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Letter

The Anthelmintic Activity of Praziquantel Analogs Correlates with Structure–Activity Relationships at $TRPM_{PZQ}$ Orthologs

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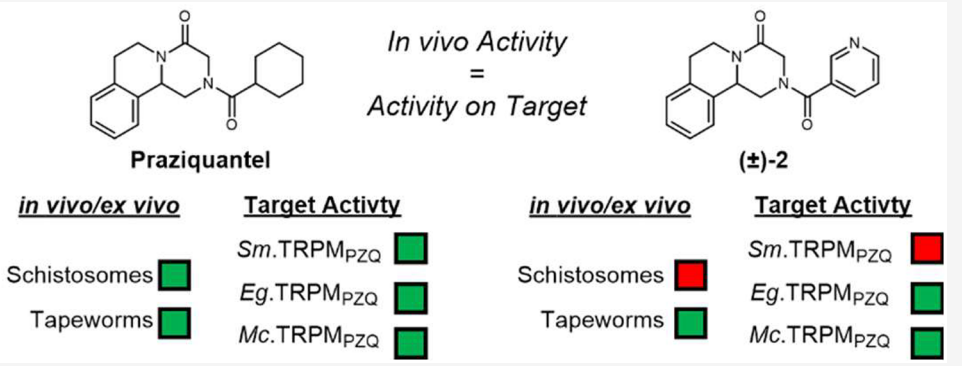

ABSTRACT: The anthelmintic drug praziquantel remains a key clinical therapy for treating various diseases caused by parasitic flatworms. The parasite target of praziquantel has remained undefined despite longstanding usage in the clinic, although a candidate ion channel target, named $TRPM_{PZQ}$, has recently been identified. Intriguingly, certain praziquantel derivatives show different activities against different parasites: for example, some praziquantel analogs are considerably more active against cestodes than against schistosomes. Here we interrogate whether the different activities of praziquantel analogs against different parasites are also reflected by unique structure–activity relationships at the $TRPM_{PZQ}$ channels found in these different organisms. To do this, several praziquantel analogs were synthesized and functionally profiled against schistosome and cestode $TRPM_{PZQ}$ channels. Data demonstrate that structure–activity relationships are closely mirrored between parasites and their $TRPM_{PZQ}$ orthologs, providing further support for $TRPM_{PZQ}$ as the therapeutically relevant target of praziquantel.

KEYWORDS: *Parasitic flatworm, Schistosome, Tapeworm, TRP channel, Ion channel*

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Exactly 40 years ago, a highly influential review on the anthelmintic activity of praziquantel (PZQ) was published by Peter Andrews and Herbert Thomas (both at Bayer AG) and Rolf Pohlke and Jürgen Seubert (both at E. Merck KG).[1] That work detailed the discovery of the anthelmintic activity of PZQ, derivatization of the scaffold, the drug's pharmacokinetic and safety profile, and the broad efficacy of this new therapeutic agent against a range of parasitic flatworms. The summary of data interrogating the activity of different pyrazino[2,1-*a*]isoquinoline derivatives against a representative trematode (*Schistosoma mansoni*) and cestode model (*Hymenolepis nana*) established both the "tightness" of the pharmacophore that underpins the efficacy of PZQ and a "structure–activity" fingerprint for the action of this drug that has long served as a reference standard for the field.

Following decades of clinical usage of PZQ for treatment of various diseases caused by parasitic flatworms,[2−5] a candidate target was recently identified[6] in *Schistosoma mansoni*. This target is a transient receptor potential (TRP) ion channel of the melastatin subfamily, named *Sm*.$TRPM_{PZQ}$,[6−8] that mirrors the structure–activity relationship (SAR) of PZQ derivatives[7] as described by Andrews et al.[1] $TRPM_{PZQ}$ is a large nonselective cation channel unique to flatworms.[6,8−10] Activation of *Sm*.$TRPM_{PZQ}$ is thought to elicit excitotoxicity through membrane depolarization, spastic contraction, and surface damage to the parasite, which then catalyzes immunological clearance from infected hosts.[11] Additional evidence from genetic association studies,[12] pharmacological screening,[13] and functional profiling of $TRPM_{PZQ}$ orthologs[9,10] add support for $TRPM_{PZQ}$ serving as the therapeutically relevant parasite target of PZQ.

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Chart 1. Structures of the PZQ Analogs Studied in This Work

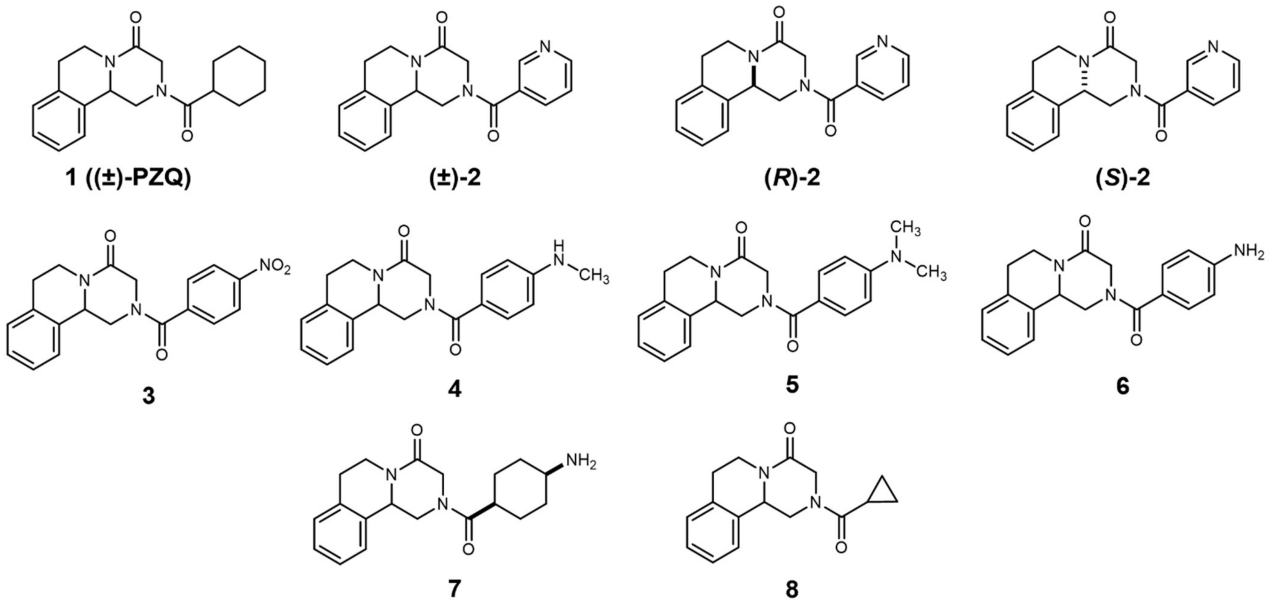

Table 1. Comparison of Antischistosomal and Anticestodal Activities of PZQ Derivatives with Their Activities at the Corresponding $TRPM_{PZQ}$ Orthologs[a]

	previous studies			this work: EC_{50} (μM)		
	S. mansoni		*H. nana*			
compound	*in vitro*	*in vivo*	*in vivo*	*Sm*.$TRPM_{PZQ}$	*Eg*.$TRPM_{PZQ}$	*Mc*.$TRPM_{PZQ}$
1 ((±)-PZQ)	+++	+++	+++	0.65 ± 0.057	0.10 ± 0.009	0.12 ± 0.016
((*R*)-PZQ)[b]	+++	+++	+++	0.28 ± 0.03	0.05 ± 0.01	0.08 ± 0.003
((*S*)-PZQ)[b]	++	+	++	28 ± 2.8	0.78 ± 0.13	1.23 ± 0.17
(±)-**2**	+	+	+++	inactive	2.3 ± 1.1	5.3 ± 1.3
(*R*)-**2**	0	++	+++	>100	1.4 ± 0.11	0.98 ± 0.088
(*S*)-**2**	0	0	++	inactive	22 ± 2.3	16 ± 2.2
3	0	+	++	inactive	inactive	inactive
4	0	++	+++	inactive	>100	>100
5	0	++	+++	inactive	inactive	inactive
6	++	++	+++	12 ± 2.6	0.96 ± 0.14	0.80 ± 0.22
7	0	0	+	inactive	>100	>100
8	0	+	++	inactive	53 ± 16	65 ± 14

[a]All presented *in vitro* and *in vivo* data on organisms (columns 2−4) were taken from ref 1. For *in vitro* studies using *S. mansoni*, "+++" indicates a maximal effect ≤3.2 μM, "++" indicates a maximal effect ≤320 μM, "+" indicates a less than maximal effect at ≤320 μM, and "0" indicates no effect at ≤320 μM. For *in vivo* studies using *S. mansoni*, "+++" indicates a complete reduction of worms at 50 mg/kg dosing (×5), "++" indicates complete reduction of worms at 500 mg/kg dosing (×5), "+" indicates less than 90% worm reduction at 500 mg/kg dosing (×5), and "0" indicates no effect at 500 mg/kg dosing (×5). For *in vivo* studies using *H. nana*, "+++" indicates a complete reduction of worms at 25 mg/kg dosing (×1), "++" indicates complete reduction of worms at 500 mg/kg dosing (×1), "+" indicates less than 90% worm reduction at 500 mg/kg dosing (×1), and "0" indicates no effect at 500 mg/kg dosing (×1). Columns 5−7 tabulate the EC_{50} values for analog activation of trematode and cestode $TRPM_{PZQ}$ orthologs *in vitro*. Data are shown as mean ± SEM for $n \geq 3$ independent transfections. *Eg*.$TRPM_{PZQ}$ = *Echinococcus granulosus* $TRPM_{PZQ}$; *Mc*.$TRPM_{PZQ}$ = *Mesocestoides corti* $TRPM_{PZQ}$. [b]EC_{50} values on the channel are reported in ref 10 and are provided for completeness.

The work of Andrews et al., however, provides an additional opportunity to interrogate the candidacy of $TRPM_{PZQ}$.[1] From the extensive list of PZQ derivatives described by Andrews et al.,[1] a small number of analogs displayed good activity against cestodes but not against schistosomes. These analogs of PZQ encompassed replacement of the cyclohexyl group by 3-pyridyl ((±)-**2**, (*R*)-**2**, and (*S*)-**2**), 4-nitrophenyl (**3**), 4-*N*-methylaniline (**4**), 4-*N*,*N*-dimethylaniline (**5**), 4-aniline (**6**), *cis*-4-aminocyclohexyl (**7**) and -cyclopropyl (**8**) groups (Chart 1). Do such analogs, which exhibit differential antiparasitic activity *versus* cestodes and schistosomes, display differential activity at schistosome and cestode $TRPM_{PZQ}$? If the specific SAR of PZQ analogs against these different parasites mimics the SAR at the corresponding $TRPM_{PZQ}$ channel, these data would provide further support for $TRPM_{PZQ}$ as the clinically relevant *in vivo* target of PZQ. To tackle this question, we synthesized eight PZQ analogs from Andrews et al.[1] that exhibited divergent bioactivity against schistosomes and cestodes and profiled them against schistosome $TRPM_{PZQ}$ and two cestode $TRPM_{PZQ}$ representatives.[10]

Table 1 (columns 2−4) reproduces data from Andrews et al.[1] that scored the activity of PZQ derivatives against *Schistosoma mansoni* and *Hymenolepis nana*. Data were collected against *S. mansoni in vitro* and *in vivo* using a

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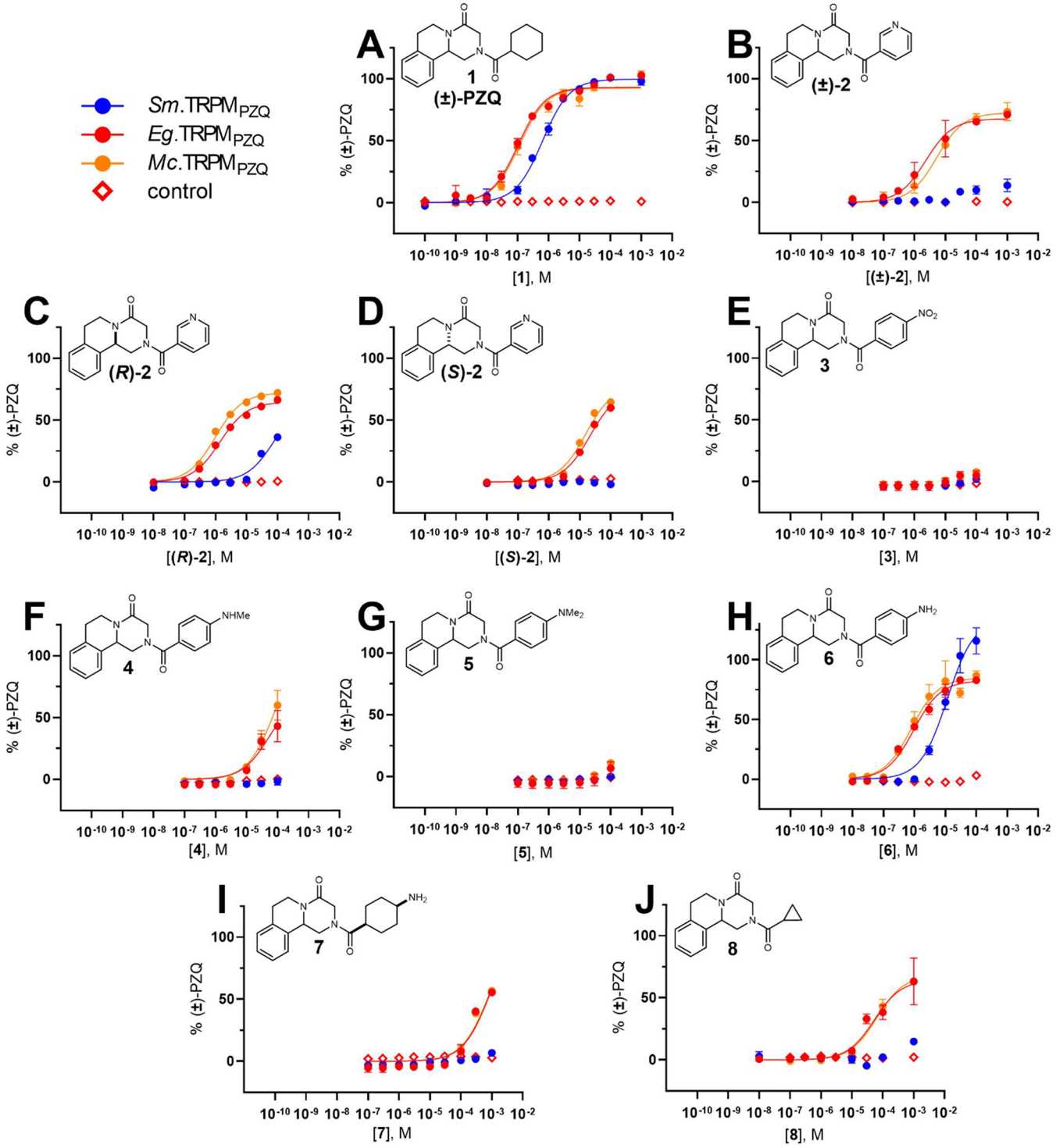

Figure 1. Functional profiling of PZQ analogs against different $TRPM_{PZQ}$ channels. Shown are concentration−response relationships for *Sm*.$TRPM_{PZQ}$ (blue circles), *Eg*.$TRPM_{PZQ}$ (red circles), and *Mc*.$TRPM_{PZQ}$ (orange circles) in response to increasing concentrations of PZQ analogs. Responses to molecules in untransfected HEK293 cells are shown as controls (red diamonds).

mouse model and also against *H. nana* using an *in vivo* mouse model.[1] Results were previously scored using broad potency ranges, graded "+++", "++", "+", or "0" (see Table 1 legend). While there are caveats in the interpretation of these data, it is evident that the selected analogs displayed appreciable activity in the cestode model (column 4) but lower or minimal activity when tested against schistosomes (columns 2 and 3). In contrast, (±)-PZQ (**1**) showed equivalent activity in phenotypic grading across all of the bioassays, with preferential stereoselectivity toward the *R* enantiomer (Table 1).

Based on Andrews' data,[1] we resynthesized compounds **2**−**8**. These analogs were then tested for activity at *Sm*.$TRPM_{PZQ}$ and two representative cestode $TRPM_{PZQ}$ orthologs (*Echinococcus granulosus* $TRPM_{PZQ}$ (*Eg*.$TRPM_{PZQ}$) and *Mesocestoides corti* $TRPM_{PZQ}$ (*Mc*.$TRPM_{PZQ}$)) that have been successfully heterologously expressed.[10] This was done using a fluo-

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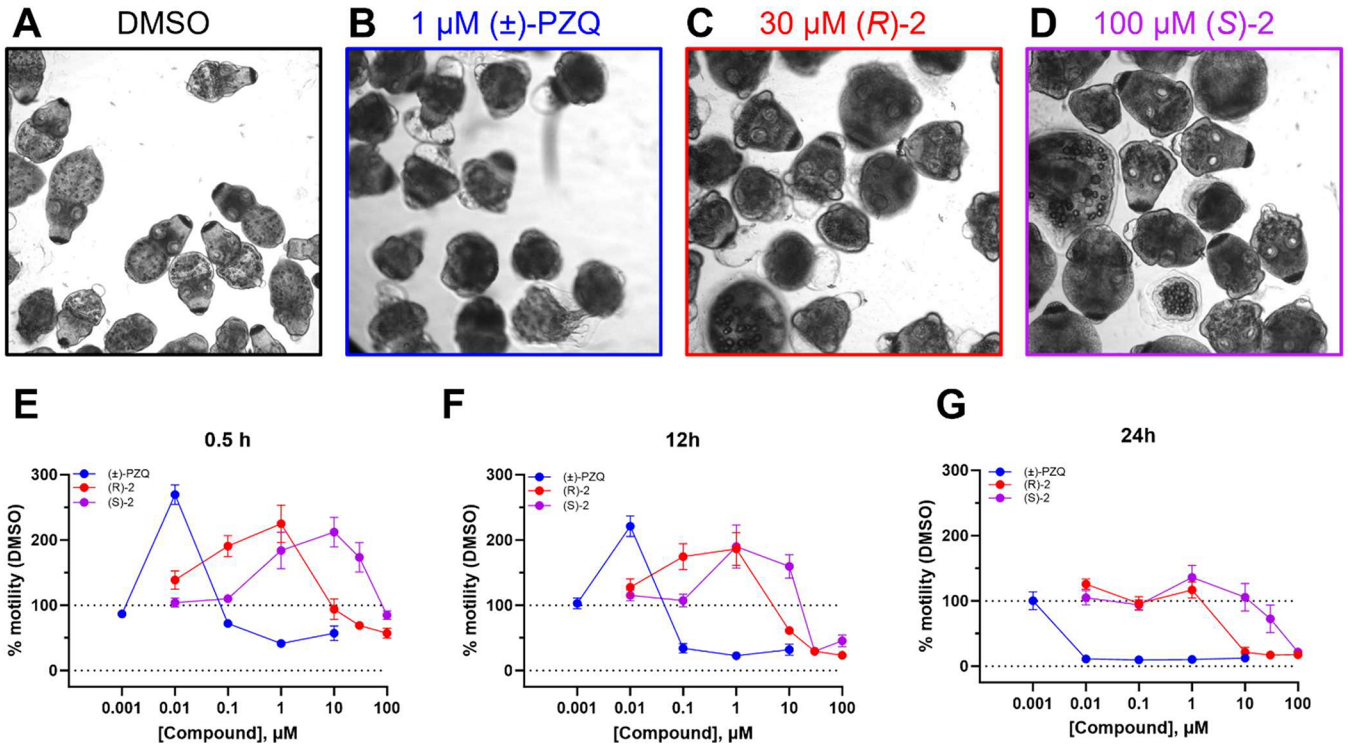

Figure 2. *E. multilocularis* protoscoleces after treatment with (A) DMSO, (B) (±)-PZQ (1 μM), (C) (*R*)-**2** (30 μM), and (D) (*S*)-**2** (100 μM). (E−G) Concentration−response motility graphs of *E. multilocularis* protoscoleces after treatment with (±)-PZQ (blue circles), (*R*)-**2** (red circles), and (*S*)-**2** (purple circles) after (E) 0.5 h, (F) 12 h, and (G) 24 h. Data are plotted as % motility *vs* vehicle (DMSO) control (mean ± SEM).

rescence-based reporter assay to measure changes in cytosolic Ca^{2+} in HEK293 cells transiently expressing the individual $TRPM_{PZQ}$ ion channels. Results from these assays are presented in Figure 1, and all EC_{50} values are tabulated in Table 1 (columns 5−7).

Data for (±)-PZQ (**1**) are shown in Figure 1A. All three $TRPM_{PZQ}$ channels, *Sm*.$TRPM_{PZQ}$ (EC_{50} = 645 ± 57 nM), *Eg*.$TRPM_{PZQ}$ (EC_{50} = 104 ± 9 nM), and *Mc*.$TRPM_{PZQ}$ (EC_{50}= 112 ± 16 nM), were potently activated by **1**. The cestode $TRPM_{PZQ}$ channels displayed ∼6-fold higher sensitivity to PZQ, consistent with previous reports and the known sensitivity of many cestode species to PZQ.[6,7,10] Figure 1B−D shows results for the 3-pyridyl PZQ analogs. The racemate, (±)-**2**, showed little activity at *Sm*.$TRPM_{PZQ}$ but displayed low-micromolar potency at both cestode channels (EC_{50} for *Eg*.$TRPM_{PZQ}$ = 2.3 ± 1.1 μM, EC_{50} for *Mc*.$TRPM_{PZQ}$ = 5.3 ± 1.3 μM; Figure 1B). Consistent with the previously detailed activity of PZQ enantiomers *in vivo*[14] that is also mirrored at $TRPM_{PZQ}$[6] (Table 1, entries 2 and 3), the *R* enantiomer (*R*)-**2** was more potent than the *S* enantiomer (*S*)-**2** at the cestode channels (Figure 1C vs Figure 1D). All activity at *Sm*.$TRPM_{PZQ}$ was attributed to enantiomer (*R*)-**2** (Figure 1C). Activation of both cestode channels, with negligible activity at the schistosome channel, was consistent with the Andrews et al. grading classification (Table 1).[1]

A series of substituted phenyl derivatives were then profiled. In previous phenotypic assays, these analogs were active against *H. nana* and *S. mansoni in vivo* but were inactive against *S. mansoni in vitro* (Table 1).[1] Consistent with these prior observations, analogs **3**−**5** showed no activity at *Sm*.$TRPM_{PZQ}$ (Figure 1E−G), and the activity of these analogs at cyclophyllidean cestode $TRPM_{PZQ}$ orthologs was also low. As previously proposed,[1] the *in vivo* activity of compounds **3**−**5** is likely caused by dealkylative metabolism to aniline **6**, and subsequent synthesis and profiling of **6** confirmed this (Figure 1H). Compound **6** activated *Sm*.$TRPM_{PZQ}$ (EC_{50} = 11.6 ± 2.6 μM), *Eg*.$TRPM_{PZQ}$ (EC_{50} = 957 ± 141 nM), and *Mc*.$TRPM_{PZQ}$ (EC_{50} = 796 ± 224 nM), and displayed an ∼8−10-fold increase in potency at cestode $TRPM_{PZQ}$ compared to schistosome $TRPM_{PZQ}$. This was similar to the ∼6-fold increase in potency for (±)-PZQ (**1**) at cestode *versus* schistosome $TRPM_{PZQ}$.

Finally, we profiled modifications of the cyclohexyl group of PZQ. Compound **7**, a 4′-aminocyclohexyl derivative, lacked activity at *Sm*.$TRPM_{PZQ}$ and was only weakly active at cestode $TRPM_{PZQ}$ orthologs (Figure 1I), corresponding to the weak *in vivo* activity against *H. nana* previously reported (Table 1).[1] Finally, cyclopropyl analog **8** activated both cestode $TRPM_{PZQ}$ representatives at concentrations >10 μM (EC_{50} = 53 ± 16 μM for *Eg*.$TRPM_{PZQ}$, EC_{50} = 65 ± 14 μM for *Mc*.$TRPM_{PZQ}$; Figure 1J). Little activity was observed at *Sm*.$TRPM_{PZQ}$. These target-based data are again consistent with the phenotypic observations of Andrews et al. (Table 1).[1]

Overall, from the profiled PZQ analogs, only a single analog (compound **6**) was sufficiently active at *Sm*.$TRPM_{PZQ}$ to derive an EC_{50} value, while five analogs displayed activity at the cestode channels (Table 1). Analog **6** was not one of the eight PZQ analogs selected based on the differential potency between cestodes and schistosomes but was synthesized to explain the *in vivo* activity of the other analogs. Therefore, the different potencies of these analogs against cestodes and schistosomes, seen in phenotypic the data of Andrews et al.[1] 40 years ago, was mirrored by the same differential potency of these analogs in target-based assays at the different $TRPM_{PZQ}$ channels. Some caveats are however appropriate.

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First, the original data did not report the activity of the PZQ derivatives against cestodes *ex vivo* (*in vitro*), so in selecting these analogs, there was no direct comparator for the action of all of these analogs between schistosomes and cestodes. Therefore, two analogs (*R*)-**2** and (*S*)-**2** were tested on *Echinococcus multilocularis* protoscoleces for comparison with PZQ (Figure 2A–D). When compared with the vehicle control, treatment with (±)-PZQ (1 μM) caused a sustained contraction of the protoscoleces (Figure 2A,B). When protoscoleces were treated with the 3-pyridyl enantiomers (*R*)-**2** (Figure 2C) and (*S*)-**2** (Figure 2D), a similar contraction was observed, with (*R*)-**2** being more potent than (*S*)-**2**. To determine IC_{50} values, concentration–response curves were obtained at multiple time points (Figure 2E–G). Low concentrations of PZQ and the 3-pyridyl analogs stimulated motility at the early time points (Figure 2E). After 24 h, the IC_{50} for (*R*)-**2** was ∼3 μM, and the IC_{50} for (*S*)-**2** was ∼30 μM (Figure 2G). Prolonged treatment with (±)-PZQ proved toxic after 24 h, and therefore, a more realistic IC_{50} value (∼100 nM) was calculated at 12 h postincubation (Figure 2F). This mirrors the EC_{50} at a cestode $TRPM_{PZQ}$ of 100 nM (Table 1). These data for activity against cestodes *ex vivo* are again consistent with the potencies of the molecules at cestode $TRPM_{PZQ}$ (Table 1).

Second, the cestode $TRPM_{PZQ}$ and motility assays derive from different cyclophyllidean cestodes (*E. granulosus*, *M. corti*, and *E. multilocularis*) than the model (*H. nana*) used by Andrews et al.[1] However, we note that the amino acid residues lining the PZQ binding pocket of $TRPM_{PZQ}$ are identical across all cyclophyllidean cestode $TRPM_{PZQ}$ orthologs examined to date, including *H. nana* $TRPM_{PZQ}$ (Figure S1).[10] This is consistent with the similarity of the functional data from *Eg*.$TRPM_{PZQ}$ and *Mc*.$TRPM_{PZQ}$.

Considering the structures of the analogs profiled here, it is evident that the cestode $TRPM_{PZQ}$ binding pocket is more tolerant to substitutions of the cyclohexyl moiety of PZQ than is the schistosome $TRPM_{PZQ}$ binding pocket. Aniline analog **6** and pyridyl analog (*R*)-**2** show submicromolar potency, and even the cyclopropyl analog **8**, which displayed no activity at *Sm*.$TRPM_{PZQ}$ at 100 μM, was clearly active at the cestode $TRPM_{PZQ}$ orthologs, consistent with the differential activity seen by Andrews et al. (Table 1).[1]

This increased tolerability to modifications of the cyclohexyl group of PZQ, a key part of the pharmacophore at *Sm*.$TRPM_{PZQ}$,[7] may provide opportunity to accommodate other cyclohexane ring modifications—notably, more metabolically stable PZQ derivatives—within the cestode $TRPM_{PZQ}$ binding pocket.[15,16] This could potentially enhance the *in vivo* efficacy of these analogs for treating cestode infections, and these data therefore highlight an opportunity to design drugs that selectively target cestode $TRPM_{PZQ}$. Whether the absolute potency of such analogs can be further improved over PZQ to yield better treatments for cestode species less sensitive to PZQ (*e.g.*, noncyclophyllidean cestodes[10,17]) or for cestode life cycle stages that are hard to treat will require further work and a better understanding of the molecular basis by which cestode-selective analogs engage the $TRPM_{PZQ}$ binding pocket. Such understanding will be aided by the recent mapping of the PZQ binding pocket in $TRPM_{PZQ}$ orthologs in different parasitic flatworms and a capacity to model these interactions.[7,10] Of likely relevance are two natural amino acid variants—a histidine residue in the S1 transmembrane helix and a serine residue in the S4/S5 linker—that are different between the PZQ binding pocket of trematode and cyclophyllidean cestode $TRPM_{PZQ}$ (compare Figure 3A and Figure 3B).[10] This natural variation within the $TRPM_{PZQ}$ binding pocket provides a possible molecular explanation underpinning the differential SAR of the PZQ analogs. Natural variation in the binding pocket has previously been shown to render *Fasciola* spp. $TRPM_{PZQ}$ insensitive to PZQ.[7,10]

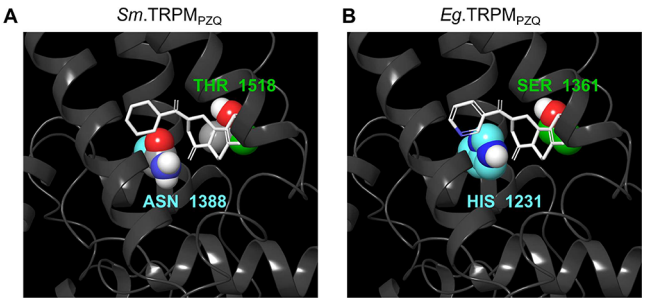

Figure 3. (A) Predicted binding pose of (*R*)-PZQ (white) in *Sm*.$TRPM_{PZQ}$. (B) Visualization of (*R*)-**2** (white) adjacent to residues showing variation in *Eg*.$TRPM_{PZQ}$, based on (A). Residues showing variation between the channels are highlighted in TM1 (Asn 1388/His 1231, teal) and TM4 (T1518/S1361, green).

The activity of (*R*)-**2** at cestode $TRPM_{PZQ}$ *versus* *Sm*.$TRPM_{PZQ}$ is noteworthy in the context of this natural variation. The transmembrane helix 1 (S1) variation occurs at a position in close proximity to the pyridyl nitrogen (Figure 3B), and it is conceivable that there is an electrostatic interaction between the histidine residue in cestode $TRPM_{PZQ}$ (*e.g.*, H1231 in *Eg*.$TRPM_{PZQ}$) and the pyridine ring that is absent with the uncharged asparagine residue in schistosome $TRPM_{PZQ}$ (*e.g.*, N1388 in *Sm*.$TRPM_{PZQ}$). Interactions between PZQ and this S1 residue are important for PZQ activation of $TRPM_{PZQ}$ across species.[7,10]

In summary, functional profiling of various PZQ derivatives on parasitic flatworms and at their respective $TRPM_{PZQ}$ orthologs shows that "the glove fits": the SAR between different parasites and different parasite $TRPM_{PZQ}$ orthologs matches well. These data provide additional support for $TRPM_{PZQ}$ serving as the relevant therapeutic target of PZQ in parasitic flatworms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00350.

Sequence alignment of the PZQ binding pocket residues in cyclophyllidean cestodes; materials and methods, synthetic procedures, characterization data, and $^{1}H/^{13}C$ NMR spectra for all previously uncharacterized molecules (PDF)

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Author Contributions

D.J.S. and J.L.H. performed chemical synthesis; S.-K.P. and D.J.S. performed pharmacological assays; M.K. performed *E. multilocularis* motility experiments, which were analyzed by M.K. and B.L.-S.; C.M.R. performed bioinformatic studies; D.M. provided chemicals to support syntheses and together with T.S. and J.S.M. supervised data evaluation and discussion; J.S.M. wrote the initial draft of the manuscript and supervised this project with B.L.-S; all of the authors worked on revisions and approved the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PZQ, praziquantel; TRP, transient receptor potential; *Sm*.TRPM$_{PZQ}$, *Schistosoma mansoni* transient receptor potential praziquantel; *S. mansoni*, *Schistosoma mansoni*; *H. nana*, *Hymenolepis nana*; *Eg*.TRPM$_{PZQ}$, *Echinococcus granulosus* transient receptor potential praziquantel; *Mc*.TRPM$_{PZQ}$, *Mesocestoides corti* transient receptor potential praziquantel; *E. multilocularis*, *Echinococcus multilocularis*; *E. granulosus*, *Echinococcus granulosus*; *M. corti*, *Mesocestoides corti*; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FLIPR, fluorescence imaging plate reader; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

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