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# Praziquantel derivatives exhibit activity against both juvenile and adult *Schistosoma japonicum*

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ABSTRACT

A praziquantel analog 10-hydroxy praziquantel and eight praziquantel/peroxide conjugates were synthesized. The biological activity of these compounds was evaluated against juvenile and adult stages of *Schistosoma japonicum*. Unlike praziquantel, 10-hydroxy praziquantel exhibits activity against both juvenile and adult *Schistosoma japonicumin*. All hybrid compounds displayed modest to significant worm killing activity. The present study has important significance for the development of hybrid antischistosomal drugs.



As one of the most concerned verminosis, schistosomiasis has been epidemic in 76 countries and territories.[1] The World Health Organization (WHO) estimates 200 million people are infected and a further 800 million people are at risk of infection. There are three major blood fluke species infect humans—*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma Japonicum*. The third one is most widely distributed and causes the highest disease burden in Asian countries.

Jointly discovered by Bayer and E. Merck in Germany in 1972,[2] praziquantel (PZQ) (Fig. 1) is the first choice of drug for the treatment of the schistosomiasis,[3,4] and has been used successfully to control Schistosomiasis in numerous countries. This drug is effective against all three major schistosome. However, its preference against the adult worm, and being much less effective against the immature schistosomulum stage,[5] makes it far from a perfect drug. Recently, Schistosome isolated with diminished sensitivity to PZQ continues to be identified.[6] Should praziquantel become less effective, no back-up drugs were developed. Moreover, WHO has classified Schistosomiasis as a category 2 disease. Taken together, development of replacements for PZQ is urgently needed.[7]

Ever since PZQ was discovered, much interest has been attracted in the elucidation of structure–activity relationship (SAR). There are five positions amenable to chemical modification in PZQ molecular (Fig. 1). Among these, only position $R^1$ was heavily investigated in the original report and patent literature.[8] Later in 2007, nitration and amination substitutes at $R^4$ were reported by Matthew group.[9] Recently, more $R^1$ position variants as well as a PZQ-ozonide hybrid were synthesized and tested against juvenile and adults stages of *S. mansoni* in vitro and in vivo.[10] Unfortunately, all the above mentioned variants shown decreased activity compared to the parent compound. Nevertheless, we believe that promising lead compounds are likely to be identified with

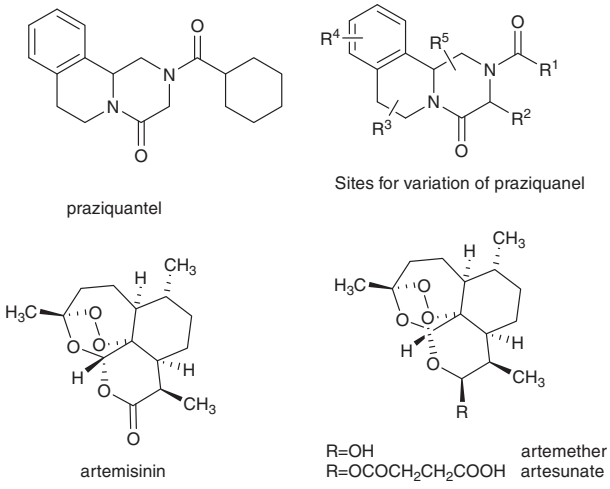


**Figure 1.** Chemical structure of antischistosomal drugs.

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extensive SAR study at other positions or with different substituent groups. Significantly, PZQ analogs with potent antischistosomal activity against both juvenile and adult stage worm are particularly needed.

In this Letter, we report our exploration of hydroxylation at position 10 in PZQ aromatic ring, and the biological evaluation of this compound as well as its peroxide hybrid conjugates against *Schistosoma J*.

A well-known antimalarial drug artemisinin, and its derivatives both artemether and artesunate (Fig. 1) are reported to show antischistosomal activity to some extent. Especially, their worm killing ability against juvenile schistosomes is superior to PZQ.[11] Later on, some synthetic compounds harboring the artemisinin's pharmocophore endoperoxide bridge, that is, 1,2,3,4-trioxane/1,2,4-trioxane motif, were discovered to show high in vitro and in vivo activity against both juvenile and adult stage of schistosomes.[12,13] We imagine that a hybrid molecular of PZQ and the endoperoxide moiety in a proper manner should generate compounds that are sensitive to both stage of worms. This conjugation strategy is also utilized by a successful antimalarial drug trioxaquines®, a dual molecules containing a quinoline and a 1,2,4-trioxane unit.[14,15] Herein, we designed eight hybrid molecules (Fig. 2). Compounds **2–5** contain two covalently linked pharmacophores: an endoperoxide bridge as in artemisinin derivative and a hydroxyl/amino-praziquentel as in praziquentel. In compounds **6–9**, the bulky artemisinin derivative was replaced by small synthetic moieties containing the endoperoxide bridge: 1,2,4,5-tetraoxane or 1,2,4-trioxane.

10-Hydroxy-PZQ (compound **1**, not reported in open patent or literature) was prepared from 10-amino-PZQ, which was prepared according to a reported procedure.[9] Treatment of 10-amino-PZQ with $NaNO_2$, and subsequent hydrolysis under strong acidic condition allowed transformation of the amino group to hydroxyl. This one-pot reaction provided compound **1** in 50% yield. Coupling of the 10-amino/hydroxyl-PZQ with artesunate through amide or ester linkage gave compound **2** and **3**. Accordingly, to synthesize compound **4–9**, intermediates **10–12** (Fig. 3) were prepared according to the reported method.[16–18] In all cases, amide/ester bond formation employed 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxybenzotriazole (HOBt) as the general coupling reagents, compounds were obtained in 45–85% yield in this step.

All prepared analogs were evaluated for their ability against both adult and juvenile *schistosomes J*. in vitro. According to previously described method,[19,20] 8–12 worms obtained from single-sex male infections were distributed in duplicate tissue culture dishes (3.5 cm) in Dulbecco's modified minimum Eagle's medium (bicarbonate buffered) supplemented with 20% newborn calf serum, 100 U $mL^{-1}$ penicillin, 100 μg $mL^{-1}$ streptomycin and 0.5 μg $mL^{-1}$

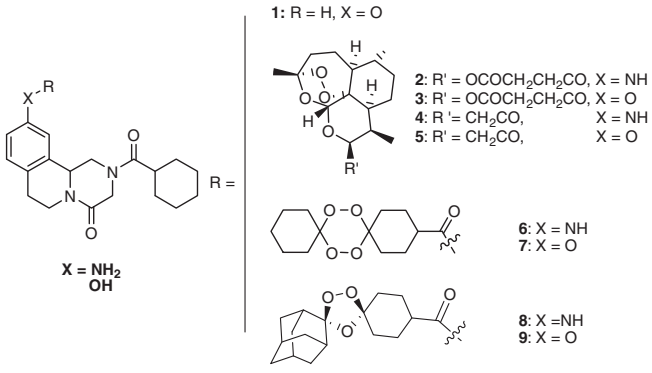


**Figure 2.** Target PZQ analogs **1**–**9**.

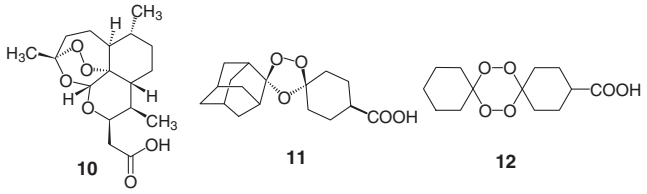


**Figure 3.** Structures of intermediates **10**–**12**.

amphotericin B. Cultures were kept at 37 °C in an atmosphere of 5% $CO_2$ in air and were observed under a Leica MZ 12.5 stereomicroscope. Worms of *S. japonicum* were exposed overnight (16 h) to various drugs, washed and subsequently cultured in drug-free medium. Juvenile worms were obtained after day 16 infection. In contrast, adult worms were obtained at day 42 infection. 10-Hydroxy-PZQ and other hybrid compounds were then added from 200 mg/mL DMSO stock solutions to achieve a series of diluted concentrations 10–50 μM. Compound activity was assessed by mortality and motility disturbances within 72 h. The score of worm vitality is illustrated as: 3 scores: the highest score, as observed in the control group during the observation period. Worms moved more actively and softly, and the body was transparent. 2 scores: worms acted all over the body, but stiffly and slowly, with the body translucent. 1 score: parasites moved partially with opaque appearance. 0 score: the worm remained contracted, did not resume movements, we could deem it 'dead'. The average score of 10–12 worms was counted for in vitro test. Data representative of repeated experiments was shown in Table 1 and Table 2.

For the in vivo screen, mice were infected with ca. 50 *schistosomiasis. J* cercariae on day 0 followed by administration of 200 mg/kg oral doses of test compounds suspended in distilled water to groups of ten mice on day 14 post-infection (juvenile stage) and 21 post-infection (adult stage). At certain days post-treatment, animals were sacrificed and dissected to assess total worm reduction as described in detail.[12]

For all compounds tested against adult *schistosomiasis. J* (Table 1), compounds **1–6** show worm killing activity superior to PZQ. The result of particular significance is for **1–3**. At concentration as low as 15–25 μM, these three compounds killed 100% worm. By comparison, worm mortality induced by 50 μM PZQ is 62.9% in 72 h. Compound **8** had the weakest activity, no worm was killed at concentration up to 50 μM, but the worm vitality score reduced to 3. The potency of compound **7** is marginally higher than compound **8**. However, the worm mortality is only 60% at 100 μM, which is obviously much lower compared to PZQ at the same concentration. Compound **9** displayed similar potency to PZQ.

Based on the adult worm killing ability, compounds displayed significant potency at 50 μM were further evaluated for their ability against juvenile worm (Table 2). Again, compounds **1–3** demonstrated significant worm killing activity, reduced worm vitality to 100% in 72 h at 15 μM. Compounds **5–7**, although less potent than 1–**3**, also displayed considerable effect against juvenile worm. Among these, compound **5** is slightly less effective compared to **1–3**, killed 100% worm at 25 μM in 72 h. Compounds **6** and **7** had modest activity against juvenile worms, reduced worm motility by 100% and 14.3% at 50 μM.

For those compounds shown significant effect against both adult and juvenile worm at 50 μM, the worm reduction activity was evaluated in vivo (Table 3). All tested compounds **1**–**3**, **5**, **6** displayed modest to high worm reduction ability, total worm reduction rate is higher than 39%. Compound **1** was able to reduce 40% worm load in vivo, which is in sharp contrast to other PZQ simple derivatives showing low level of activities,[9,10] suggesting variation in the aromatic ring may serve a good choice for development

**Table 1**
Effects of compound **1**–**9** on adult *Schistosoma j.* in ex vivo culture

| Compound | Conc (μM) | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|
| | | % Worm survival | Vitality score | % Worm survival | Vitality score | % Worm survival | Vitality score |
| Vehicle | | 100 | 3 | 100 | 3 | 100 | 3 |
| **1** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 20 | 1 | 20 | 1 |
| | 5 | 60 | 1 | 80 | 1 | 80 | 1 |
| **2** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 0 | 0 | 60 | 1 | 40 | 1 |
| | 10 | 100 | 3 | 100 | 3 | 100 | 3 |
| **3** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| **4** | 50 | 20 | 0 | 80 | 2 | 100 | 2 |
| **5** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 40 | 1 | 0 | 0 |
| | 10 | 100 | 3 | 80 | 1 | 0 | 0 |
| | 5 | 0 | 0 | 40 | 1 | 20 | 1 |
| **6** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 60 | 1 | 0 | 0 | 40 | 1 |
| | 20 | 100 | 2 | 100 | 3 | 100 | 3 |
| | 15 | 100 | 3 | 100 | 3 | 100 | 3 |
| **7** | 50 | 40 | 2 | 80 | 1 | 80 | 0 |
| | 80 | 80 | 2 | 80 | 1 | 40 | 0 |
| | 100 | 80 | 2 | 20 | 1 | 0 | 0 |
| **8** | 50 | 100 | 3 | 100 | 3 | 100 | 3 |
| **9** | 50 | 40 | 1 | 40 | 1 | 40 | 1 |
| | 60 | 40 | 1 | 40 | 1 | 40 | 1 |
| | 70 | 20 | 1 | 40 | 1 | 20 | 0 |
| | 80 | 0 | 0 | 0 | 0 | 0 | 0 |
| PZQ | 50 | 62.9 | 3 | 44.4 | 3 | 36.3 | 2 |
| | 80 | 28.8 | 2 | 19.1 | 1 | 10.0 | 1 |
| | 100 | 10.0 | 1 | 4.4 | 1 | 1.4 | 1 |

**Table 2**
Effects of 10–50 μM concentration of **1**–**3**, **5**–**7** on juvenile *Schistosoma j.* in ex vivo culture

| Compound | Conc. (μM) | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|
| | | % Worm survival | Vitality score | % Worm survival | Vitality score | % Worm survival | Vitality score |
| Vehicle | | 100 | 3 | 100 | 3 | 100 | 3 |
| **1** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 45.5 | 1 | 54.5 | 2 |
| | 5 | 0 | 0 | 27.3 | 1 | 27.3 | 2 |
| **2** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 11.1 | 1 | 0 | 0 | 0 | 0 |
| | 10 | 9.1 | 1 | 45.5 | 2 | 54.5 | 2 |
| | 5 | 9.1 | 1 | 9.1 | 1 | 9.1 | 1 |
| **3** | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 16.7 | 1 | 33.3 | 1 | 33.3 | 1 |
| **5** | 50 | 14.3 | 1 | 0 | 0 | 0 | 0 |
| | 25 | 28.6 | 1 | 25.0 | 1 | 0 | 0 |
| | 15 | 28.6 | 1 | 28.6 | 1 | 28.6 | 1 |
| **6** | 50 | 33.3 | 2 | 0 | 0 | 0 | 0 |
| | 25 | 37.5 | 3 | 33.3 | 2 | 12.5 | 1 |
| **7** | 50 | 0 | 0 | 14.3 | 1 | 14.3 | 1 |
| PZQ | 100 | 100 | 3 | 100 | 3 | 100 | 3 |

of promising antischistosomal PZQ analogs. Significantly, the 10-OH-PZQ and artesunate hybrid compound **3** demonstrated good worm killing ability against both adult and juvenile worm. Especially, the superior effect against juvenile worm is noteworthy.

**Table 3**
Effect of compound **1**, **2**, **3**, **5**, **6** to mice harboring 14-day-old juvenile and 21-day-old adult *S. japonicum* infection

| Compound | Adult | | Juvenile | |
|---|---|---|---|---|
| | Number of detected worms/mice ($\bar{x} \pm s$) | Total worm reduction rate | Number of detected worms/mice ($\bar{x} \pm s$) | Total worm reduction rate |
| Vehicle | 50.2 ± 3.9 | | 50.2 ± 3.9 | |
| **1** | 30.0 ± 6.6 | 40.2 | 42.1 ± 6.7 | 16.1 |
| **2** | 29.2 ± 3.6 | 41.8 | 48.3 ± 6.9 | 3.8 |
| **3** | 22.0 ± 4.3 | 56.2 | 14.9 ± 2.9 | 70.3 |
| **5** | 25.3 ± 2.6 | 49.6 | 39.9 ± 4.2 | 20.5 |
| **6** | 30.8 ± 3.9 | 38.7 | 3.76 ± 3.2 | 25.1 |
| PZQ | 20.0 ± 2.1 | 66.7 | 35.3 ± 2.2 | 19.8 |

This result is significant for the development of antischistosomal agents possessing therapeutic as well as preventive properties. Whether this compound exerts its effect as a whole compound, or the possible in vivo hydrolyzed product 10-OH-PZQ and artesunate interplay separately, needs to be further investigated.

In summary, we have identified new PZQ derivatives with activity against both adult and juvenile *S. japonicum*. Some of these compounds demonstrated superior activity to PZQ. Their effect on juvenile worm is especially noteworthy. The simple molecule 10-OH-PZQ was recognized as a new PZQ analog and being the most active one among all aromatic ring modified PZQ derivatives.[8,9] This result implied that promising drug candidate is likely to be discovered with extensive SAR exploration in the aromatic ring, which position was somewhat ignored in the previous research. This active PZQ analog also provided a choice for the attachment of dyes or linker moieties in the elucidation of PZQ's in vivo target. The present study also revealed several important facts for the SAR trend of these PZQ hybrid derivatives: The hybrids of PZQ with artemisinin analog displayed higher worm killing effect compared to that with artemisinin replacement peroxide moieties. Therefore, other peroxide structural motif should be investigated in the future study; Considering the hybrids **2** and **3** possess different linker space than compounds **4** and **5**, we deduce that coupling of PZQ with another pharmacophore through a proper linker would influence the hybrid biological activity. Hybrids of PZQ with 1,2,4,5-tetraoxanes showed higher potency compared to with 1,2,4-trioxanes, which could be in consistence with the suggestion that 1,2,4-trioxane was much less stable than 1,2,4,5-tetraoxanes.[21]

The in vivo study of these compounds revealed that the hybrid of PZQ with artesunate provided an ideal drug lead possessing adult and juvenile worm killing capability, suggesting the significance of the conjugation design strategy toward antischistosomal drugs. However, optimization of the juvenile sensitive moiety, and the conjugation position in PZQ should be further investigated.

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