	16.7 ±8.3
		♂	100	33.3	0	0	0	0
25		♀	100	33.3	0	0	0	0
		♂	50.0 ±25.0	16.7 ±16.7	50.0 ±25.0	16.7 ±16.7	0	0
			50.0 ±25.0	16.7 ±16.7	50.0 ±25.0	16.7 ±16.7	25.0 ±25.0	8.3 ±8.3

[†]For each group, 4–5 male or female worms were used.
[‡]Experiments were performed once. The others were done three times, the average ±SD is given. The most efficient compounds are shown in bold font. See supporting information for percent mobile worm and mobility score definitions.
 PZQ: Praziquante.

Table 2. Effects of compounds on 14 day juvenile <i>Schistosoma japonicum</i> <i>in vitro</i> .							
Compound (No.)	Concentration (μ M)	24 h		48 h		72 h	
		Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
Control		100	100	100	100	100	100
7	50 [†]	100	55.6	33.3	11.1	0	0
	25	100	50.0 \pm 25.0	50.0 \pm 10.0	16.7 \pm 8.4	16.7 \pm 16.7	5.6 \pm 5.6
	10	100	33.3 \pm 16.5	100	33.3 \pm 6.5	40 \pm 20	13.3 \pm 6.7
8	50 [†]	100	33.3	100	33.3	100	33.3
9	50 [†]	55.6	27.8	0	0	0	0
	25	22.2	7.4 \pm 3.5	22.4 \pm 7.5	7.4 \pm 3.8	5.6 \pm 5.6	1.9 \pm 1.9
	10	100	63.3 \pm 10.3	84.6 \pm 4.5	48.7 \pm 24.3	50 \pm 10	16.7 \pm 3.3
10	50	100 [†]	58.3 [†]	100 [†]	33.3 [†]	100 [†]	33.3 [†]
11	50 [†]	37.5	12.5	0	0	0	0
	25	100	66.7 \pm 11.1	100	55.6 \pm 24.5	66.7 \pm 11.1	33.3 \pm 3.7
	10	100	52.4 \pm 26.2	100	52.4 \pm 26.2	100	52.4 \pm 14.2
12	50 [†]	100	33.3	100	33.3	100	33.3
13	50 [†]	100	100	100	100	100	100
14	50 [†]	100	66.7	100	66.7	100	55.6
15	50 [†]	83.3	55.6	50.0	22.2	50.0	22.2
16	50 [†]	100	33.3	100	33.3	100	33.3
17	50 [†]	37.5	12.5	0	0	0	0
	25	50	36.7 \pm 3.5	50.0 \pm 10.0	20.0 \pm 5.0	10.0 \pm 10.0	3.3 \pm 6.6
	10	100	48.5 \pm 10.2	54.5 \pm 27.2	18.2 \pm 4.3	0	0
18	50 [†]	0	0	0	0	0	0
	25	56.3 \pm 10.3	33.3 \pm 12.3	56.3 \pm 18.7	31.1 \pm 12.3	50.0 \pm 6.7	22.2 \pm 6.7
	10	100	33.3 \pm 14.5	100	33.3 \pm 13.5	84.0 \pm 8.0	28.0 \pm 4.0
19	50 [†]	57.1	19	0	0	0	0
	25	100	66.7 \pm 11.1	72.7 \pm 28.3	39.4 \pm 32.4	36.4 \pm 18.2	21.2 \pm 9.1
	10	100	66.7 \pm 22.2	100	60.0 \pm 10.0	60.0 \pm 20.0	33.3 \pm 6.7
20	50 [†]	0	0	0	0	0	0
	25	100	55.6 \pm 15.6	100	44.4 \pm 12.6	100	44.4 \pm 5.6
	10	100	66.7 \pm 12.3	100	66.7 \pm 12.3	100\pm	66.7 \pm 4.8
29	50 [†]	100	100	100	100	100	66.7
30	50 [†]	100	33.3	100	33.3	100	33.3
31	50 [†]	60.0	40.0	40.0	20.0	0	0
	25	84.6 \pm 5.2	71.8 \pm 10.5	46.2 \pm 21.3	17.9 \pm 25.6	30.8 \pm 15.4	10.8 \pm 7.7
	10	100	90.5 \pm 3.2	100	71.4 \pm 18.2	85.7 \pm 14.3	52.4 \pm 9.5
32	50 [†]	100	56.7	100	43.3	100	43.3
33	50 [†]	100	45.8	75.0	12.5	12.5	4.2

[†]Experiments were performed once. The others were done three times, the average \pm SD is given. The most efficient compounds are in bold font. See supporting information for mobile worm and mobility score definitions. For each group, 6–8 worms were used. Art: Artesunate; PZQ: Praziquantel.

Table 2. Effects of compounds on 14 day juvenile *Schistosoma japonicum* in vitro (cont.)

Compound (No.)	Concentration (μ M)	24 h		48 h		72 h	
		Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
34	50 [†]	100	38.1	100	38.1	100	38.1
36	50 [†]	100	83.3	50.0	16.7	0	0
	25	100	93.3 \pm1.2	100	66.7 \pm15.8	10.0 \pm20.0	3.3 \pm16.7
	10	100	84.8 \pm5.6	100	56.0 \pm26.0	100	45.5 \pm13.6
37	50 [†]	100	70.8	93.8	56.3	87.5	52.1
38	50 [†]	21.4	7.1	0	0	0	0
	25	100	98.6 \pm 0.2	100	85.5 \pm 3.0	65.2 \pm 13.0	55.1 \pm 8.7
39	50 [†]	80.0	26.7	60.0	12.0	0	0
	25	66.7 \pm22.3	29.6 \pm32.0	55.6 \pm28.0	25.9 \pm32.5	33.3 \pm22.2	11.1 \pm11.1
	10	100	33.3 \pm12.6	100	33.3 \pm12.6	85.7 \pm14.2	42.9 \pm9.5
40	50 [†]	20.0	6.7	0	0	0	0
	25	100	66.7 \pm11.1	85.7 \pm4.3	38.1 \pm11.6	42.9 \pm28.6	19.0 \pm19.0
	10	100	66.7 \pm33.3	100	66.7 \pm33.3	100	33.3 \pm20.0
41	50 [†]	33.3	11.1	0	0	0	0
	25	100	33.3 \pm14.3	92.3 \pm5.1	30.8 \pm23.8	53.8 \pm15.4	17.9 \pm10.3
	10	100	37.5 \pm12.5	100	37.5 \pm12.5	100	37.5 \pm12.5
42	50 [†]	100	66.7	90.0	60.0	70.0	26.7
43	50 [†]	100	66.7	100	66.7	100	66.7
44	50 [†]	14.2	4.8	0	0	0	0
	25	100	33.3 \pm17.7	10.0 \pm5.0	3.3 \pm3.3	0	0
	10	100	83.3 \pm6.7	100	66.7 \pm15.7	93.8 \pm12.5	47.9 \pm8.3
45	50 [†]	100	51.9	100	51.9	100	51.9
PZQ	50	100	33.3 \pm 0.0	100	33.3 \pm 0.0	100	33.3 \pm 0.0
	25	100	33.3 \pm 0.0	100	33.3 \pm 0.0	100	33.3 \pm 0.0
Art	50	100	100	100	100	100	100

[†]Experiments were performed once. The others were done three times, the average \pm SD is given. The most efficient compounds are in bold font. See supporting information for mobile worm and mobility score definitions. For each group, 6–8 worms were used.
Art: Artesunate; PZQ: Praziquante.

to achieve higher worm killing efficacy. Meanwhile, the *in vivo* metabolic stability or pharmacokinetic parameters of the compounds should be further optimized.

Conclusion

In summary, we have designed and prepared a series of endoperoxide and PZQ conjugates. The *in vitro* activity of these compounds against both adult and juvenile *S.*

japonicum, and against *S. mansoni* as well, was thoroughly investigated. In general, these compounds exhibited consistent activity to both species of worms. Although some conjugates exhibited high efficacy at low concentration, the promising *in vitro* efficacy leads to significant but moderate worm burden reduction activity *in vivo*. Nevertheless, this research provided important insights into antischistosomal agents which executed the hybrid drug design rationale.

Key terms:

Peroxide: Compound containing the oxygen–oxygen single bond.

Future perspective

Schistosomiasis is listed as one of the 17th NTDs. Since the 1970s, praziquantel has been the only drug

Table 3. Effects of the compounds at 50 μ M on adult *Schistosoma mansoni* *in vitro*.

Compound (No.)	24 h		48 h		72 h	
	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)	Mobile worm (%)	Mobility score (%)
7	93.8 \pm 6	53.1 \pm 9	12.5 \pm 13	6.3 \pm 6	0	0
8	0	0	0	0	0	0
9	6.3 \pm6	3.1 \pm3	0	0	0	0
10	0	0	0	0	0	0
11	12.5 \pm13	6.3 \pm6	0	0	0	0
12	0	0	0	0	0	0
13	100	59.4 \pm 3	75.0 \pm 25	37.5 \pm 13	43.86	21.9 \pm 3
14	100	71.9 \pm 9	87.5 \pm 13	56.3 \pm 6	68.8 \pm 6	46.9 \pm 9
15	100	90.6 \pm 9	100	87.5 \pm 6	100	87.5 \pm 6
16	100	100	100	100	93.8 \pm 6	93.8 \pm 6
17	100	84.4 \pm9	0	0	0	0
18	100	100	100	68.8 \pm 6	62.5 \pm 13	31.3 \pm 6
19	100	100	25 \pm 13	12.5 \pm 6	0	0
20	100	100	50 \pm 0	25 \pm 0	18.8 \pm 6	9.4 \pm 3
29	100	71.9 \pm 3	25.0 \pm 0	12.5 \pm 0	12.5 \pm 13	6.3 \pm 6
30	100	87.5 \pm 13	18.8 \pm 19	9.4 \pm 9	0	0
31	37.5 \pm13	18.8 \pm6	0	0	0	0
32	100	71.9 \pm 9	31.3 \pm 6	15.6 \pm 3	25 \pm 13	12.5 \pm 6
33	100	59.4 \pm 9	12.5 \pm 13	6.3 \pm 6	0	0
34	12.5 \pm13	6.3 \pm6	0	0	0	0
36	87.5 \pm 0	62.5 \pm 6	68.8 \pm 19	43.8 \pm 13	12.5 \pm 13	6.3 \pm 6
37	100	100	87.5 \pm 13	75 \pm 19	75 \pm 19	50 \pm 0
38	31.3 \pm 6	15.6 \pm 3	50 \pm 13	25 \pm 6	37.5 \pm 0	18.8 \pm 0
39	100	100	100	90.6 \pm 3	100	87.5 \pm 0
40	100	87.5 \pm 13	68.8 \pm 6	40.6 \pm 3	25.0 \pm 13	12.5 \pm 6
41	100	100	100	84.4 \pm 3	100	71.9 \pm 3
42	87.5 \pm 13	43.8 \pm 6	81.3 \pm 19	40.6 \pm 19	75 \pm 13	37.5 \pm 6
43	100	100	37.5 \pm 0	21.9 \pm 3	25 \pm 0	12.5 \pm 0
44	50.0 \pm 13	28.1 \pm 3	37.5 \pm 0	18.8 \pm 0	6.3 \pm 6	3.1 \pm 3
45	62.5 \pm38	37.5 \pm25	0	0	0	0
Praziquante	0	0	0	0	0	0
Control	100	100	100	100	100	100

The most efficient compounds are in bold. See supporting information for mobile worm and mobility score definitions. For each group, eight worms were used. Experiments were performed twice, the averages \pm SD are given.

of choice for the treatment of this disease. Recently, *Schistosoma* with low sensitivity or resistance to praziquantel have been observed and reported. Therefore, there is an urgent need for the discovery of new and effective antischistosomal drugs.

The present study reported the design, synthesis and evaluation a series of synthetic **peroxide** and

praziquantel conjugates with high worm inhibition activity against both *S. japonicum* and *S. mansoni*. Although highly efficient *in vitro*, these compounds did not display promising worm killing ability *in vivo*. This research provided insights into antischistosomal drug discovery using the hybrid drug design rationale. Consequently, efforts to search for new

Table 4. Effect of single 400 mg/kg oral dose of the compounds on mice harboring 35 day-old adult *Schistosoma japonicum*.

Compounds	Number of detected worms/mice (mean \pm SD)	Worm burden reduction rate (%)
Control	43 \pm 3.9	0
9	41 \pm 1.7	3.9
11	32 \pm 3.5	25.1**
15	30 \pm 4.9	29.3**
16	39 \pm 4.0	10.0
17	29 \pm 1.6	32.2**
19	37 \pm 2.8	14.7*
31	31 \pm 3.0	28.6**
44	37 \pm 3.9	14.4*
Praziquante	14 \pm 3.9	67.7**

Five mice were used in each group. T-test was used to compare each compounds with the control group (*p < 0.05, **p < 0.01).

Table 5. Effect of single 400 mg/kg oral dose of the compounds on mice harboring 14 day-old juvenile *Schistosoma japonicum*.

Compounds	Number of detected worms/mice (mean \pm SD)	Worm burden reduction rate (%)
Control	39 \pm 5.9	0
7	23 \pm 4.4	42.8**
9	26 \pm 2.3	35.2**
11	31 \pm 3.9	21.5*
17	32 \pm 3.6	20.2
19	36 \pm 1.1	9.4
44	39 \pm 3.6	0
Artesunate	10 \pm 0.8	74.7**

Five mice were used in each group. T-test was used to compare each compounds with the control group (*p < 0.05, **p < 0.01).

hybrid molecules as antischistosomal agents need to be considered. Meanwhile, further studies concerning the compound pharmacokinetic property as well as bioavailability should be pursued and combined in the following research.

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Executive summary

- Praziquantel and synthetic peroxide conjugates displayed high worm killing activity against both juvenile and adult *Schistosoma*.
- The type of the linker and the peroxide moiety in the conjugates play a significant role for their antischistosomal activity. Compounds with amide linker and 1,2,4,5-tetraoxane as pharmacophore exhibited higher inhibitory activity.
- These conjugates exhibited consistent worm killing profile against *Schistosoma japonicum* and *Schistosoma mansoni*.
- Although highly efficient *in vitro*, these conjugates show low to moderate worm reducing activity *in vivo*.

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