Screening of a PDE-focused library identifies imidazoles with \textit{in vitro} and \textit{in vivo} antischistosomal activity

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\textbf{ABSTRACT}

We report the evaluation of 265 compounds from a PDE-focused library for their antischistosomal activity, assessed \textit{in vitro} using \textit{Schistosoma mansoni}. Of the tested compounds, 171 (64\%) displayed selective \textit{in vitro} activity, with 16 causing worm hypermotility/spastic contractions and 41 inducing various degrees of worm killing at 100μM, with the surviving worms displaying sluggish movement, worm unpairing and complete absence of eggs. The compounds that did not affect worm viability (n=72) induced a complete cessation of ovipositing. 82\% of the compounds had an impact on male worms whereas female worms were barely affected. \textit{In vivo} evaluation in \textit{S. mansoni}-infected mice with the \textit{in vitro} ‘hit’ NPD-0274 at 20 mg/kg/day orally for 5 days resulted in worm burden reductions of 29\% and intestinal tissue egg load reduction of 35\% at 10 days post-treatment. Combination of praziquantel (PZQ) at 10 mg/kg/day for 5 days with NPD-0274 or NPD-0298 resulted in significantly higher worm killing than PZQ alone, as well as a reduction in intestinal tissue egg load, disappearance of immature eggs and an increase in the number of dead eggs.

\textbf{1. Introduction}

Schistosomiasis has among the highest morbidity of the world’s neglected tropical diseases, causing an estimated 4,026,000 disability-adjusted life years (DALYs). In socio-economic terms, public health importance and prevalence in the developing world, it ranks second only to malaria. More than 780 million people are at risk and approximately 261 million are infected in 78 countries of which 85\% reside in sub-Saharan Africa (WHO, 2017). No vaccine is yet available and almost all control initiatives rely on praziquantel (PZQ), which has been considered the drug of choice since the 1970s because it is highly effective after a single oral dose against all \textit{Schistosoma} species that are pathogenic to humans. In Egypt, as in most African countries, control measures rely on campaigns of mass PZQ administration targeting high-risk groups (WHO, 2012). PZQ possesses positive features with respect to safety, efficacy, cost and ease of distribution (Cioli et al., 2014) but its efficacy is dependent on the age of the infection, the sex of the worms and their paired or unpaired status (Pica-Mattoccia and Cioli, 2004). Immature worms (between 1 and 5 weeks after infection) are much less sensitive to PZQ and hence such infections require re-treatments. Moreover, findings denoting a possible threat of resistance development were reported in mice under laboratory conditions (Fallon and Doenhoff, 1994; Ismail et al., 1994; Sabra and Botros, 2008; Liang et al., 2011) and during the intra-molluscan phase (Couto et al., 2011). At the clinical level, reduced susceptibility to PZQ in \textit{S. mansoni} field isolates has been reported (Gryseels et al., 2001; William et al., 2001; Cioli et al., 2004; Doenhoff et al., 2008; Melman et al., 2009; Mwangi et al., 2014) but goes largely unnoticed in the mass-administration
campaigns, in part because of the insufficient efforts to monitor PZQ resistance. For the above reasons, the search for new antischistosomal agents represents an overriding priority. To date, a large number of compounds have been identified as potential antischistosomal agents but none has yet represented a suitable alternative to PZQ.

This study phenotypically evaluated the antischistosomal activity potential of 265 compounds from an acyclic nucleotide phosphodiesterase (PDE) focused library constructed by the PDE4NPD consortium (www.PDE4NPD.eu), which focused on exploring PDEs as potential drug targets for several parasite species, including *S. mansoni*. Several PDE inhibitors are currently in use as therapeutic agents for various human conditions, acting by targeting specific PDE isoenzymes and thereby the breakdown of cyclic nucleotides (cAMP, cGMP) and thus prolonging their biological effects (Ghosh et al., 2009). Pharmacologically, PDEs gained great interest as drug targets for a large variety of clinical conditions including their antiparasitic potential (Shakur et al., 2011).

The in vitro screening campaign used *S. mansoni* worm killing as the primary parameter with in vivo follow-up evaluation of selected actives in a mouse model.

## 2. Materials and methods

### 2.1. Drugs and compounds

Praziquantel (PZQ) tablets (Distocide®) were obtained from Egyptian International Pharmaceutical Industries Company (EIPICO). The experimental compounds were prepared at University of Amsterdam (VUA), University of Antwerp (UA) and Centro de Investigaciones Biológicas (CIB-CSIC) and have a purity ≥95% by HPLC. Synthesis of the imidazoles tested in vivo (NPD-0274, NPD-0298 and NPD-0264) was performed according to described procedures (García et al., 2017).

### 2.2. Cytotoxicity on human lung fibroblasts

MRC-5 human lung fibroblasts were cultured in MEM medium supplemented with 20 mM L-glutamine, 16.5 mM NaHCO₃ and 5% fetal calf serum (FCS). Assays were performed at 37°C and 5% CO₂ in 96-well tissue culture plates with confluent monolayers. After 7 days of incubation, cell viability was assessed after addition of resazurin and

## Table 1

| Serial No | PDE inhibitors | Worm killing at different concentrations (% of total; male + female) | EC₅₀ (μM) | Uncoupling & complete absence of eggs | Reduction in number of eggs (%) | MRC-5 EC₅₀ (μM) |
|-----------|----------------|---------------------------------------------------------------|-----------|-------------------------------------|--------------------------------|----------------|
| 1         | NPD-0274*      | 56 47 13 0 0 0 72 No No No 79 50 33 >64                      |           |                                     |                                |                |
| 2         | NPD-0029       | 77 27 0 0 0 69 No No No 42 50 33 9                           |           |                                     |                                |                |
| 3         | NPD-0356       | 50 39 14 0 0 89 No No No 60 10 0 60                           |           |                                     |                                |                |
| 4         | NPD-0048       | 63 25 19 0 0 79 No No No 42 50 33 9                           |           |                                     |                                |                |
| 5         | NPD-1012       | 62 0 0 0 0 99 No No No 67 32 38 ND                           |           |                                     |                                |                |
| 6         | NPD-1246       | 100 53 0 0 0 50 Yes No No 100 20 10 >64                       |           |                                     |                                |                |
| 7         | NPD-1253       | 50 37 0 0 0 91 Yes No No 100 21 29 35                       |           |                                     |                                |                |
| 8         | NPD-2904       | 50 0 0 0 0 100 Yes Yes Yes 100 100 100 >64                   |           |                                     |                                |                |
| 9         | NPD-1014       | 56 37 0 0 0 81 No No No 0 0 0 ND                           |           |                                     |                                |                |
| 10        | NPD-1211       | 52 30 0 0 0 91 Yes No No 100 13 8 >64                       |           |                                     |                                |                |
| 11        | NPD-1085       | 50 7 0 0 0 100 No No No 25 17 8 >64                       |           |                                     |                                |                |
| 12        | NPD-1013       | 56 0 0 0 0 99 No No No 25 0 0 ND                           |           |                                     |                                |                |

All compounds listed displayed 100% reduction in egg numbers with uncoupling at 100 μM and 50 μM. a Compounds selected for in vivo testing. Yes: uncoupling and complete absence of eggs. No: no uncoupling and incomplete absence of eggs. ND, not determined.

## Table 2

| Serial No | PDE inhibitors | % Worm killing | Male worms | Female worms | EC₅₀ male (μM) | EC₅₀ female (μM) |
|-----------|----------------|----------------|------------|--------------|---------------|-----------------|
| 1         | NPD-0274*      | 100 88 25 0 0 0 32 0 0 0 0 0 NE |           |              |               |                |
| 2         | NPD-0029       | 91 33 0 0 0 59 0 0 0 0 0 0 NE |           |              |               |                |
| 3         | NPD-0356       | 88 71 29 0 0 36 0 0 0 0 0 0 NE |           |              |               |                |
| 4         | NPD-0048       | 100 50 33 0 0 41 14 0 0 0 0 0 >100 |           |              |               |                |
| 5         | NPD-1012       | 60 50 0 0 0 68 0 0 0 0 0 0 NE |           |              |               |                |
| 6         | NPD-1246       | 100 50 0 0 0 50 100 57 0 0 0 50 |           |              |               |                |
| 7         | NPD-1253       | 100 66 0 0 0 49 0 0 0 0 0 0 NE |           |              |               |                |
| 8         | NPD-2904       | 73 0 0 0 0 97 0 0 0 0 0 0 NE |           |              |               |                |
| 9         | NPD-1014       | 100 67 0 0 0 49 0 0 0 0 0 0 NE |           |              |               |                |
| 10        | NPD-1211       | 88 56 0 0 0 48 56 13 0 0 0 93 |           |              |               |                |
| 11        | NPD-1085       | 78 13 0 0 0 76 0 0 0 0 0 0 NE |           |              |               |                |
| 12        | NPD-1013       | 70 0 0 0 0 98 0 0 0 0 0 0 NE |           |              |               |                |
| 13        | NPD-0298*      | 33 0 0 0 0 0 0 >100 0 0 0 0 0 NE |           |              |               |                |
| 14        | NPD-0264*      | 12 0 0 0 0 0 0 >100 0 0 0 0 0 NE |           |              |               |                |

Controls: PZQ was 100% effective against both male and female worms at all concentrations tested (100, 50, 25, 10 and 5 μM), and DMSO (negative control, solvent; 0.125%-2%) had no effect on worm viability. NE, no effect.
fluorescence reading.

2.3. In vitro studies

2.3.1. Preparation of compounds

5mM stock solutions of PZQ and test compounds were prepared in DMSO. At the day of experiment, 100μM, 50μM, 25μM, 10μM and 5μM concentrations were freshly prepared in RPMI-1640 medium. All compounds were initially tested at 100 and 50μM; those showing worm killing were further tested at 25, 10 and 5μM.

2.3.2. Worm killing

Six to eight worms (obtained from Schistosome Biology Supply Center (SBSC) of the Theodor Bilharz Research Institute (TBRI)), including a minimum of one worm couple, were placed in each well of a 12-well tissue culture plate containing 2 ml of fresh RPMI-1640 medium supplemented with glutamine, 20% newborn calf serum and antibiotics (streptomycin, penicillin (2 ml/100 ml) and gentamicin (200 μl/100 ml)), and the indicated concentration of the test compounds (Pica-Mattoccia and Cioli, 2004; Botros et al., 2005). The plates were incubated overnight at 37 °C and 5% CO₂. Worms were examined by phase-contrast microscopy, 24h after the start of the incubation, washed thrice with sterile saline, fresh medium was added and the incubation was continued. After 48 h, worm motility was observed and 72 h later, medium was changed again. After 96 h (end of observation period) worms were microscopically examined for motility and appearance. Each concentration was tested in duplicate. The final recording of percent worm mortality was determined as the number of dead worms [contracted and opaque] divided by the total number of worms × 100. Negative controls using pure medium without test compound or medium with DMSO (2%), and positive control media containing parallel concentrations of PZQ were tested in parallel. Immature, early mature and mature S. mansoni worms were 3 weeks, 4 weeks and 6–7 weeks old respectively.

2.3.3. Worm ovipositing

Compounds showing worm killing, sluggish movement and/or unpairing were tested for inhibitory effect on ovipositing using 12-well tissue culture plates with each well containing at least one worm couple. Each concentration was tested in duplicate and eggs were counted a first time after 72 h, upon which the eggs were discarded and the medium changed. After 96 h (end of observation period), the newly deposited eggs were counted and the total number was calculated for each concentration tested.

Fig. 1. Effect of PDE inhibitors NPD-1012 and NPD-0356 on worm killing of different S. mansoni maturity stages.
2.4. In vivo antischistosomal activity

2.4.1. Experimental infection of animals

Male Swiss albino mice (CD-1) obtained from SBSC and weighing 18–20 g were housed under environmentally controlled room temperature of 20–22°C, 12h light/dark cycle and 50–60% humidity with food and water ad libitum throughout the acclimatization and experimental periods. Mice were infected with *S. mansoni* cercariae (provided by SBSC) using body immersion (Liang et al., 1987) by exposure to 80 ± 10 cercariae/mouse. All the animal experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Institutional Review Board of TBRI.

2.4.2. Test compounds and experimental design

NPD-0274, NPD-0298, NPD-0264 and PZQ were freshly suspended in 2% Cremophore-EL (Sigma-Aldrich, St Louis, MO, USA). Infected mice were divided into 8 groups: the first three groups were treated with NPD-0274, NPD-0298 and NPD-0264, respectively, at 20mg/kg/day for 5 days. Group 4 was treated with PZQ at 10mg/kg/day for 5 days. Groups 5, 6 and 7 were treated with each of the test compounds combined with PZQ, each at 10mg/kg/day for 5 days starting from the 7th week post-infection. Group 8 was the vehicle control. To minimize first-pass elimination, the CYP450 inhibitor aminobenzotriazole (ABT) was administered at 100mg/kg/day for 5 days 2h prior to each compound administration. All drug administrations were performed orally.

2.4.3. Parasitological criteria for cure

Ten days post-treatment, all mice were sacrificed and perfused, and the number of worms recovered (worm burden) was quantified and sexed (Duvall and De Witt, 1967). The number of eggs per gram of liver or intestinal tissue was counted (Cheever, 1968). The percentage of egg developmental stages (oogram pattern) was studied (Pellegrino et al., 1962), in which eggs at different stages of maturity (from I to IV) were identified and counted. Mature eggs and dead eggs (granular, dark, and semi-transparent) were also counted in three fragments of intestine and the mean number of each stage was calculated.

2.5. Statistical analysis

The percentage reduction of worm or egg burden in each treated group was calculated. The 50% effective concentration (EC$_{50}$) or dose (ED$_{50}$) was calculated using Prism (GraphPad; Version 5.0) software using a variable slope for the sigmoidal curve with an upper limit of 100%. Results are expressed as mean ± SEM. A two-tailed, unpaired Student’s t-test was used to detect the significance of difference between the means of different groups. Results are considered significant if *P* value is < 0.05.

3. Results

3.1. In vitro activity against *S. mansoni*

265 compounds from a PDE-targeting library were initially screened for worm killing at 100μM. Antischistosomal effects were recorded in 171 compounds (64%) as worm killing, sluggish worm movement, unpairing, and absence or reduction in egg numbers; 16 resulted in worm hypermotility/spastic contraction. Based on these *in vitro* findings, the compounds were categorized into five classes: Class-I and II showed worm killing while the other three classes did not show worm killing but impacted ovipositing, resulting in either a reduction or the complete absence of eggs. Class-I compounds (12/171) showed worm killing of 50–100% with EC$_{50}$ values in the range of 50–100μM (Table 1). Class-I consisted of known PDE-like scaffolds, including phthalazinones (4 × ), imidazoles (2 × ) and a quinazoline (Table S1).
These 12 hits were further investigated for dose-related worm unpairing, and reduction or complete absence of eggs. The Class-II compounds NPD-0298 and NPD-0264, displaying strong effects on ovipositing and slight effects on worm killing, were also included in the in vitro follow-up study (Table 2).

All 14 compounds displayed 100% uncoupling of worm pairs with complete absence of eggs at both 100μM and 50μM, and several even impacted on egg numbers at 5μM, a concentration with no effect on worm viability at all. NPD-0298 and NPD-0264, which only had a slight effect on viability at 100μM, strongly reduced ovipositing down to 5μM. For NPD-0298 this may be mostly explained through worm uncoupling; for NPD-0264, however, there was no uncoupling below 50μM while egg production was still severely affected. A similar pattern was observed with NPD-0274 and some other compounds, indicating a specific effect on egg depositing rather than on viability or coupling (Table 1).

The above screening was conducted on mixed groups of males and females where it was noted that the male worms were generally much more severely affected than the female worms. We therefore reanalyzed worm killing for the hits in Table 1, this time performing parallel incubations with male and female populations. Out of the 14 compounds (Table 1), 11 displayed no activity on females even at 100μM. However, NPD-1246 had 100% worm killing on both sexes while NPD-1211 displayed moderate effects at 100μM (Table 2).

One of the major shortcomings of PZQ is its limited efficacy against immature worms, which prompted us to look at the effect of the Class-I compounds NPD-1012 and NPD-0356 against 3 week-old immature and 4 week-old early mature worms in comparison to the effects on mature 6 week-old parasites (Table 1). More prominent effects on early mature and to a lesser extent on immature worms were noted at concentrations of 50μM and 25μM compared to parallel findings in mature worms (Fig. 1).

3.2. In vivo activity in the S. mansoni mouse model

Guided by the in vitro findings, three in vitro active 2, 4-arylimidazoles compounds, NPD-0274, NPD-0264 and NPD-0298 (Fig. 2), were tested in S. mansoni-infected mice. Because these imidazole derivatives had been reported to be metabolically unstable (Sebastián-Pérez et al., 2018), concomitant dosing with the CYP450 inhibitor amino-benzotriazole (ABT) (Watanabe et al., 2016) was carried out with each experimental treatment (NPD, PZQ or combination of both). In a separate experiment, ABT itself had no significant effect on worm burden compared to untreated control mice (31.3 ± 1.3 (n=3) vs 29.0 ± 1.2 (n=6), p = 0.28).

Treatment of S. mansoni-infected mice with the Class-I compound
NPD-0274 at 20 mg/kg/day for 5 days significantly decreased the worm load and the intestinal tissue egg burden by 29% ($P < 0.05$) and 35% ($P < 0.05$), respectively, as compared to the untreated controls (Fig. 3A and B). Combination treatment with NPD-0274 and PZQ in reduced doses of 10 mg/kg/day each for 5 days revealed significantly higher worm killing (96% vs 60%; $P < 0.05$), and higher intestinal tissue egg burden reduction (83% vs 67%; $P < 0.05$) compared to PZQ-treated alone (Fig. 3A and B). Interestingly, the maturation of eggs did not change upon treatment with NPD-0274 alone (almost half the eggs were immature or mature), but after the combination treatment only very few mature eggs remained and the rest were dead (Fig. 3C).

With monotherapy of Class-I compound NPD-0247 yielding only moderate relief of worm and egg burdens, it was decided also to evaluate the Class-II compounds NPD-0264 and NPD-0298, particularly since in vitro NPD-0298 had impacted greatly on oviposition and coupling (Table 1). Infected mice were given the same treatment dose and schedule as the Class-I compounds. NPD-0264 did not reveal significant antischistosomal activity, either alone or when combined with PZQ (Fig. 4). NPD-0298 alone also did not reveal significant antischistosomal activity. However, when co-administered with PZQ, worm killing was significantly enhanced compared to PZQ alone (88% vs 60%; $P < 0.05$), intestinal tissue egg load was significantly reduced (83% vs 67%) and the percentage of dead eggs was markedly increased with complete absence of immature eggs (Fig. 5).

4. Discussion

From a PDE-focused library, 265 compounds were phenotypically screened for antischistosomal efficacy and 171 (64%) displayed various degrees of antischistosomal action revealed by worm mortality, motor activity alterations (sluggish worm movement or spastic contractions), reduced ovipositing and unpairing. The 171 ‘hits’ were sorted into five classes based on their primary in vitro profile at 100 μM and 50 μM. At 100 μM, 12 (7%) displayed 50–100% killing (Class-I), and 29 (17%) displayed <50% worm killing but with worm unpairing, sluggish movement of survivors and absence of eggs (Class-II). The reason why the percentage of worm killing barely exceeded 50% was due to the fact that almost all compounds mainly affected males with little or no impact on the females, whereas PZQ was not sex-specific over the dose range tested. As such, the EC$_{50}$ values for males were substantially lower than those for the mixed populations (Tables 1 and 2) with 32 μM as the lowest EC$_{50}$ value, for NPD-0274. Disparities in in vitro drug susceptibility between male and female _S. mansoni_ worms have indeed been previously reported. Higher susceptibility of male over female schistosomes has previously been reported for PZQ, oxamniquine, ginger extract, and some essential oils (Mikhail et al., 1978; Pica-
Mattoccia and Cioli., 2004; Mostafa et al., 2011; Tonuci et al., 2011).

Absolute killing of male worms only was also reported by Fernandes et al. (2013) when the antischistosomal activity of different synthetic preparations of N-alkylated diamines, amino alcohols, and glycosylated amino alcohols were examined in vitro. Preferential killing of female schistosomes has also been reported for some compounds, including 2-(butylamino)-1-phenyl-1-ethanethiosulfuric acid, amino alkanethiosulfuric acids and artesunate (de Araújo et al., 2007; de Oliveira Penido et al., 2008; Mitsui et al., 2009).

Many of the 171 test compounds, including those in Class-I and Class-II, also induced motor activity alterations, which were usually manifested as sluggish movement of the male worms. Spastic worm contractions were observed with 16 compounds including only 1 from Class-I (NPD-1014, a pyridazinone), but these contractions did not result in death during the in vitro observations. This phenotype has recently been linked to inhibition of SmPDE4A (Longet al., 2017) and the fact that few compounds in our PDE-focused library induced spastic contractions may indicate that this particular PDE was not the main target of most of the compounds. Indeed, the imidazole series that includes the hits NPD-0274, NPD-0264 and NPD-0298 is associated with inhibition of the PDE10 family and has potential utility in Parkinson’s disease (García et al., 2017). Motor activity alterations are considered important indicators of schistosomicidal activity, disturbing not only the whole worm’s muscle function and movement but also the muscles of the suckers essential to attach to the host vessels and the tight pairing of male and female worms (Ribeiro and Patocka, 2013; Patocka et al., 2014).

Possibly the most important observation was the high impact on ovipositing at very low, sub-lethal concentrations. Complete absence of eggs is obviously expected in the presence of 100% worm killing or separated worms (Magalhães et al., 2012). Reduction or absence of eggs can also be expected when severe motor activity alterations are recorded as these disturb the muscle lining of the reproductive excretory organs (Ribeiro and Patocka, 2013; Patocka et al., 2014).

![Fig. 5.](image)

(A) Worm burden, (B) tissue egg load and (C) oogram pattern of NPD-0298 sacrificed 10 days post end of treatment when used alone at 20 mg/kg/day or in combination with PZQ at 10 mg/kg/day for 5 days. *significantly different from infected control at P < 0.05, †significantly different from PZQ group at P < 0.05. Numbers above columns and between parentheses represent percentage change from infected control group. ABT (100 mg/kg orally) was administered 2 h prior to administration of NPD-0298 and PZQ, whether alone or in combination. Error bars represent SEM.
imidazole scaffold. The phthalazinones are related to the well-known T. brucei PDE81 and B2 inhibitors NPD-001 and NPD-008 (De Koning et al., 2012; Veerman et al., 2016; Blaazer et al., 2017), while the imidazoles are chemically related to previously described human PDE10A inhibitors (García et al., 2017). As the imidazole scaffold yielded several of the most active compounds and, as mammalian PDE inhibitors, may be among the most likely inhibitors of helminth PDEs, three imidazole derivatives were selected for evaluation in S. mansoni-infected mice: one from Class-I (NPD-0274) and two from Class-II (NPD-0264 and NPD-0298) (Fig. 2). The selection took into account factors such as cytotoxicity and observed adverse effects in mice at fixed dose regimens. Particular significance was given to the % reduction of egg production, since the eggs cause the clinical pathology of schistosomiasis, as well as disease transmission. If ovipositing could be irreversibly affected, the killing of the worm becomes of secondary importance.

NPD-0274 showed a modest but significant reduction in worm burden in vivo. In line with the in vitro effect on ovipositing rather than worm viability, the other two imidazoles (NPD-0264, NPD-0298) showed no significant worm killing in vivo. When administered at reduced doses with PZQ, NPD-0274 and NPD-0298 significantly enhanced worm killing (96% and 88% respectively, compared to 60% killing for PZQ alone). The same combinations also showed a higher reduction in intestinal egg load (both 83%, vs 67% for PZQ); of the eggs that were still present, the vast majority was dead and none were immature (i.e. viable and recently deposited). An increase of >50% of mature eggs with absence of one or more of the immature stages is definite proof that a drug possesses antischistosomal activity (Pellegrino et al., 1962). Combination therapy of PZQ and oxamniquine, omeprazole or melphalan in experimental schistosomiasis demonstrated increased worm lethality (Botros et al., 1989; El-Lakkany et al., 2011; Keiser et al., 2011; Almeida et al., 2015). Information as to whether drug combinations provide increased therapeutic efficacy over monotherapy is scarce, although in the studies conducted to date no curative advantages over single treatment were reported (Kamel et al., 2000; Utzinger et al., 2003; Cui and Su, 2009).

In conclusion, this study enabled successful selection of several antischistosomal compounds, based on in vitro findings such as worm killing, unpairing, alterations of motor activity (sluggish worm movement, spastic contractions) and ovipositing. Guided by these criteria, three imidazole derivatives were administered alone and in combination with PZQ to S. mansoni infected mice, providing enhanced therapeutic efficacy in combination with PZQ. When administered alone in vitro, there was a clear reduction to complete absence of eggs with no recovery over 7 days in the absence of drug, suggesting long-term disabling of ovipositing. The near-complete eradication of viable eggs with the PZQ/NPD-0274 and PZQ/NPD-0298 combinations is particularly promising as it would greatly diminish or eliminate pathology and above all transmission. It is the rapid reinfestation caused by the failure to break the transmission cycle that remains the most important factor in the persistently high infection rates in endemic areas.

These imidazoles are structurally related to a family of human PDE10A inhibitors and have also shown phenotypic activities in trypanosomatids (Sebastián-Pérez et al., 2018; de Araújo et al., 2019). PDE10A inhibitors and have also shown phenotypic activities in trypanosomatids (Sebastián-Pérez et al., 2018; de Araújo et al., 2019). Synergy of omeprazole and praziquantel in vitro treatment against Schistosoma mansoni adult worms. PLoS Neglected Trop. Dis. 9, e0004086. Blaazer, A.R., Singh, A.K., Edink, E., Orling, K.M., Veerman, J., Van den Bergh, T., Jansen, C., Balabanraman, E., Mooij, W.J., De Heuvel, E., Tagoe, D.A.N.A., Munday, J.C., Huijtman, H., Mathews, S., Simões, M., De Graaf, C., Maat, E., De Koning, H., Bailey, D., Steer, G.J., De Esch, I.P., Brown, D.G., Leurs, R., 2017. Targeting a subepithelium in Trypanosoma brucei B1 assists structure-based drug discovery for African sleeping sickness. J. Med. Chem. 60, 3870–3888. Botros, S., Pica-Mattoccia, L., Wilson, D., 2005. 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