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Praziquantel derivatives I: Modification of the aromatic ring

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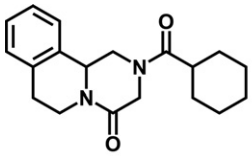
Abstract—Several analogues of the potent anthelmintic praziquantel were prepared with variation in the aromatic ring. The biological activity of these analogues was evaluated and compared against known analogues. Amination of the ring was tolerated while other variations were not. These results have important implications for drug development for schistosomiasis.

Schistosomiasis is one of the most burdensome of the neglected tropical diseases.[1,2] The World Health Organisation estimates 200 million people are infected and it is calculated that a further 600 million people are at risk of infection. The intermediate host of the parasite is a snail widespread in fresh water, and it is skin contact with this water that permits infection. It is because of this that schistosomiasis represents such an enduring public health challenge. The estimate of the level of public health burden of schistosomiasis (expressed in what is known as disability-adjusted life-years, or DALYs) was recently greatly increased to reflect the enormous impact of this 'silent pandemic' on the economic and social development of the affected societies.[3,4]

Thankfully, a highly effective drug exists to treat this disease. Praziquantel (**1**) exhibits high cure rates, low toxicity and low cost.[5] The drug is being distributed on a mass scale by the Schistosomiasis Control Initiative, funded by the Gates Foundation.[6] Other countries, such as Cameroon, have since initiated their own control programmes.[7] There are no back-up drugs for the treatment of schistosomiasis should praziquantel become less effective. Since the introduction of PZQ in the 1970s, there have been suggestions, but no definitive reports, of resistance or tolerance.[8] However, it can be argued that resistance is inevitable: mass consumption of any drug for an infectious disease is *a priori* likely to increase resistance to that drug, and PZQ has already shown low efficacy in the treatment of trematodes in Vietnam.[9,10] Tolerance was seen after only a few iterations of a simple laboratory-based artificial selection.[11] With wider deployment of PZQ, the pressure for the development of resistance is much higher. We take the view that replacements for PZQ need to be developed in advance, rather than after they are needed.

The development of replacements for PZQ could operate in three ways: (a) synthesis of analogues of PZQ, (b) the rational design of new pharmacophores and (c) the discovery of new compounds from large-scale screening programmes. Approach (b) is hampered by the lack of knowledge of the vivo target of PZQ.[12] Screening programmes are underway in several locations, for example, WHO's TDR programme and the Sandler Centre at UCSF. This paper describes approach (a), the synthesis of novel derivatives of PZQ.



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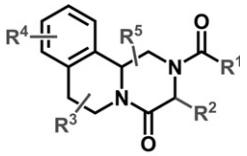

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There are five distinct positions of variation possible in PZQ's structure (**2**). Position R^1 was varied heavily in the original report and patent literature, though it is not clear how many of these compounds were

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synthesized and tested.[13–15] Few of the original QSAR data are available. What is clear is that some variation is acceptable (e.g., 3-fluorobenzoyl, nicotinoyl)[16] while other groups (e.g., isobutyryl)[14] eliminate activity when present in this position. A single report of amination in R^1 (the (4-aminomethyl)cyclohexyl analogue of PZQ) is known in the literature (towards the preparation of anti-PZQ antibodies), although no details were published on the characterization and purity of the compound, nor its biological activity.[17] What is perhaps surprising, given the >30-year lifespan of PZQ, is that none of the other positions of the PZQ pharmacophore have been properly investigated for activity against schistosomes.

In this paper, we describe the first explorations of amination in position R^4 (not explored in patent or open literature), and the biological evaluation of these analogues against *Schistosoma mansoni*. The analogues are synthesized directly from commercial PZQ. Variation in the aromatic ring of PZQ is of interest for the production of analogues. We are also interested in the production of fluorescent versions of PZQ as a possible means to elucidating PZQ's mechanism of action in vivo. An amine in the aromatic ring would, *prima facie*, be an ideal starting point for the attachment of fluorescent dyes or the synthesis of photoaffinity probes based on PZQ. In addition, three known samples of PZQ analogues with variation in position R^1 were donated to us by Bayer, and we here re-evaluate them as a comparison with the novel analogues described herein.

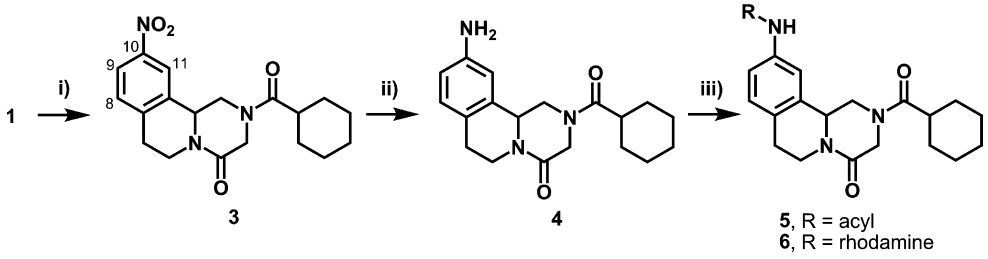
Treatment of PZQ with a standard combination of reagents for nitration gave the nitro-PZQ derivative **3** in a modest 39% yield (91% based on conversion, Scheme 1). Longer reaction times saw the appearance of a small amount of a second product spot on TLC that was not isolated, but was assumed to be either a dinitro derivative or regioisomer. There are four possible positions of nitration in the aromatic ring. The splitting pattern in the aromatic region of the ^{1}H NMR spectrum includes a singlet, ruling out positions 8 and 11. NOE irradiation of the downfield proton at the 1 position (methylene next to the stereocentre) clearly indicated a proximity to the aromatic singlet, indicating that the nitro group has been installed at position 10 (see supporting information).

The nitro-PZQ could be easily reduced with either a catalytic hydrogenation or tin chloride (the latter giving a higher yield of 95%) to give the amino-PZQ (**4**). To investigate whether substitution of the type required for the attachment of a label is tolerated with respect to schistosomal activity in this position, we first carried out a simple acetylation to give **5**. We also coupled 10-amino-PZQ to rhodamine to produce the fluorescently tagged derivative **6**. We have previously examined the behaviour of this molecule in *S. mansoni* with real-time fluorescence spectroscopy.[18]

Analogues **3**, **4**, **5** and **6** were evaluated for their ability to paralyse and kill schistosomes. Analogues **7**–**9**, obtained from Bayer, were also evaluated (Table 1). Paralysis caused by this class of compounds is spastic, that is, it is accompanied by marked muscular contraction (as shown in Fig. 1).

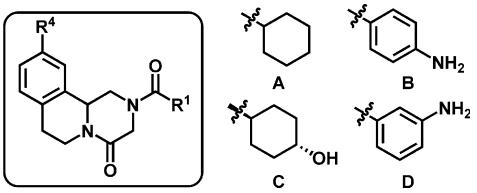
The results are significant for the future development of antischistosomal agents. The results of particular significance are for compounds **4** and **7** (shown in bold). Amino-PZQ analogue **4** displays decreased potency versus PZQ, but the nitro and acylated versions of the drug are essentially inactive against the parasite. This implies that the aromatic ring, or at least the 10 position of this ring, is a poor place to attach dyes for investigation of the in vivo activity of PZQ. However, the nature of the substituent, and not just the position of attachment, is important, since the amine analogue **4** is much more active than the essentially inactive nitro derivative **3**. Our previous observations[18] of the in vivo activity appeared to suggest that the fate of these fluorescent derivatives may have been dictated by the nature of the dye, rather than by the PZQ moiety, and the low biological activity of compound **6** could explain this. We have also previously attached compound **4** to an aqueous-compatible support (Affigel) and performed affinity chromatography with parasite proteins.[19] The results from this experiment were inconclusive, again in line with a reduced level of interaction between **4** and any potential in vivo target of PZQ.

Of the other analogues tested, the most active were compounds **7** and **9**, where potency is between one quarter and one sixth that of PZQ. These results indicate that the southeast portion of PZQ may be a more promising position of attachment for a fluorescent dye or tracer molecule than the northwest, but not by a significant margin. We are currently investigating this strategy. These results correlate with a study on praziquantel analogues for the treatment of *Clonorchis sinensis*, where



Scheme 1. Synthesis of novel PZQ analogues. Reagents and conditions: (i) H_2SO_4/HNO_3 (39% yield); (ii) $SnCl_2{\cdot}2H_2O$ (95% yield) or Pd/C, H_2, MeOH (90% yield); (iii) acetyl chloride, pyridine (for **5**) or rhodamine B, EDC, HOBt, DMAP (for **6**).

Table 1. Biological potency against *S. mansoni* for analogues **3**–**9** versus PZQ, **1**



Compound	R^1	R^4	Killing[a] (μM)	Relative potency	Paralysis[b] (μM)	Relative paralysis potency
1	A	H	3	100	1	100
3	A	NO_2	>300	<1	>300	<0.3
4	**A**	**NH_2**	**28**	**10.7**	**10**	**10.0**
5	A	NHAc	>300	<1	>300	<0.3
6	A	NHRh	>80[c]	<3.7	>80	<1.2
7	**B**	**H**	**18**	**16.6**	**7**	**14.3**
8	C	H	200	1.5	75	1.3
9	D	H	12	25.0	7	14.3

[a] Minimum concentration for 100% killing.
[b] Minimum concentration for complete spastic paralysis.
[c] Insoluble at higher concentrations.

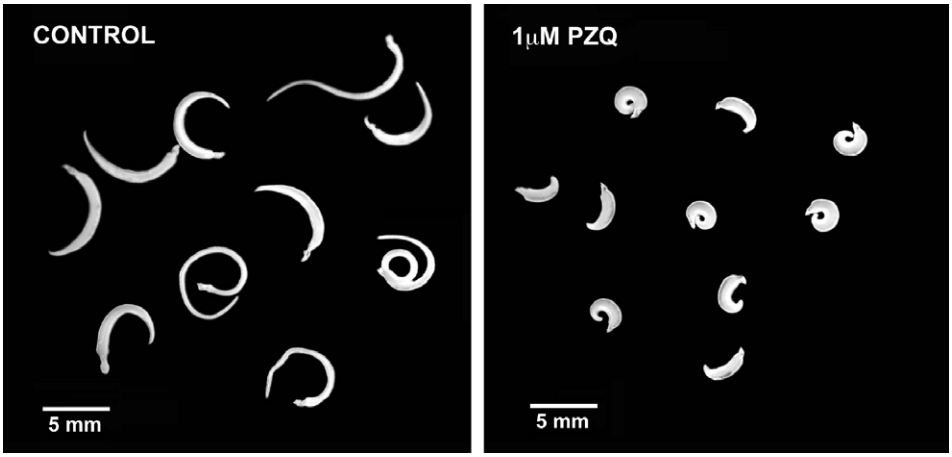


Figure 1. Contraction of *S. mansoni* upon treatment with PZQ; untreated worms (left) versus worms treated overnight with 1 μM PZQ (right).

methoxy groups in the aromatic ring were poorly tolerated, perhaps suggesting a similar in vivo target.[20]

We have introduced several novel modifications in the aromatic portion of praziquantel and evaluated the activity of four analogues against *S. mansoni*. The low levels of activity of these analogues indicate that this portion of the structure may be a poor choice for the attachment of dyes or linker moieties in the elucidation of PZQ's in vivo target, as well as being a poor choice for variation in the development of novel analogues to replace PZQ. Additionally, other amino moieties in the southeast portion of the molecule, while more compatible with biological activity, still give a significant reduction in the molecule's potency. Remaining possible positions of variation of PZQ (**2**) are currently being investigated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.063.

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