The anthelmintic praziquantel is a human serotoninergic G-protein-coupled receptor ligand.
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Schistosomiasis is a debilitating tropical disease caused by infection with parasitic blood flukes. Approximately 260 million people are infected worldwide, underscoring the clinical and socioeconomic impact of this chronic infection. Schistosomiasis is treated with the drug praziquantel (PZQ), which has proved the therapeutic mainstay for over three decades of clinical use. However, the molecular target(s) of PZQ remain undefined. Here we identify a molecular target for the antischistosomal eutomer — (R)-PZQ — which functions as a partial agonist of the human serotoninergic 5HT2B receptor. (R)-PZQ modulation of serotoninergic signaling occurs over a concentration range sufficient to regulate vascular tone of the mesenteric blood vessels where the adult parasites reside within their host. These data establish (R)-PZQ as a G-protein-coupled receptor ligand and suggest that the efficacy of this clinically important anthelmintic is supported by a broad, cross species polypharmacology with PZQ modulating signaling events in both host and parasite.
Hundreds of millions of people are infected with parasitic worms. One of the most burdensome infections is the neglected tropical disease schistosomiasis (Bilharzia) caused by parasitic flatworms of the genus *Schistosoma*. *Schistosoma* infections result from contact with fresh water containing cercariae, the free-swimming larval stage of the parasite. Cercariae penetrate the host’s skin, and then transform and mature into sexualized forms while undergoing a remarkable intravascular migration to defined vascular beds, where paired male and female worms complete their maturation and commence egg laying.
The debilitating impact of schistosomiasis results from the host’s immune response to schistosome eggs, deposited in prolific numbers in the liver, intestine and/or bladder where they elicit granulomatous inflammation, perportal fibrosis and hypertension. Clinical outcomes span gastrointestinal and liver pathologies, genitourinary disease, anemia, malnutrition, and a heightened risk for comorbidities and HIV transmission. The associated disease burden encumbers third world economies with an annual loss of up to 70 million disability-adjusted life years. Effective drug therapy for schistosomiasis is therefore a key healthcare priority.

Praziquantel, a tetracyclic tetrahydroisoquinoline derivative administered as a racemic mixture (\((\pm)\)-PZQ), is the main drug therapy for combating schistosomiasis. PZQ causes rapid paralysis of schistosome musculature and subsequent tegumental damage that promotes immunological clearance of worms from the host. The World Health Organization estimates a considerable future demand for PZQ of 2 billion tablets over 5 years to...
support mass drug administration initiatives\textsuperscript{5}. Clearly, the continued efficacy of PZQ is essential for this strategy and reports of PZQ-resistant worm isolates in both laboratory and field highlight an urgency in resolving how PZQ works\textsuperscript{6–8}. Such knowledge would catalyze the development of next generation anthelmintics.

Here we employ a variety of experimental approaches to demonstrate that the antischistosomal eutomer (\textit{R})-PZQ acts as a partial agonist of the human 5-HT\textsubscript{2B} receptor, establishing (\textit{R})-PZQ as a GPCR ligand. These data will prioritize future screening of flatworm GPCRs for a (\textit{R})-PZQ target and underscore an activity of (\textit{R})-PZQ on 5-HT\textsubscript{2B}R signaling in the human host, manifest within the vascular beds where the adult parasites reside.

**Results**

**Mammalian GPCR target(s) of PZQ.** Prior work has demonstrated an ergomimetic like action of PZQ to engage flatworm vascular beds, where the adult parasites reside.

**Fig. 3 (\textit{R})-PZQ constricts mouse mesenteric vasculature.**

\textbf{a} Image of mesentery surrounding mouse intestine, showing blood vessel containing a schistosome worm-pair (inset). \textbf{b} Representative image of a surgically isolated piece of mouse mesenteric artery held under tension between two clamped wires. Scalebar, 1 mm. \textbf{c} Changes in tension triggered by varied manipulations. Left, sustained contraction of vessels in KPSS and 5-HT (10 \textmu M). Right, tension in individual arteries incubated in the presence of vehicle (DMSO, open circle) or SB204741 (10 \textmu M, closed circle), and challenged with (\textit{R})-PZQ (50 \textmu M) or (\textit{S})-PZQ (50 \textmu M). W, wash; complete media exchange. Preincubation of strips with SB204741 (10 \textmu M) attenuated (\textit{R})-PZQ evoked vasoconstriction. \textbf{d} Cumulative data set from experiments reporting effects of ligands relative to KPSS(5)-evoked contractile tension, concentrations of ligands: 5-HT (1 \textmu M), (\textit{R})-PZQ (50 \textmu M), (\textit{S})-PZQ (50 \textmu M), SB204741 (10 \textmu M). Replicate number for each measurement as indicated, where individual measurements reflect the response from a naïve artery isolated from different mice. Data are presented as mean ± s.d. Probability, $p < 0.05$ (*), $p < 0.01$ (**). \textbf{e} Schematic model (not to scale) depicting (\textit{R})-PZQ action on both parasite (contraction) and host vasculature (contraction, increased perfusion pressure) facilitating hepatic shift of worms.
bioaminergic signaling pathways. Specifically, PZQ exposure caused a bipolar regeneration phenotype in regenerating free-living planarian flatworms that was phenocopied by ergot alkaloids acting as serotonergic antagonists, but opposed by 5-hydroxytryptamine (5-HT9). Similarly, in both adult schistosome worms (Supplementary Fig. 1) and schistosomules9, PZQ evoked a rapid paralysis counteracted by exogenous 5-HT. On the basis of these observations, we considered whether PZQ acts as a direct serotonergic ligand to oppose 5-HT evoked G-protein-coupled receptor (GPCR) signaling.

To investigate the possibility that PZQ engages serotonergic GPCRs, in silico docking approaches were first applied. The resolved PZQ enantiomers—(R)-PZQ (the active, antischistosomal euomer, Fig. 1a) and (S)-PZQ (Fig. 1b)—were screened against a panel of 3436 computational models, representing 1465 protein targets mostly of human origin, (MolScreen). Both ligands were docked and scored into ensemble 4D10 models of various targets assembled in the Pocketome database11. The top candidate from the docking to pocket classification (dpc models) was the human 5-HT2B receptor (5-HT2BR), with (R)-PZQ possessing the highest predicted affinity (Supplementary Table 1). Figure 1c depicts the orientation of both (R)-PZQ and (S)-PZQ within the 5-HT2BR orthosteric binding pocket from modeling simulations. Different binding orientations for both PZQ enantiomers were predicted with the (R)-isomer exhibiting a more favorable hydrogen bond with the backbone amine group of L209 in extracellular loop 2 of 5-HT2BR (Fig. 1c).

With these in silico predictions in hand, we profiled a broad panel of human GPCRs through the NIMH Psychostimulatory Drug Screening Program (PDS12) first using racemic (+)-PZQ (10 µM) and then the resolved enantiomers ((R)-PZQ and (S)-PZQ)13; Supplementary Fig. 2). (+)-PZQ exhibited a polypharmacological profile (Supplementary Fig. 3) consistent with predictions from the in silico modeling data (Fig. 1b). The ability of PZQ to engage host GPCRs likely explains prior reports of (+)-PZQ action on heart and smooth muscle14,15, and potentially other known activities of PZQ such as the bitterness of (S)-PZQ16, a taste transduced through GPCR signaling. This screen was then repeated using the individual enantiomers with the criterion for a positive screening hit defined as stereoselective inhibition of radioligand binding by (R)-PZQ, but not (S)-PZQ. This was observed at only one GPCR in the primary screen—the human 5-HT2B receptor (5-HT2BR)—where inhibition seen with (+)-PZQ was attributable solely to (R)-PZQ (Fig. 1d, Supplementary Fig. 3). These data support the in silico modeling data (Fig. 1b) consistent with predictions from the in silico modeling (Fig. 1b). The ability of PZQ to engage host GPCRs likely explains prior reports of (+)-PZQ action on heart and smooth muscle14,15, and potentially other known activities of PZQ such as the bitterness of (S)-PZQ16, a taste transduced through GPCR signaling. This screen was then repeated using the individual enantiomers with the criterion for a positive screening hit defined as stereoselective inhibition of radioligand binding by (R)-PZQ, but not (S)-PZQ. This was observed at only one GPCR in the primary screen—the human 5-HT2B receptor (5-HT2BR)—where inhibition seen with (+)-PZQ was attributable solely to (R)-PZQ (Fig. 1d, Supplementary Fig. 3). These data support the in silico modeling, although demonstrating more biological selectivity for (R)-PZQ over (S)-PZQ at 5-HT2BR.

PZQ interaction with 5-HT2BR was then validated using radioligand binding and functional assays. Analysis of [3H]-LSD displacement by (±)-PZQ revealed complete displacement of specific [3H]-LSD binding at 5-HT2BR by unlabeled (±)-PZQ (Kd = 5.3 µM, Fig. 1e). For functional activity, Ca2+ flux assays were performed in HEK293 cells expressing individual 5HT2R isoforms. (R)-PZQ evoked Ca2+ release in 5-HT2BR expressing cells at concentrations >1 µM (Fig. 1f). The peak amplitude of (R)-PZQ-evoked Ca2+ release was consistently less than evoked by 5-HT (Fig. 1d), suggesting action as a 5-HT2BR partial agonist. No Ca2+ release activity was observed using (S)-PZQ over a comparable concentration range (Fig. 1f). No Ca2+ release activity was observed in uninduced cells lacking 5-HT2BR expression, or cells expressing either 5-HT2AR or 5-HT2CR (Fig. 1f), indicating that (R)-PZQ activity was specific and selective for the 5-HT2BR. At other tested GPCRs, including several predicted by the modeling simulations (Supplementary Table 1), (±)-PZQ lacked Ca2+ release activity (Supplementary Fig. 4).

Further characterization of PZQ-evoked Ca2+ release was performed in a 5-HT2BR inducible cell line by confocal imaging. Neither (±)-PZQ, nor 5-HT, evoked cellular Ca2+ transients in the absence of 5-HT2B induction, whereas acetylcholine (ACh) elevated cytoplasmic Ca2+ through endogenous GPCRs (Fig. 2a, b). Following 5-HT2BR induction, both (±)-PZQ and 5-HT triggered Ca2+ signals (Fig. 2a, b). Prior incubation of induced cells with serotonergic blockers ritanserin, SB204747, or mesulergine blocked PZQ and 5-HT evoked Ca2+ transients, but failed to impact ACh-evoked Ca2+ signals (Fig. 2c). Dose–response relationships revealed (±)-PZQ activity over the micromolar range (EC50 ≈ 8.1 µM; Fig. 2d) with Ca2+ release activity solely caused by the (R)-enantiomer (Fig. 2e, f). The 5-HT2BR blocker LY272015 also inhibited PZQ-evoked Ca2+ signals (Supplementary Fig. 5).

Next, we examined PZQ-evoked signaling using a transcriptional reporter assay, designed to monitor Ca2+-dependent NFAT translocation (Supplementary Fig. 6A). In HEK293 cells expressing the reporter construct alone, neither (±)-PZQ nor 5-HT increased the basal luminescence signal (Supplementary Fig. 6B). In cells transiently transfected with 5-HT2BR, both PZQ and 5-HT elicited NFAT translocation and this response was blocked by ritanserin (Supplementary Fig. 6B). (R)-PZQ activation of NFAT occurred over the micromolar range (Supplementary Fig. 6C), again less potent and penetrant than observed with 5-HT (Figs. 1d, 2d). Therefore, multiple experimental approaches demonstrated (R)-PZQ acted as a partial agonist at human 5-HT2BR.

**Action of (R)-PZQ at host GPCRs.** These data are significant for two reasons. First they identify PZQ as a bona fide GPCR ligand at a defined molecular target. This discovery prioritizes future screening of flatworm GPCRs (~120 GPCRs in schistosomes17) for a receptor selectively engaged by (R)-PZQ that could be a key target for future antischistosomal drug development18,19. Second, these data suggest PZQ—conventionally viewed as a selective antiparasitic therapy—can interact with endogenous 5-HT2BRs in the host human. One pathophysiologically relevant effect would be an activity of (R)-PZQ on host mesenteric vascular beds, a destination of the mature, paired adult S. mansoni and S. japonicum blood flukes and sites where egg laying commences2,3 (Fig. 3a). After drug treatment, worms are rapidly displaced from their mesenteric habitat to the liver where elimination occurs. This hepatic shift is a frequently used assay for drug efficacy and has been attributed to loss of worm muscle tone evoked by antischistosomal agents20,21 as seen in vitro (Supplementary Movie 1). Serotonergic ligands are well known regulators of the tone of arteries and veins22,23, including mesenteric vessels23, such that PZQ-evoked changes in mesenteric blood flow may help ‘flush’ PZQ-paralyzed worms toward the liver. Levels of PZQ within the splanchnic vasculature likely reach levels an order of magnitude higher than peak plasma concentrations that are measured after first-pass metabolism of the drug (>4 µM for 40 mg/kg human dosing, even higher in mouse models24,25). Therefore, PZQ concentration within mesenteric vessels falls well within the concentration range for causing host serotonergic effects demonstrated in vitro (Figs. 1, 2).

The activity of PZQ within the mesenteric vasculature of uninfected mice was assessed by measuring mesenteric artery tone using wire myography. Mounted vessel segments (Fig. 3b) exhibited a rapid, sustained contraction to high K+ media which reversed upon media exchange (Fig. 3c). Addition of 5-HT to resting vessel segments also elicited a protracted, contractile response that declined over time (Fig. 3c). Addition of (R)-PZQ to naïve vessels mimicked the profile of 5-HT evoked contractions (Fig. 3c), and this effect was prevented by preincubation with SB204741, a 5-HT2BR antagonist (Fig. 3c). No changes in resting tone were observed with (S)-PZQ. PZQ also evoked contraction of hepatic portal vein segments (Supplementary Fig. 7), again
mimicking 5-HT action. The cumulative data set demonstrates (R)-PZQ caused mesenteric artery vasoconstriction, mimicking the action of 5-HT (Fig. 3d).

In conclusion, we demonstrate that PZQ is a GPCR ligand, with antischistocidal activity. (R)-PZQ acting as a low micromolar affinity partial agonist at human 5-HT_{2A}Rs. Activity at both parasite and host receptors—both targets potentially being GPCRs—likely contribute to clinical efficacy of PZQ by combining a deleterious paralytic effect on the parasite (Supplementary Movie 1) with beneficial host effects that promote worm clearance. We propose PZQ causes contraction of both the parasite and host mesenteric vessels to increase perfusion pressure and flush paralyzed worms to the liver (Fig. 3e). PZQ activity at host 5-HT_{2BR} receptors may be further enhanced in individuals infected with chronic schistosomiasis disease mitigating actions in both host and parasite. In conclusion, these data support a strategy for developing novel schistosomiasis pathologies that extend beyond the vasculature given a role for 5HT_{2BR} in computational models designed to predict activities of arbitrary chemical compounds. PZQ enantiomers ((R)- and (S)-) were resolved using HPLC methods by Woele et al.13.

### Mammalian GPCR profiling.

PZQ (racemic and enantiomers) were screened against human GPCRs using primary and secondary assays coordinated through the NIH Psychonaut Drug Screening Program (PDSP). Full details on these methods are available in the PDSP Assay Protocol Book available online (https://pdspdb.unc.edu/pdspWeb/). PZQ was dissolved in 100% ethanol at a concentration of 100 µM to achieve a maximal assay concentration (up to 100 µM) for both binding and functional assays. Ca^2+ flux experiments utilized tetracycline-inducible stable cell lines for 5HT_{1AR}, 5HT_{3AR}, and 5HT_{2CR} receptors initially generated using the Flp-In 293 T-Rex tetracycline inducible system (gift from the Roth Lab, Invitrogen). Cells were maintained in DMEM (ThermoFisher, 10569010) supplemented with 10% dialyzed fetal bovine serum (Gibco, 26400044), 50 µM Penicillin-Streptomycin (ThermoFisher Scientific, 15140122), 100 µg/ml BactoLigin (Research Products International, 8050930.1) and 100 µg/ml Hygromycin B (ThermoFisher Scientific, 10687010) at 37°C and 5% CO2. For Ca^2+ flux assays, receptor expression was induced with 2 µg/ml tetracycline, and cells were plated into white 384 well plates. Cells were then cultured with drug solutions or buffer and read for an additional 110 s. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. The data were normalized to maximum peak fold over basal fluorescence by 5-HT (100%) and baseline fluorescence (0%). The data were analyzed using the sigmoidal dose-response function built into GraphPad Prism 5.0.

### In silico modeling.

(R)-PZQ and (S)-PZQ were screened against a panel of 3436 computational models designed to predict activities of arbitrary chemical compounds, representing 1465 proteins targets mostly of human origin (so called ‘MolScreen’ panel). The earlier version of this set of models included different classes of targets and described and validated the panel. Two main types of three dimensional docking models were used: ‘dpc’ models (349 models representing the conformations emerging from binding pocket conformations from the Pocketome database13) and ‘lda’ models (pharmacophoric field models based on the continuous three dimensional pharmacophoric fields of the diverse co-crystallized ligands from the Pocketome database, enhanced by training on the activity data from the ChEMbl database2,3). Confocal Ca^2+ imaging. For single-cell confocal Ca^2+ imaging, the 5HT_{3AR} inducible cell line was seeded onto collagen-coated, 35 mm glass bottom dishes (MatTek, P35GCOL-0.14-C) at a density of 1 x 10^4 cells three days prior to imaging. Two days prior to imaging, 5-HT receptor expression was induced by supplementing the growth media with 1 µg/ml doxycycline (Sigma-Aldrich, D3477). Two hours prior to conducting experiments, growth medium was exchanged for EMEM supplemented with 1% dialyzed FBS, 100 µg/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml neomycin. The cells were washed twice with Hank’s balanced salt solution (HBBS), and incubated with Fluoro-4-AM (4 µM) and Fluoron F127 (0.4%) for 25 minutes at room temperature. The cells were then washed twice with HBBS, and left at room temperature (30 min) for de-esterification. Dishes were mounted on an Olympus BX41 microscope with high fluorescence collection (Leica, L = 488 nm, 0.5x, Olympus, Japan). Confocal images were collected using a Leica TCS SP5 confocal microscope (Leica, Wetzlar, Germany) and processed using Leica LAS AF software. For quantitative analysis of mean fluorescence intensity, ImageJ was used. To identify the optimal pre-stretch value for experiments, a normalization factor (IC75/IC50) was calculated for individual test strips, defined as the ratio of the internal circumference at which the maximum response to vasoconstriction (KCl, plus 40 µM norepinephrine) was observed (IC50), divided by the internal circumference at which a transmural wall pressure of 100 mm of Hg is attained on a length-tension plot overlayed with a La Place transformation isobar (IC75).

### Wire myography.

Swiss Webster mice (female, aged 10–13 weeks) were obtained from Charles River Laboratories. Measurements of mouse mesenteric vessel tone were conducted using a multiwire myograph (FLEXITETRA, Molecular Devices). Vessels were isolated from second order mesenteries and equilibrated for 30 min in gassed (95% O2, 5% CO2) physiological saline solution (PSS, 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH2PO4, 1.17 mM MgSO4, 14.9 mM NaHCO3, 5.5 mM dextrose, 0.026 mM EDTA, 1.6 mM CaCl2, pH 7.4 at 37°C). To identify the optimal pre-stretch value for experiments, a normalization factor (IC75/IC50) was calculated for individual test strips, defined as the ratio of the internal circumference at which the maximum response to vasoconstriction (KCl, plus 40 µM norepinephrine) was observed (IC50), divided by the internal circumference at which a transmural wall pressure of 100 mm of Hg is attained on a length-tension plot overlayed with a La Place transformation isobar (IC75).

### Adult Schistosoma mansoni mobility assays.

Infected mice were provided by the Biomedical Research Institute (33) from which adult worms were collected by portal perfusion 6–8 week-post infection. Mobility experiments were conducted 24–48 h after worm collection using a compound microscope equipped with a digital video camera to acquire video recordings (3 frames per second for 1–2 min) of worms exposed to various drugs. Analysis was performed in ImageJ after file import using the Bio-Formats plugin. Differences in illumination were corrected using the stack deflicker function of the wrMTrck plugin. Images were processed by converting to binary format, and mobility was assessed by measuring the average difference in pixels resulting from subtracting two consecutive frames, providing a measurement of the worms displacement over that period (~0.3 s). This calculation was performed for each frame in the video, and the results were averaged over the length of the recording to provide a metric of worm movement. Unless otherwise noted, values reported represent the mean ± standard error of at least three independent experiments.

### Mammalian GPCR profiling.

Primary GPCR screening assays were coordinated through the NIH Psychonaut Drug Screening Program (PDSP). Methods and statistical analyses for radioligand and functional assays are available in the PDSP Assay Protocol Book available online (https://pdspdb.unc.edu/pdspWeb/). Ca^2+ flux assays were performed as similarly described, except experiments utilized co-transfection with the NFAT reporter assay. For experiments measuring CAMP, experiments utilized co-transfection with the cAMP split-luciferase reporter GloSensor(R)-22F plasmid (Promega, E151) and were read for luminescence on a TriLux Microbeta (Perkin Elmer). For Gi/o mediated CAMP inhibition, forskolin (1 µM) was used to stimulate CAMP via adenyl cyclase activation.

### NFAT reporter assay.

The Ca^2+ sensitive transcriptional luciferase reporter plgLE30(luc2P/NFAT-RE/Hygro) (Promega, E8481) was transiently transfectioned into the 5HT_{3AR} stable cell line. Briefly, 3 x 10^5 cells were plated in a Nunc Cell Culture Treated flask (25 cm², ThermoFisher) and transfected with lipofectamine 2000 (ThermoFisher) plus 1 µg of plasmid DNA according to the manufacturer's protocol. The following day, culture media was replaced with induction media (DMEM + 10% dialyzed FBS supplemented with 1 µg/ml doxycycline), and 24 h later cells were re-plated into 96 well, solid white plates (Costar, 3917). After allowing 3 h for cells to adhere, drugs were added at 20× concentration. For agonist experiments, cells were incubated with 5HT_{3AR} antagonists for 2 h prior to supplementation addition of drugs. After 18 h of culture, plates were assayed using the ONE-Glo Luciferase Assay System (Promega, E6120) and read on a GloMax-Multi Detection System plate reader (Promega).

### Statistical analysis.

Except for the myography experiments, data (PDSP data, confocal calcium imaging) were analyzed using the two tailed Student’s t-test, and are reported except where explicitly indicated as mean ± s.e.m for independent biological replicates (defined as a single subject). For myography experiments, where the amounts of resolved enantiomers were limiting, measurements were made in vessels isolated from 3–6 mice, a sample size
providing a α-significance criterion of 0.05 and a β of 0.9. No randomization or blinding was used in the animal studies, as no comparative analyses were involved. The statistical significance between experimental groups in myography experiments was determined using the Mann–Whitney test and data are presented as mean ± s.d. for these assays. Replicate numbers for individual experiments are outlined within Figure Legends or Figures as appropriate for clarity. Probability values of p < 0.05 were considered statistically significant.

Data availability. The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Data Files or from the corresponding author on reasonable request.

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Author contributions
J.D.C., P.M.C., G.S.G., and J.D.M. performed experiments, analyzed data and contributed figures for the manuscript. K.S., T.R.W., P.I.D. performed chemical isolation and validated purity of PZQ enantiomers. Y.Y and R.A. performed in silico modeling analyzes. B.L.R., R.A., C.C., and J.S.M established hypothesis and supervised experimental studies. J.D.C. and J.S.M. co-wrote the manuscript with help from other authors.

Additional information
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