A drug repurposing screen for whipworms informed by comparative genomics

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Abstract

Hundreds of millions of people worldwide are infected with the whipworm *Trichuris trichiura*. Novel treatments are urgently needed as current drugs, such as albendazole, have relatively low efficacy. We have investigated whether drugs approved for other human diseases could be repurposed as novel anti-whipworm drugs. In a previous comparative genomics analysis, we identified 409 drugs approved for human use that we predicted to target parasitic worm proteins. Here we tested these *ex vivo* by assessing motility of adult worms of *Trichuris muris*, the murine whipworm, an established model for human whipworm research. We identified 14 compounds with $EC_{\_}$ values of $\leq 50\ \mu M$ against *T. muris ex vivo*, and selected nine for testing *in vivo*. However, the best worm burden reduction seen in mice

was just 19%. The high number of *ex vivo* hits against *T. muris* shows that we were successful at predicting parasite proteins that could be targeted by approved drugs. In contrast, the low efficacy of these compounds in mice suggest challenges due to their chemical properties (e.g. lipophilicity, polarity, molecular weight) and pharmacokinetics (e.g. absorption, distribution, metabolism, and excretion) that may (i) promote absorption by the host gastrointestinal tract, thereby reducing availability to the worms embedded in the large intestine, and/or (ii) restrict drug uptake by the worms. This indicates that identifying structural analogues that have reduced absorption by the host, and increased uptake by worms, may be necessary for successful drug repurposing against whipworms. Therefore, we recommend that prior to *in vivo* studies, future researchers first assess drug absorption by the host, for example, using human intestinal organoids or cell lines, and drug uptake by whipworms using intestinal organoids infected with *T. muris*.

Key words

Trichuris trichiura, *Trichuris muris*, whipworms, trichuriasis, anthelmintic, drug repurposing, drug screening

Introduction

An estimated 316-413 million people worldwide are infected with whipworms (*Trichuris trichiura*), the cause of the neglected tropical disease trichuriasis (GBD 2019 DISEASES AND INJURIES COLLABORATORS 2020). Whipworms infect the large intestine, causing abdominal pain, tiredness, colitis, anaemia and *Trichuris* dysentery syndrome (ELSE *et al.* 2020). In addition, chronic infection of children with whipworms is associated with impaired cognitive and physical development (ELSE *et al.* 2020). There is an urgent need for new treatments to fight whipworm infection because no vaccines are available and single doses of mebendazole or albendazole, the mainstay of mass drug administration programmes, do not achieve complete deworming (SPEICH *et al.* 2015).

Drug repurposing aims to test currently approved drugs for new uses such as trichuriasis, so that any promising hits can progress relatively quickly through the drug development pipeline, since a lot is already known about their safety and pharmacology (OPREA AND OVERINGTON 2015). One drug repurposing approach, taken by the ReFRAME project, is to test all approved drugs as well as compounds that have undergone significant pre-clinical studies, to find potential new drugs against pathogens (JANES *et al.* 2018).

We have previously taken an alternative approach to identify potential drugs for repurposing (INTERNATIONAL HELMINTH GENOMES CONSORTIUM 2019). Comparative genomics of many different parasitic worms, including *Trichuris* spp., was used to identify proteins that are homologous to known drug targets from other species and then to select known ligands of those targets as candidates with possible activity against worms. Making use of the ChEMBL database of bioactive molecules and their targets (MENDEZ *et al.* 2019), we identified 817 drugs approved for human use that we predicted to interact with protein targets in parasitic worms. Here, we screened 409 of these 817 drugs against adult worms of *T. muris*, the natural mouse whipworm and model of the closely related human parasite *T. trichiura*. Our screen was based upon quantifying worm motility *ex vivo* in the presence of the drugs, using the Invertebrate Automated Phenotyping Platform (INVAPP) (PARTRIDGE *et al.* 2018; BUCKINGHAM *et al.* 2021). For the highest scoring hits, we then performed *ex vivo* dose-response experiments to estimate EC_{50} (half maximal effective concentration). The most

active compounds were subsequently tested in *T. muris*-infected mice. Interestingly, there were a high number of hits *ex vivo*, but no significant hits *in vivo*; we discuss possible reasons for this finding, and its implications for future drug screens against whipworms.

Methods

Drugs. Based on a comparative genomics analysis, we previously proposed a screening set for parasitic worms, of 817 drugs approved for human use (INTERNATIONAL HELMINTH GENOMES CONSORTIUM 2019). Taking into account price, availability and chemical class, 409 compounds were obtained from Sigma-Aldrich in 10 mM dimethylsulfoxide (DMSO) solutions, and stored at -20 °C (**Supplementary Table 1**; **Figure 1**). For *in vivo* testing, we obtained high-purity compounds from Sigma-Aldrich: econazole nitrate (catalogue number E0050000), prazosin hydrochloride (BP399), flunarizine dihydrochloride (F0189900), butoconazole nitrate (1082300), felodipine (BP777), cyproheptadine hydrochloride (1161000), terfenadine (T00710000), pimozide (BP682 or P1793), nicardipine hydrochloride (1463224 or N7510), mebendazole (1375502), as well as Tween 80 (P6474) and ethanol (PHR1373) for dissolving the drugs.

Ethics statement. Mouse experimental infections were conducted under the UK Home Office Project Licence No. PPL (P77E8A062). All protocols were revised and approved by the Animal Welfare and Ethical Review Body (AWERB) of the WSI. The AWERB is constituted as required by the UK Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012.

Mice. NOD SCID mice (NOD.*Cg-Prkdc*^{scid} Il2rg^{tm1Wjl}/SzJ) were used to maintain the life cycle of *T. muris* and for *in vivo* testing of drugs. All animals were housed in GM500 Individually Ventilated Cages or IsoCage N-Biocontainment Systems (Tecniplast) under environmentally-controlled conditions (temperature: 19-23°C, humidity: 45-65%, light/dark cycle 12h/12h), with access to water and rodent food. No more than five animals were housed per cage. Welfare assessments were carried out daily, and abnormal signs of behaviour or clinical signs of concern were reported.

Parasites. Mouse infections to maintain *T. muris* were conducted as previously described (WAKELIN 1967). Female NOD SCID mice (6 wk old) were infected with a dose of 400 infective embryonated *T. muris* eggs via oral gavage. Thirty-five days later, the mice were euthanised and their caecae and proximal colons removed. Intestines were opened longitudinally, washed with pre-warmed Roswell Park Memorial Institute (RPMI)-1640 media supplemented with penicillin (500 U/mL) and streptomycin (500 μg/mL) (all from Sigma-Aldrich), and adult worms were carefully removed using forceps. Worms were maintained in RPMI-1640 media supplemented with penicillin (500 U/mL) and streptomycin (500 μg/mL) at approximately 37°C. On the same day of collection, worms were sent by courier in flasks at 37°C to UCL.

Ex vivo T. muris *drug screen*. At UCL, individual adult worms were placed in wells of 96-well plates containing 100 μL of RPMI-1640 media supplemented with penicillin (500 U/mL) and streptomycin (500 μg/mL), plus the tested compound dissolved in 1% v/v DMSO final concentration. Levamisole (10 μM, Sigma-Aldrich) was used as a positive control, and 1% DMSO as a negative control.

Plates were incubated at 37°C and 5% CO_2. Motility was determined after 24 h using the Invertebrate Automated Phenotyping Platform (INVAPP) (PARTRIDGE *et al.* 2018;

BUCKINGHAM *et al.* 2021), which recorded 200-frame movies of the whole plate at 100 ms intervals. The Paragon algorithm (PARTRIDGE *et al.* 2018) was used to detect changes in motility by analysing changes in pixel variance.

In the primary library screen, the test drug concentration was 100 μM, and each compound was screened in triplicate using three adult worms in three individual wells, carried out over three separate occasions. In the confirmatory re-screen, each of the top 50 hits from the primary screen was tested again at 100 μM in six replicates (using six adult worms in six individual wells) (**Figure 1**). Each replicate used worms obtained from independent mice, and the re-screen was carried out over two occasions. To help prioritise the hit compounds for further investigation, their activity was evaluated at 5 and 20 μM, with six replicates for each compound.

Ex vivo C*aenorhabditis* elegans *drug screen*. To obtain *C. elegans* worms for screening, synchronised L1 worms were prepared as described in (PARTRIDGE *et al.* 2018). In the screen, worms were cultured at 20°C for 6 days in 96-well plates containing *E. coli* food, and 100 μM of tested drug (1% v/v DMSO) or a diluted DMSO control, as above. The worms were then imaged using INVAPP and a motility score calculated using Paragon, as for the *T. muris* screen. The top 14 hits from the primary library screen based on the lowest mean motility score were re-screened at 100 μM, with six replicates for each compound.

Cheminformatics analysis of the top 50 hits. The top 50 hits from the primary library screen in *T. muris* (based on the lowest mean motility score) were assigned to chemical classes, based on information in DrugBank (WISHART *et al*. 2018), ChEBI (HASTINGS *et al*. 2016), Wikipedia, and PubMed (**Supplementary Figure 1**). We identified additional approved drugs in ChEMBL v25 (MENDEZ *et al.* 2019) that were similar to our top 50 hits, using two approaches in DataWarrior v5.0.0 (SANDER *et al.* 2015): (i) the 'Similarity analysis' function, and (ii) the 'Substructure search' function, to search for compounds with substructures present in our top 50 hits (**Supplementary Figure 2**). The additional drugs identified were filtered to only retain over-the-counter or inexpensive drugs. Drugs were considered inexpensive if they cost ≤ US $5 per vial/capsule according to DrugBank v5.1.4 (WISHART *et al*. 2018), while over-the-counter drugs (which are usually very safe) were identified from ChEMBL (MENDEZ *et al*. 2019). We discarded antipsychotics, general anaesthetics and anti-clotting agents, due to possible low suitability for repurposing. This led us to obtain an additional 71 drugs from Sigma-Aldrich for testing (**Figure 1**).

Ex vivo T. muris *drug screen on additional approved drugs.* The 71 additional drugs were screened by testing four adult worms in four individual wells: one at 100 μM, two at 50 μM, and one at 20 μM. 17 compounds of interest were selected to be re-tested at 100 μM and 50 μM for 24 h, with six replicates each.

EC_{50} values ex vivo *in* T. muris. To determine the relative potency of the most promising drugs, activity was measured at eight concentrations (typically between 100 µM and 10 nM). The motility of five individual worms obtained from different mice was measured at each point. Using the R package drc (RITZ *et al.* 2015), concentration-response curves were fitted using the three-parameter log-logistic model, and EC_{50} values estimated for the drugs.

In vivo *drug screen against* T. muris. Nine drugs were tested in mice, following a similar protocol to (KEISER *et al*. 2016). Briefly, female NOD SCID mice (6 wk old) were infected with a low dose (20 eggs) of *T. muris* eggs via oral gavage, with six mice per treatment group. To confirm establishment of infection, on day 35 post-infection (D35 p.i.), faecal

pellets from individual mice were collected and faecal smears performed to check for the presence of *T. muris* eggs. Treatment with mebendazole (positive control), vehicle-only (negative control), or candidate drugs, was performed by oral gavage, for three consecutive days on D36, D37 and D38 p.i.. We used a three-day treatment regimen (following (HOULDEN *et al*. 2015)) because mebendazole treatment for three days is far more effective in humans than a single dose (PALMEIRIM *et al*. 2018).

The compounds were administered at dosages of 15-100 mg/kg of body weight, which were equivalent to ~2-16% of their LD_{50}, and were previously reported to cause no adverse effects on mice (**Supplementary Table 2**). A dose of 50 mg/kg body weight was used for mebendazole. The drug vehicle consisted of 7% Tween 80, 3% ethanol and 90% water (v/v/v), following previous mouse drug screens (COWAN AND KEISER 2015; PANIC *et al*. 2015). At D44 p.i. the mice were culled and intestines dissected for adult worm collection and counting. The worm burden (WB) of treated mice was compared with the WB of control (vehicle-only) mice. The worm burden reduction (WBR) was calculated as: WBR (%) = 100% - (100% * WB-treatment/WB-control).

Statistics. For the confirmatory *ex vivo* re-screens in *T. muris* and *C. elegans*, a P-value was calculated for each compound using a Mann-Whitney test to compare motility scores in drug-treated wells to the DMSO control wells. These P-values were corrected for multiple testing using the Bonferroni correction. For the *in vivo* drug testing, the Wilcoxon test (in R) was used to determine the statistical significance of WBRs.

Results

Worm motility ex vivo *was greatly reduced by the top 50 hit compounds.* We screened 409 drugs approved for human use by evaluating their effect on motility of *T. muris* adult worms *ex vivo*, using the automated system INVAPP (PARTRIDGE *et al.* 2018) and a drug concentration of 100μM for 24 h (**Figure 1**). The distribution of effects of the drugs on motility are shown in **Figure 2A**, and the motility score for each drug given in **Supplementary Table 1**. Complete loss of motility was observed for 26 drugs, while another 24 compounds induced a partial reduction in motility.

The chemical and pharmacological features of these top 50 drugs are summarised in **Table 1** (with additional details in **Supplementary Table 3**). They included compounds in 24 broad chemical classes (**Supplementary Figure 1**).

To confirm activity, the top 50 hits were re-screened at 100 μM for 24 h. Of the 50, 46 showed a significant reduction in parasite motility compared with the negative (DMSO-only) control (P<0.05) (**Figure 2B**; **Supplementary Table 3**). The effects on motility for the DMSO-only control and two exemplar hits that block movement, astemizole and pimozide, are shown in the form of time-lapse frames in **Figure 2D**, and as a full recording in **Supplementary Movie 1**. To help prioritise the hit compounds for further investigation, their relative potency was assessed by also estimating their activity at lower concentrations (5 and 20 μM for 24 h). Only pimozide, nicardipine, terfenadine, thonzonium, nicotine, paraoxon and dicumarol were clearly associated with reduced motility (P<0.05) at these lower concentrations (**Supplementary Table 3**), although the latter three were excluded from subsequent screens due to unfavourable properties (paraoxon, incorrectly classified as an approved drug; nicotine, neurotoxic; dicumarol, blood clotting effects).

Expansion screen using structurally related approved drugs for hit compounds. Having identified hit compounds from a variety of chemical classes, we wanted to ensure that we had

found the most active compounds from each class to study further. We therefore searched ChEMBL (MENDEZ *et al.* 2019) for additional approved drugs that are structurally related to our top 50 hits. This led us to obtain an additional 71 approved drugs (**Supplementary Table 4**; **Figure 1**). Amongst the top 50 hits were several antihistamines, which tend to be very safe, so we chose to further investigate two antihistamines (azelastine, cyproheptadine) that were just below our cut-off for the top 50 hits in the primary library screen.

We prioritised the 71 additional compounds and two antihistamines, by first estimating their relative activity against *T. muris ex vivo* in a small-scale pilot experiment at 100 μM, 50 μM and 20 μM (data not shown). Based on this, 17 compounds were selected to be re-tested at 100 μM and 50 μM (**Supplementary Figure 3**). Of the 17 drugs, 13 significantly reduced motility (P<0.05) at 100 μM; and 2 significantly reduced motility at 50 μM, with flunarizine and nonoxynol-9 just above the significance threshold (P=0.056 and P=0.061, respectively) (**Supplementary Table 4**; **Figure 2C**).

Only two hit compounds were effective against both C. elegans *and* T. muris. *C. elegans* is a free-living nematode but commonly used as a model for parasitic nematodes (BURNS *et al.* 2015; PARTRIDGE *et al.* 2020)**.** The 409 approved drugs were screened at 100 μM in a *C. elegans* assay that followed worm development from L1 to L4/young adult stages to identify those compounds that either slowed/blocked larval development or reduced motility. Far fewer hits were detected than in the *T. muris* screen; the top 14 candidates were re-screened at 100 μM, and five significantly reduced the growth/motility of *C. elegans* (P<0.05): ponatinib, chlorhexidine, clotrimazole, sorafenib, and paroxetine. Thus, of the 409 approved drugs screened, only clotrimazole and chlorhexidine were hits in both *T. muris* and *C. elegans*. Three antihistamines were hits in *T. muris*, but there were no antihistamine hits in *C. elegans*.

Ex vivo *potency guided the selection of drugs for* in vivo T. muris *efficacy tests*. Based on the *ex vivo* motility results, we selected the seven most active drugs (pimozide, astemizole, thonzonium, tamoxifen, nicardipine, terfenadine, and chlorprothixene) and determined their relative potency by measuring concentration-response curves across eight concentrations using the *T. muris ex vivo* motility assay. The EC_{50} of six drugs was below 50 μM and that of the seventh, chlorprothixene, was 52 ± 9 μM (**Figure 2E, Supplementary Table 5**). For an additional seven drugs (econazole, cyproheptadine, butoconazole, flunarizine, clemastine, felodipine, and prazosin) we did not measure the EC50 using a full concentration-response curve. However, based on single-concentration activity measurements at 5, 20, 50 and 100 μM in the drug screens described earlier (see previous paragraphs), we estimated that the EC50 of these drugs was at or below 50 μM (Supplementary Table 5).

Of the 14 compounds with EC_{50} of $\leq$50 μM, we considered thonzonium unsuitable for testing in mice because it is only approved for topical use in humans; and tamoxifen and chlorprothixene unsuitable because of the potential for serious side effects. Clemastine was previously tested against *T. muris* in mice by (KEISER *et al.* 2016), who found it resulted in a worm burden reduction of 20.1%. The remaining ten drugs (pimozide, astemizole, nicardipine, terfenadine, econazole, cyproheptadine, butoconazole, flunarizine, felodipine, and prazosin) were considered good candidates to test in mice. Although econazole and butocondazole are only approved for topical use in humans, they have been previously tested orally in mice (e.g. (DONG *et al.* 2017)). We were unable to obtain astemizole, so tested nine drugs in mice (**Supplementary Table 5**; **Figure 3**).

Pimozide was the most efficacious drug tested against T. muris *in mice*. In three independent experiments, the positive control mebendazole caused a 100% reduction in worm burden

(one-sided Wilcoxon tests: *P*=0.008, 0.008 and 0.001, respectively; **Table 2**; **Figure 4**). Pimozide reduced the worm burden by 19.0% in one experiment (see 'pim-c' versus 'ctl-c' in **Figure 4**), which was borderline statistically significant (one-sided Wilcoxon test: *P*=0.08). However, two replicate experiments using the same dose of pimozide (35 mg/kg) did not show statistically significant reductions, nor did using a higher dose of pimozide (70 mg/kg). All mice dosed with cyproheptadine had to be culled due to adverse effects, and none of the other drugs showed statistically significant reductions in worm burden (**Table 2**; **Figure 4**).

Discussion

Despite the millions of people worldwide affected by trichuriasis (GBD 2019 DISEASES AND INJURIES COLLABORATORS 2020), and the pressing need for more effective drugs to treat whipworm infections, this disease receives little research attention, which makes it a major 'neglected tropical disease' (HOTEZ 2013). Given the lack of investment in developing new drugs to treat trichuriasis, repurposing drugs that are currently approved for other uses is particularly attractive. While some drug repurposing libraries such as ReFRAME 'cast a wide net' by including all approved drugs as well as compounds that have undergone significant pre-clinical studies (JANES *et al.* 2018), we have used a comparative genomics approach to narrow our focus to a smaller set of drugs most likely to have key targets in whipworms. Specifically, we have prioritised whipworm targets that have *C. elegans*/*Drosophila melanogaster* orthologues with lethal/sterile phenotypes, and made use of curated data on drugs and their targets from ChEMBL (MENDEZ *et al.* 2019) to predict 409 drugs that will target those whipworm proteins (INTERNATIONAL HELMINTH GENOMES CONSORTIUM 2019). Our automated high-throughput screening platform INVAPP (PARTRIDGE *et al.* 2018; BUCKINGHAM *et al.* 2021) enabled us to screen these 409 drugs at 100 μM against *T. muris* adults *ex vivo*. Our high hit rate, ~12% (50/409), demonstrated the utility of our comparative genomics approach to predict parasite proteins for targeting by approved drugs.

T. muris is relatively expensive and labour-intensive to maintain, so it is important to explore whether the same hits can be found by screening against *C. elegans*, which is far cheaper and easier to maintain. *C. elegans* is often used as a proxy for whipworms and other parasitic nematodes in drug screens (KWOK *et al.* 2006; BURNS *et al.* 2015), and functional assays in *C. elegans* have aided the development of new anthelmintics such as the amino-acetonitrile derivative monepantel (KAMINSKY *et al.* 2008). However, when our drug library was screened against *C. elegans*, only two of the top 50 hits against whipworm were also hits against *C. elegans*. In fact, *C. elegans* had only 14 hits compared to 50 in *T. muris*. This may reflect a greater ability of *C. elegans* to detoxify the particular compounds in our screening library (LINDBLOM AND DODD 2006). Despite the low number of the top 50 *T. muris* hits also shared by *C. elegans* in our screen, 14 out of 50 have previously been reported as having activity against *C. elegans* and/or other nematodes in *ex vivo* screens that detected a variety of phenotypes (**Table 1**). This was the case for four of the nine highly active compounds that we tested in mice (pimozide, econazole, cyproheptadine, felodipine), suggesting these drugs may cause phenotypic effects in a broad range of nematodes.

For a promising *ex vivo* hit to be chosen as a lead compound for further drug development, one would require a worm burden reduction of at least ~60% *in vivo* (PANIC *et al.* 2015). Despite their high *ex vivo* activities, the nine drugs tested resulted in low and non-statistically significant worm burden reductions in mice. A previous screen against *T. muris* by (COWAN *et al.* 2016) also reported promising *ex vivo* hits, but low worm burden reductions ($\leq$24%)

for seven drugs tested in mice. Why do many drugs have high efficacy against *T. muris ex vivo,* but poor activities in mice? One possibility is that the ADME (absorption, distribution, metabolism, excretion) properties of the drugs mean that only a low concentration of drug (or a high concentration for only a short time) is available to *T. muris* in the large intestine. (COWAN *et al.* 2016) pointed out that this has a high probability of occurring in repurposing screens, because most drugs approved for human use have been optimised for high absorption by the human gastrointestinal tract. For example, more than 50% of orally delivered pimozide is absorbed by the human gastrointestinal tract, compared to only 5-10% of mebendazole (from DrugBank (WISHART *et al*. 2018)). Another possibility is that, even if a drug is available at a high concentration in the large intestine for a relatively long time, there may be poor uptake by *T. muris*. It is thought likely that the crucial pathway of mebendazole uptake into *T. muris* is by diffusion across the cuticle of the worms' posterior end, which lays freely in the intestinal tract (COWAN *et al*. 2017), rather than uptake by the worm's long anterior end (including its mouth), which is embedded inside host intestinal epithelial cells. However, little is known about optimising this process for mebendazole or other drugs.

A bottleneck in drug discovery *in vivo* testing in mice, which is expensive and sacrifices research animals. There is a clear need for development of intestinal *ex vivo* models and assays to assess, further optimise and select hits, before performing *in vivo* testing. Based on the intestinal location of the whipworm parasite, and likely uptake of compounds by diffusion across the worms' posterior end, future screens for anti-whipworm compounds should try to increase understanding of how to optimise exposure of the worms to the drug, as this would likely improve the rate of success in mouse experiments. We suggest that one strategy would be to take the initial hits from a screen as lead compounds, and then identify structural analogues that are predicted to have lower absorption (compared to the lead compounds) by the human gastrointestinal tract, by modifying their lipophilicity, molecular weight and polarity (WILS *et al*. 1994; EL-KATTAN AND VARMA 2012). The anti-whipworm activity of the structural analogues could be assessed in a second *ex vivo* screen, and their absorption by the human gastrointestinal tract predicted using human intestinal organoids or cell lines (WILS *et al*. 1994; DUQUE-CORREA *et al*. 2022). After optimising for low absorption, top compounds could then be assessed for anti-whipworm activity against *T. muris* embedded in intestinal organoids, to determine whether compounds are readily taken up by and have a phenotypic effect on *T. muris* embedded in the host gastrointestinal tract (DUQUE-CORREA *et al*. 2022). Strong hits from this latter step could be tested in mice. By adding these additional steps in the drug discovery pipeline for trichuriasis, we will better understand how to increase exposure of the worms to the compounds, and this should help us to better select compounds likely to give a higher worm burden reduction in mice. A bonus of optimising compounds for low absorption by the human gastrointestinal tract would be reduced side-effects and tolerance of higher doses by patients.

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Author contribution

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Frederick A. Partridge: conceptualisation, data curation, project administration, formal analysis, investigation, software, methodology, validation, visualisation, supervision, writing – reviewing and editing.
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Competing interests

We do not have any competing interests to disclose.

Figure legends

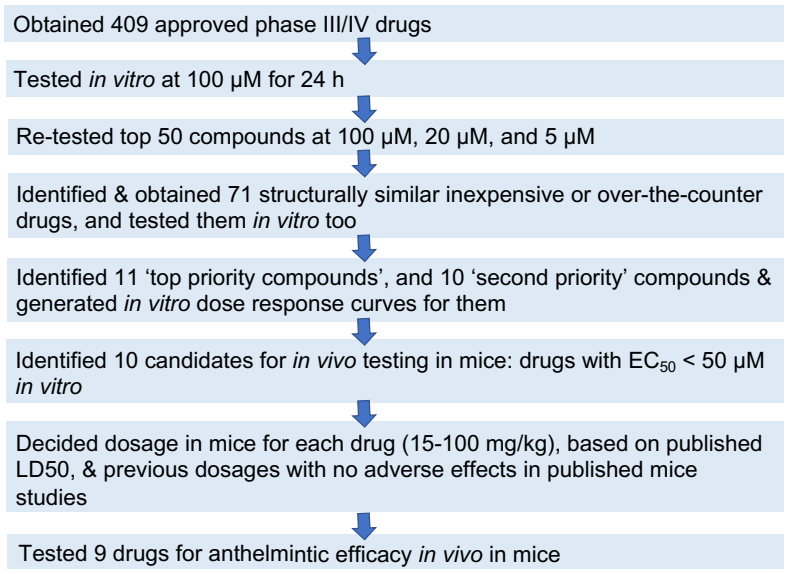


Figure 1. Study flow for testing 409 approved drugs against *T. muris.*

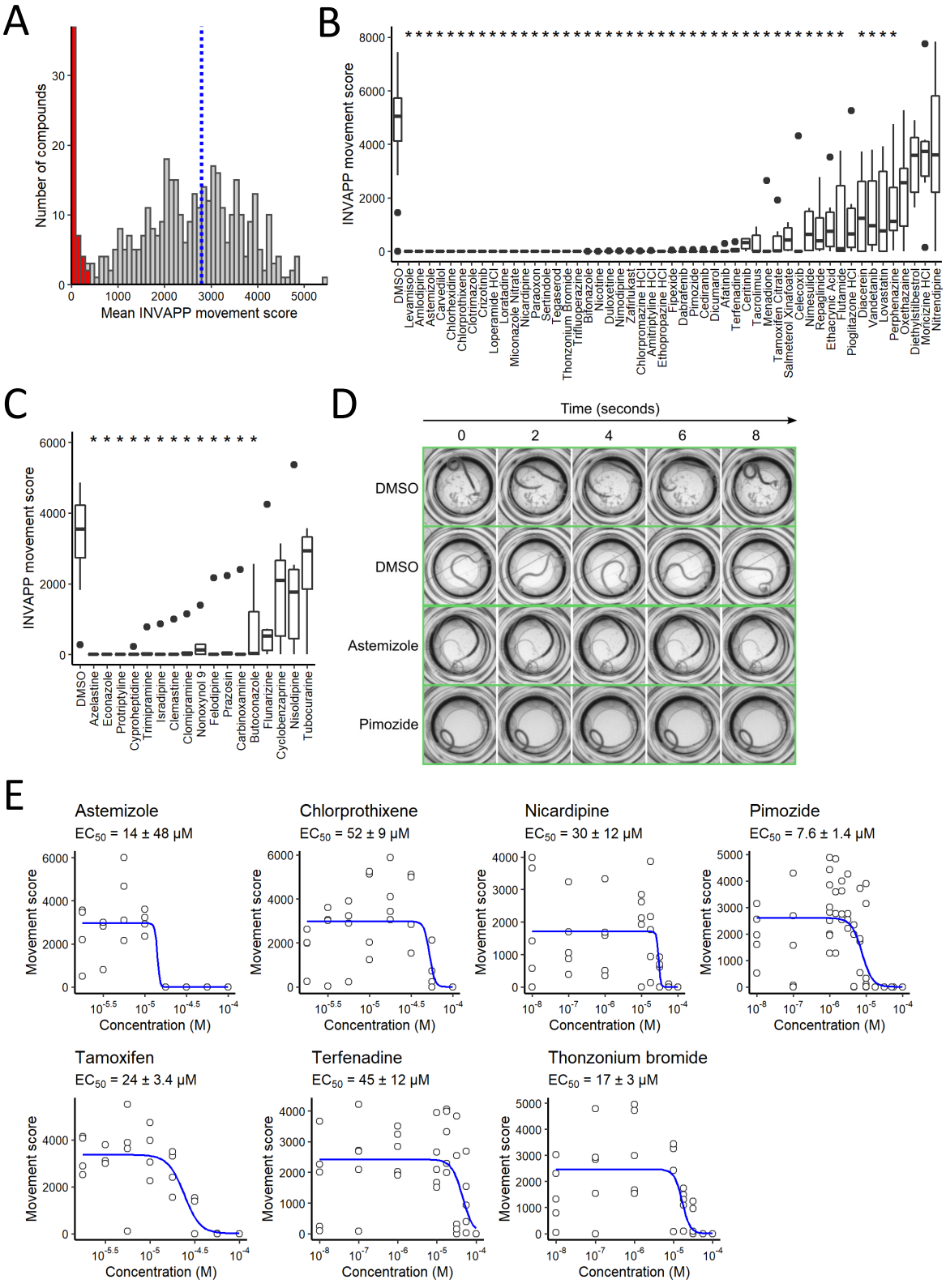


Figure 2. (A) Histogram showing the mean effect on *T. muris* adult motility of each of the 409 approved drugs tested in the primary screen at 100 µM. Blue dotted line indicates the mean movement score for DMSO-only negative controls. The 50 compounds with the greatest reduction in mean movement score, indicated in red, were selected as candidate hits. (B) Secondary screen confirmed the activity of 46 drugs at 100 µM. * indicates significant reduction in *T. muris* adult movement score compared to the DMSO-only control (Mann-

Whitney test adjusted for multiple comparisons by the Bonferroni method, $P < 0.05$, n=6). (C) Identification of an additional 13 active drugs by testing structurally-related drugs in the same *T. muris* adult motility assay at 100 µM. * indicates significant reduction in *T. muris* adult movement score compared to the DMSO-only control (Mann-Whitney test adjusted for multiple comparisons by the Bonferroni method, $P < 0.05$, n=6). (D) Montage of frames selected from time-lapse recording of worms treated with DMSO alone, or with astemizole or pimozide. A movie of this data is presented in **Supplementary Movie 1**. (E) Concentration-response curves for selected active drugs with EC_{50} values at or below 50 µM. Curves fitted with the three-parameter log-logistic model.

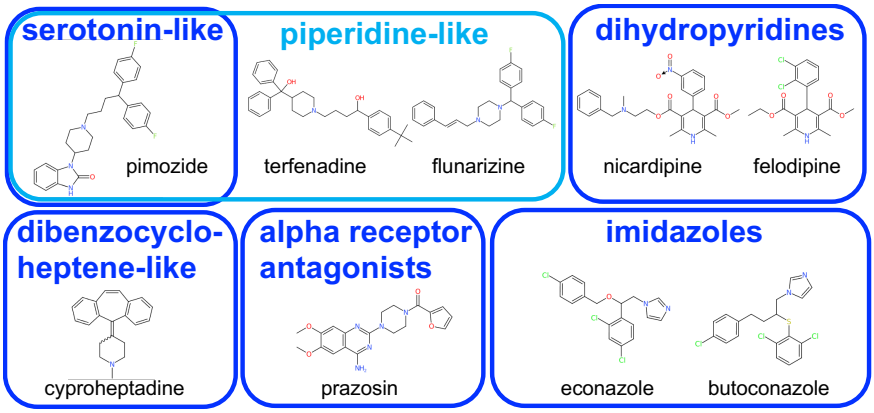


Figure 3. The nine compounds tested *in vivo* in mice. The images of compounds were generated using the CDKDepict website (WILLIGHAGEN *et al.* 2017). The salt form tested is given in **Supplementary Table 2**.

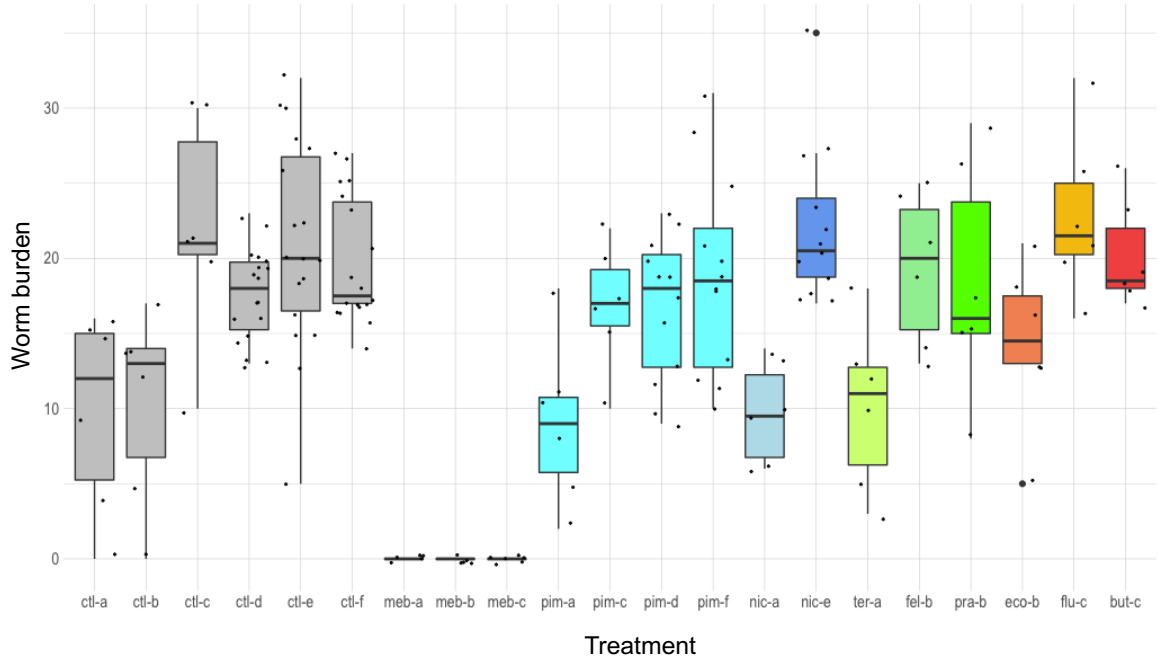


Figure 4. Results of the *in vivo* screen in mice. Each data point is one drug-treated or control mouse. The order of the treatments is the same as that in **Table 2**, and 'ctl' is vehicle-only control, 'meb' mebendazole, 'pim' pimozide, 'nic' nicardipine, 'ter' terfenadine, 'fel'

felodipine, 'pra' prazosin, 'eco' econazole, 'flu' flunarizine, 'but' butoconazole. The letters 'a', 'b', 'c', 'd', 'e' beside the treatment names refer to the respective control groups.

Tables

Table 1. The top 50 hits in our initial *ex vivo* screen of approved drugs (at 100 μM for 24 hr) against *T. muris* adults, and the top 17 hits in our screen of structurally related drugs. The drugs above the horizontal line are the top 50 hits in the initial screen, and those below the line are the top 17 hits in our screen of structurally related drugs. Column E says whether the mode of action of the current use in human affects the nervous system or muscle. In column F, **Top** means only approved for topical use (based on data in ChEMBL and DrugBank (WISHART *et al*. 2018)); **B** means a 'black box' warning; **W** means withdrawn; **Tox** denotes highly toxic compounds; **In** denotes inexpensive compounds (≤US $5 per vial/capsule, based on data in DrugBank); **WHO** denotes a compound on the WHO list of essential medicines (from https://www.who.int/medicines/publications/essentialmedicines/en/); and **OC** a drug sold over-the-counter (data from ChEMBL). The salt form tested is given in **Supplementary Table 1**. In column G, nematode species are shown in orange, and flatworm species in purple. Species of helminths are abbreviated as: Sm=*Schistosoma mansoni*, Ofel=*Opisthorchis felineus*, Egr=*Echinococcus granulosus*, Acy=*Ancylostoma ceylanicum*, Acan=*Ancylostoma caninum*, Cel=*Caenorhabditis elegans*, Tmu=*Trichuris muris*, Hpol=*Heligmosomoides polygyrus*, Sdig=*Setaria digitata*.

A. Chemical class	B. Name of drug	C. Use in humans	D. Target for use in humans	E. Nerve/ muscle	F. Properties	*G. In vitro* anthelmintic activity
Dihydropyridines	nicardipine	hypertension	blocks Ca^{2+} channels	yes	In	Sm (MENDONCA-SILVA *et al.* 2006; PANIC *et al.* 2015)
Dihydropyridines	nitrendipine	hypertension	blocks Ca^{2+} channels	yes		
Dihydropyridines	nimodipine	hypertension	blocks Ca^{2+} channels	yes	B	Acy (ELFAWAL *et al.* 2019)
Dihydropyridines	amlodipine	hypertension	blocks Ca^{2+} channels	yes	B, In, WHO	Sm (PANIC *et al.* 2015)
Serotonin-like	fipexide	senile dementia	may increase neurotransmitter activity	yes	W	
Serotonin-like	astemizole	allergic disorders	histamine receptor antagonist	yes	W	
Serotonin-like	tegaserod	irritable bowel syndrome	serotonin receptor agonist/antagonist	yes	W	
Serotonin-like	sertindole	psychotic disorders	dopamine/serotonin receptor antagonist	yes	W	
Serotonin-like; Piperidines	pimozide	psychotic disorders	dopamine/serotonin receptor antagonist	yes	In	Cel (KWOK *et al.* 2006), Sm (TOMOSKY *et al.* 1974; PANIC *et al.* 2015)
Piperidines	terfenadine	allergic disorders	histamine receptor antagonist	yes	W	Sm (PANIC *et al.* 2015)
Piperidines	loperamide	diarrhoea	blocks Ca^{2+} channels; opioid receptor agonist	yes	B, In, WHO, OC	Egr (NICOLAO *et al.* 2014)
Surfactants	thonzonium	in skin/nasal drops	disperses cellular debris		Top	Acy (KEISER *et al.* 2016), Sm (PANIC *et al.* 2015)
Alpha/beta receptor ligands	duloxetine	depression; anxiety	inhibits noradrenaline/serotonin re-uptake	yes	B, In	
Alpha/beta receptor ligands	carvedilol	cardiovascular disorders	alpha/beta receptor antagonist	yes	In, WHO	
Alpha/beta receptor ligands	salmeterol	asthma	beta receptor agonist	yes	Top, B, In	
Imidazoles	clotrimazole	fungal infections	inhibits fungal ergosterol production		In, WHO, OC	Cel (KWOK *et al.* 2006), Ofel (MORDVINOV *et al.* 2017)
Imidazoles	bifonazole	fungal infections	inhibits fungal ergosterol production		Top	Acy (KEISER *et al.* 2016)
Imidazoles	miconazole	fungal infections	inhibits fungal ergosterol production		B, In, WHO, OC	Cel (WEEKS *et al.* 2018), Sm (ABDULLA *et al.* 2009; ZINIEL *et al.* 2015), Ofel (MORDVINOV *et al.* 2017)

Phenothiazines	perphenazine	psychotic disorders	dopamine receptor antagonist	yes	**B, In**	**Sm** (TAFT *et al.* 2010)
Phenothiazines	chlorpromazine	psychotic disorders	dopamine/serotonin/ histamine receptor antagonist	yes	**B, In, WHO**	**Cel** (KWOK *et al.* 2006), **Acan**, **Tmu**, **Sm** (ABDULLA *et al.* 2009; TAFT *et al.* 2010; WEEKS *et al.* 2018), **Acy** (KEISER *et al.* 2016)
Phenothiazines	chlorprothixene	psychotic disorders	dopamine/serotonin/ histamine receptor antagonist	yes		**Cel** (KWOK *et al.* 2006), **Acy** (KEISER *et al.* 2016), **Sm** (TAFT *et al.* 2010; PANIC *et al.* 2015)
Phenothiazines	trifluoperazine	psychotic disorders	dopamine/serotonin receptor antagonist	yes	**B, In**	**Cel** (KWOK *et al.* 2006), **Sm** (HILLMAN *et al.* 1978; TAFT *et al.* 2010)
Phenothiazines	ethopropazine	Parkinson's disease	histamine and muscarinic receptor antagonist	yes	**In**	**Acy**, **Hpol**, **Tmu** (KEISER *et al.* 2016)
Phenothiazines	moricizine	irregular heartbeats	blocks Na^+ channels	yes	**B**	
Oestrogen receptor ligands	diethylstilbestrol	prostate cancer	oestrogen receptor agonist		**W**	
Oestrogen receptor ligands	tamoxifen	breast cancer	inhibits oestrogen binding to its receptor		**B, In, WHO**	**Sm** (PANIC *et al.* 2015)
Kinase inhibitors	crizotinib	non-small cell lung cancer	inhibitor of receptor tyrosine kinases			**Sm** (COWAN AND KEISER 2015)
Kinase inhibitors	afatinib	non-small cell lung cancer	inhibitor of tyrosine kinases		**WHO**	**Sm** (COWAN AND KEISER 2015)
Kinase inhibitors	vandetanib	thyroid cancer	inhibitor of tyrosine kinases			**Sm** (COWAN AND KEISER 2015)
Kinase inhibitors	cediranib	cancers e.g. liver cancer	inhibitor of receptor tyrosine kinases			
Kinase inhibitors	ceritinib	non-small cell lung cancer	inhibitor of receptor tyrosine kinases			
Kinase inhibitors	dabrafenib	thyroid cancer; melanoma	inhibitor of kinases			
Biguanides	chlorhexidine	skin antiseptic agent	disrupts microbial cell membranes		**Top, In, WHO, OC**	**Acy** (KEISER *et al.* 2016)
Local anaesthetics	oxethazaine	pain from stomach ulcers	local anaesthetic effect on the gastric mucosa	yes		**Sm** (PANIC *et al.* 2015)
Meglitinides	repaglinide	type 2 diabetes mellitus	closes K^+ channels in pancreatic beta-cells		**In**	
Dibenzocycloheptenes	amitriptyline	depression	inhibits noradrenaline/serotonin re-uptake	yes	**B, In, WHO**	**Acy** (KEISER *et al.* 2016), **Sm** (ABDULLA *et al.* 2009; TAFT *et al.* 2010)
Dibenzocycloheptenes	loratadine	allergic disorders	histamine receptor antagonist	yes	**In, WHO, OC**	
Loop diuretics	ethacrynic acid	hypertension	inhibits the Na^+ K^+ Cl^- cotransporter 2		**In**	**Sdig** (SRINIVASAN *et al.* 2009)
Benzenesulfonamides	zafirlukast	asthma	leukotriene receptor antagonist	yes	**In**	
Vitamin K-like	menadione	nutritional supplement	precursor of vitamin K			**Sm** (PANIC *et al.* 2015)
Vitamin K-like	dicumarol	decreasing blood clotting	inhibits vitamin K reductase			
Anthraquinones	diacerein	osteoarthritis	reduces interleukin-1 beta activity			
Macrolides	tacrolimus	organ transplants	inhibits immunophilin FKBP		**B, In**	
COX-2 inhibitors	celecoxib	arthritis pain	inhibits COX-2		**W**	**Sm** (COWAN AND KEISER 2015)
COX-2 inhibitors	nimesulide	acute pain; osteoarthritis	inhibits COX-2		**W**	
Antiandrogens	flutamide	carcinoma of the prostate	androgen receptor antagonist		**B, In**	**Cel** (KWOK *et al.* 2006)
Thiazolidinediones	pioglitazone	type 2 diabetes	nuclear receptor PPAR-gamma agonist		**B**	
Statins	lovastatin	to lower risk of heart attack	inhibits HMG-CoA reductase		**In**	**Sm** (ARAUJO *et al.* 2002)

Toxic alkaloids	nicotine	nicotine withdrawal	nicotinic cholinergic receptor agonist	yes	**Tox**	did not search literature
Insecticides	paraoxon	not a drug; insecticide	inhibits acetylcholinesterase s	yes	**Tox**	did not search literature
Piperidine-like	flunarizine	migraine; vascular disease	calcium channel blocker	yes	**In**	**Sm** (PANIC *et al.* 2015)
Piperidine-like	carbinoxamine	allergic disorders	histamine receptor antagonist	yes	**In**	
Piperidine-like	clemastine	allergic disorders	histamine receptor antagonist	yes	**OC**, **In**	**Acy**, **Hpol**, **Tmu** (KEISER *et al.* 2016)
Dibenzocycloheptenes	clomipramine	depression; OCD	inhibitor of serotonin re-uptake	yes	**In**, **WHO**, **B**	
Dibenzocycloheptenes	cyclobenzaprine	muscle spasm	serotonin receptor antagonist	yes	**In**	
Dibenzocycloheptenes	cyproheptadine	allergic disorders	serotonin/histamine receptor antagonist	yes	**In**	**Acy** (KEISER *et al.* 2016)
Dibenzocycloheptenes	trimipramine	depression	serotonin/histamine receptor antagonist	yes	**In**, **B**	**Acy**, **Hpol**, **Tmu** (KEISER *et al.* 2016)
Dibenzocycloheptenes	protriptyline	depression	inhibitor of serotonin/noradrenaline re-uptake	yes	**In**, **B**	**Acy**, **Tmu** (ELFAWAL *et al.* 2019)
Imidazoles	butoconazole	fungal infections	inhibits fungal ergosterol production		**OC**, **In**, **Top**	
Imidazoles	econazole	fungal infections	inhibits fungal ergosterol production		**In**, **Top**	**Cel** (KWOK *et al.* 2006); **Acy**, **Tmu** (ELFAWAL *et al.* 2019)
Dihydropyridines	isradipine	hypertension	blocks Ca^{2+} channels	yes	**In**	**Cel** (PARTRIDGE *et al.* 2018)
Dihydropyridines	felodipine	hypertension	blocks Ca^{2+} channels	yes	**In**	**Cel** (KWOK *et al.* 2006)
Dihydropyridines	nisoldipine	hypertension	blocks Ca^{2+} channels	yes	**In**	
Alpha/beta receptor antagonists	prazosin	hypertension	alpha receptor antagonist	yes	**In**	**Sm** (PANIC *et al.* 2015)
Kinase inhibitor-like	tubocurarine	muscle relaxation	Ach receptor antagonist	yes	**In**; **by injection**	
Antihistamine	azelastine	rhinitis; conjunctivitis	histamine receptor antagonist	yes	**In**; **Top**	
Thonozonium-like	nonoxynol 9	surfactant in spermicide	surfactant		**OC**; **Top**	

Table 2. Worm burden reductions of *T. muris*-infected mice treated with drugs.

The a, b, c, d, e in square brackets beside the drug names refer to the respective control groups. A one-sided Wilcoxon test was used to calculate the P-value for the worm burden reduction, comparing to the worm burden in the corresponding control worms. Note that one pimozide-treated mouse had to be culled early when treated with pimozide at 70 mg/kg.

Treatment	Salt form tested in mice	Dosage tested in mice (mg/kg body weight)	No. of mice	Median worm burden	Worm burden reduction	P-value for worm burden reduction
control a (vehicle-only)	NA	NA	6	12.0	NA	NA
control b (vehicle-only)	NA	NA	6	13.0	NA	NA
control c (vehicle-only)	NA	NA	6	21.0	NA	NA
control d (vehicle-only)	NA	NA	18	18.0	NA	NA
control e (vehicle-only)	NA	NA	18	20.0	NA	NA
control f (vehicle-only)	NA	NA	18	17.5	NA	NA
mebendazole [a]	mebendazole	50 mg/kg	5	0.0	100.0%	0.008
mebendazole [b]	mebendazole	50 mg/kg	5	0.0	100.0%	0.008
mebendazole [c]	mebendazole	50 mg/kg	6	0.0	100.0%	0.001
pimozide [a]	pimozide	35 mg/kg	6	9.0	25.0%	0.5
pimozide [c]	pimozide	35 mg/kg	6	6.0	19.0%	0.08
pimozide [d]	pimozide	35 mg/kg	12	18.0	-	0.8
pimozide [f]	pimozide	70 mg/kg	12	18.5	-	-
nicardipine [a]	nicardipine hydrochloride	50 mg/kg	6	9.5	20.80%	0.4
nicardipine [e]	nicardipine hydrochloride	50 mg/kg	12	20.5	-	-
terfenadine [a]	terfenadine	100 mg/kg	6	11.0	8.30%	0.5
felodipine [b]	felodipine	35 mg/kg	6	20.0	-	-

prazosin [b]	prazosin hydrochloride	100 mg/kg	6	16.0	-	-
econazole [b]	econazole nitrate	50 mg/kg	6	14.5	-	-
flunarizine [c]	flunarizine dihydrochloride	35 mg/kg	6	21.5	-	-
butoconazole [c]	butoconazole nitrate	100 mg/kg	6	18.5	12.0%	0.2

Supplementary information

Supplementary Table 1. The 409 compounds obtained for the initial screen.

Supplementary Table 2. The 9 approved drugs that were tested in mice.

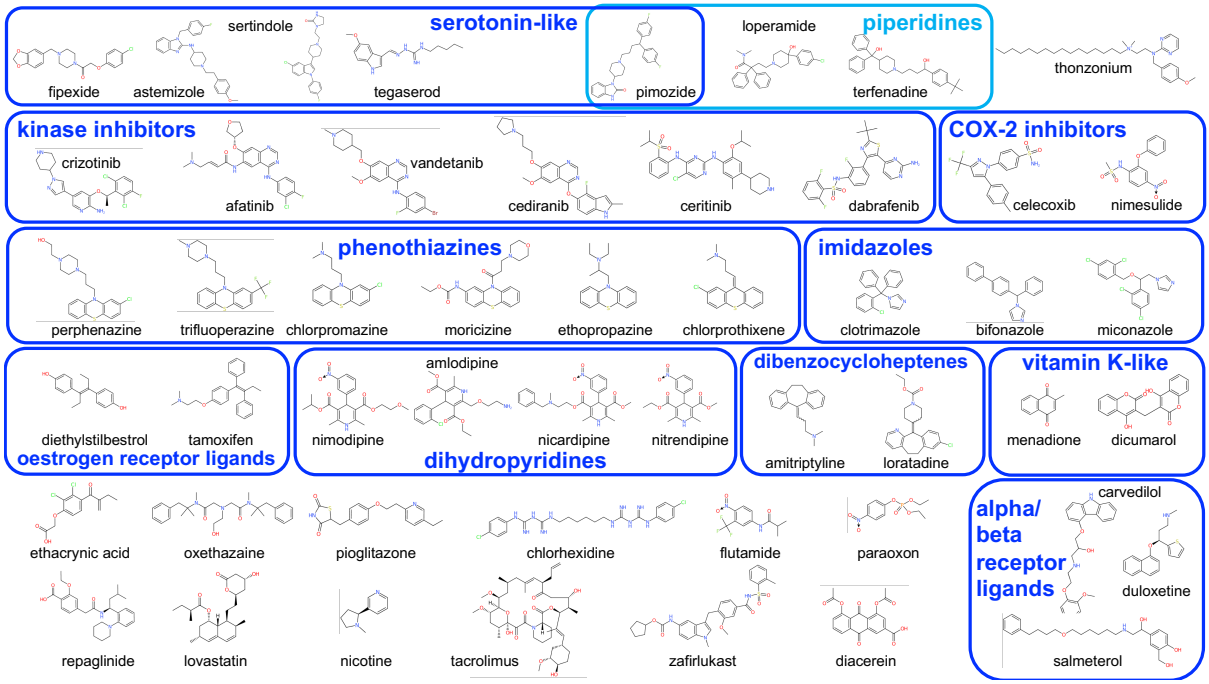
Supplementary Table 3. Results for the top 50 hits from the screen at 100 μM for 24 hours.

Supplementary Table 4. The 71 additional compounds obtained for the screen, which were structurally related to our top 50 hits in the initial screen.

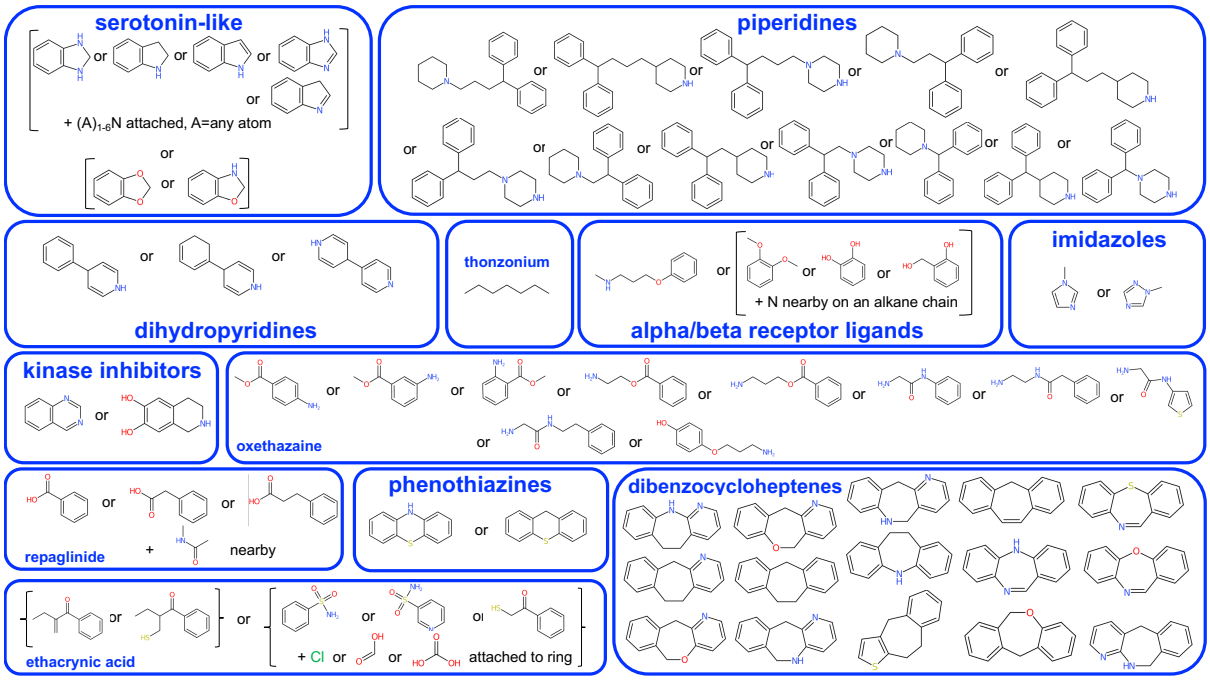
Supplementary Table 5. The 14 approved drugs that have estimated EC_{50} values of ≤50 μM.

Supplementary Movie 1. Recordings of *T. muris* adults treated with DMSO alone, or with astemizole or pimozide at 100 µM for 24 hours.
https://doi.org/10.6084/m9.figshare.22193977.v1

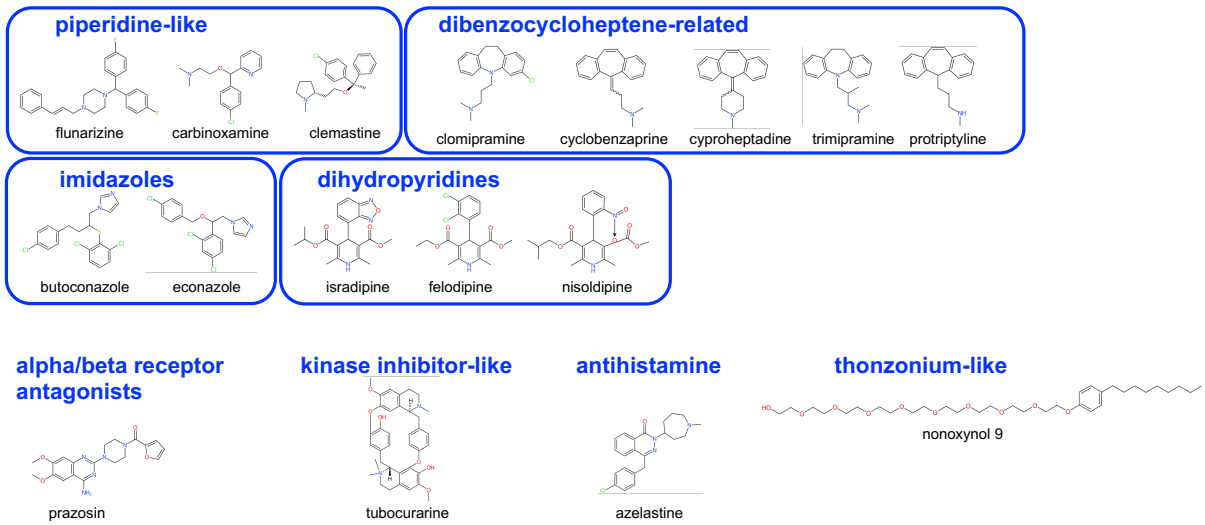
Supplementary figure legends



Supplementary Figure 1. The top 50 hits in our initial *ex vivo* screen. The images of compounds were generated using the CDKDepict website (WILLIGHAGEN *et al.* 2017). The salt form tested is given in **Supplementary Table 3**.



Supplementary Figure 2. The substructures that were used in the 'substructure search function' in DataWarrior, to search for additional approved drugs with substructures present in our top 50 hits. Images of compounds were generated using the CDKDepict website (WILLIGHAGEN *et al.* 2017).



Supplementary Figure 3. The top 17 hits in our screen of additional approved drugs that were structurally related to our initial top 50 hits. The images of compounds were generated using the CDKDepict website (WILLIGHAGEN *et al.* 2017). The salt form tested is given in **Supplementary Table 4**.

References

Abdulla, M. H., D. S. Ruelas, B. Wolff, J. Snedecor, K. C. Lim *et al.*, 2009 Drug discovery for schistosomiasis: hit and lead compounds identified in a library of known drugs by medium-throughput phenotypic screening. PLoS Negl Trop Dis 3**:** e478.

Araujo, N., A. Kohn, A. A. Oliveira and N. Katz, 2002 [Schistosoma mansoni: the action of lovastatin on the murine model]. Rev Soc Bras Med Trop 35**:** 35-38.

Buckingham, S. D., F. A. Partridge, B. C. Poulton, B. S. Miller, R. A. McKendry *et al.*, 2021 Automated phenotyping of mosquito larvae enables high-throughput screening for novel larvicides and offers potential for smartphone-based detection of larval insecticide resistance. PLoS Negl Trop Dis 15**:** e0008639.

Burns, A. R., G. M. Luciani, G. Musso, R. Bagg, M. Yeo *et al.*, 2015 Caenorhabditis elegans is a useful model for anthelmintic discovery. Nat Commun 6**:** 7485.

Cowan, N., and J. Keiser, 2015 Repurposing of anticancer drugs: in vitro and in vivo activities against Schistosoma mansoni. Parasit Vectors 8**:** 417.

Cowan, N., C. Meier, A. Neodo and J. Keiser, 2017 Exposure of Heligmosomoides polygyrus and Trichuris muris to albendazole, albendazole sulfoxide, mebendazole and oxantel pamoate in vitro and in vivo to elucidate the pathway of drug entry into these gastrointestinal nematodes. Int J Parasitol Drugs Drug Resist 7**:** 159-173.

Cowan, N., A. Raimondo and J. Keiser, 2016 Approved oncology drugs lack in vivo activity against Trichuris muris despite in vitro activity. Parasitol Res 115**:** 4443-4446.

GBD 2019 Diseases and Injuries Collaborators, 2020 Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396**:** 1204-1222.

Dong, C., R. Yang, H. Li, K. Ke, C. Luo *et al.*, 2017 Econazole nitrate inhibits PI3K activity and promotes apoptosis in lung cancer cells. Sci Rep 7**:** 17987.

Duque-Correa, M. A., D. Goulding, F. H. Rodgers, J. A. Gillis, C. Cormie *et al.*, 2022 Defining the early stages of intestinal colonisation by whipworms. Nat Commun 13**:** 1725.

El-Kattan, A., and M. Varma, 2012 Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability in *Topics on Drug Metabolism*, edited by J. Paxton. InTechOpen.

Elfawal, M. A., S. N. Savinov and R. V. Aroian, 2019 Drug Screening for Discovery of Broad-spectrum Agents for Soil-transmitted Nematodes. Sci Rep 9**:** 12347.

Else, K. J., J. Keiser, C. V. Holland, R. K. Grencis, D. B. Sattelle *et al.*, 2020 Whipworm and roundworm infections. Nat Rev Dis Primers 6**:** 44.

Hastings, J., G. Owen, A. Dekker, M. Ennis, N. Kale *et al.*, 2016 ChEBI in 2016: Improved services and an expanding collection of metabolites. Nucleic Acids Res 44**:** D1214-1219.

Hillman, G. R., A. M. Gibler and J. W. Anderson, 1978 Scanning microfluorimetric studies of anticholinergic drugs in Schistosoma mansoni. J Pharmacol Exp Ther 207**:** 992-997.

Hotez, P. J., 2013 *Forgotten People, Forgotten Diseases: The Neglected Tropical Diseases and their Impact on Global Health and Development*. ASM Press.

Houlden, A., K. S. Hayes, A. J. Bancroft, J. J. Worthington, P. Wang *et al.*, 2015 Chronic Trichuris muris Infection in C57BL/6 Mice Causes Significant Changes in Host Microbiota and Metabolome: Effects Reversed by Pathogen Clearance. PLoS One 10**:** e0125945.

International Helminth Genomes Consortium, 2019 Comparative genomics of the major parasitic worms. Nat Genet 51**:** 163-174.

Janes, J., M. E. Young, E. Chen, N. H. Rogers, S. Burgstaller-Muehlbacher *et al.*, 2018 The ReFRAME library as a comprehensive drug repurposing library and its application to the treatment of cryptosporidiosis. Proc Natl Acad Sci U S A 115**:** 10750-10755.

Kaminsky, R., P. Ducray, M. Jung, R. Clover, L. Rufener *et al.*, 2008 A new class of anthelmintics effective against drug-resistant nematodes. Nature 452**:** 176-180.

Keiser, J., G. Panic, R. Adelfio, N. Cowan, M. Vargas *et al.*, 2016 Evaluation of an FDA approved library against laboratory models of human intestinal nematode infections. Parasit Vectors 9**:** 376.

Kwok, T. C., N. Ricker, R. Fraser, A. W. Chan, A. Burns *et al.*, 2006 A small-molecule screen in C. elegans yields a new calcium channel antagonist. Nature 441**:** 91-95.

Lindblom, T. H., and A. K. Dodd, 2006 Xenobiotic detoxification in the nematode Caenorhabditis elegans. J Exp Zool A Comp Exp Biol 305**:** 720-730.

Mendez, D., A. Gaulton, A. P. Bento, J. Chambers, M. De Veij *et al.*, 2019 ChEMBL: towards direct deposition of bioassay data. Nucleic Acids Res 47**:** D930-D940.

Mendonca-Silva, D. L., E. Novozhilova, P. J. Cobbett, C. L. Silva, F. Noel *et al.*, 2006 Role of calcium influx through voltage-operated calcium channels and of calcium mobilization in the physiology of Schistosoma mansoni muscle contractions. Parasitology 133**:** 67-74.

Mordvinov, V. A., A. G. Shilov and M. Y. Pakharukova, 2017 Anthelmintic activity of cytochrome P450 inhibitors miconazole and clotrimazole: in-vitro effect on the liver fluke Opisthorchis felineus. Int J Antimicrob Agents 50**:** 97-100.

Nicolao, M. C., G. M. Denegri, J. G. Carcamo and A. C. Cumino, 2014 P-glycoprotein expression and pharmacological modulation in larval stages of Echinococcus granulosus. Parasitol Int 63**:** 1-8.

Oprea, T. I., and J. P. Overington, 2015 Computational and Practical Aspects of Drug Repositioning. Assay Drug Dev Technol 13**:** 299-306.

Palmeirim, M. S., S. M. Ame, S. M. Ali, J. Hattendorf and J. Keiser, 2018 Efficacy and Safety of a Single Dose versus a Multiple Dose Regimen of Mebendazole against Hookworm Infections in Children: A Randomised, Double-blind Trial. EClinicalMedicine 1**:** 7-13.

Panic, G., M. Vargas, I. Scandale and J. Keiser, 2015 Activity Profile of an FDA-Approved Compound Library against Schistosoma mansoni. PLoS Negl Trop Dis 9**:** e0003962.

Partridge, F. A., A. E. Brown, S. D. Buckingham, N. J. Willis, G. M. Wynne *et al.*, 2018 An automated high-throughput system for phenotypic screening of chemical libraries on C. elegans and parasitic nematodes. Int J Parasitol Drugs Drug Resist 8**:** 8-21.

Partridge, F. A., R. Forman, C. J. R. Bataille, G. M. Wynne, M. Nick *et al.*, 2020 Anthelmintic drug discovery: target identification, screening methods and the role of open science. Beilstein J Org Chem 16**:** 1203-1224.

Ritz, C., F. Baty, J. C. Streibig and D. Gerhard, 2015 Dose-Response Analysis Using R. PLoS One 10**:** e0146021.

Sander, T., J. Freyss, M. von Korff and C. Rufener, 2015 DataWarrior: an open-source program for chemistry aware data visualization and analysis. J Chem Inf Model 55**:** 460-473.

Speich, B., S. M. Ali, S. M. Ame, Bogoch, II, R. Alles *et al.*, 2015 Efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, albendazole plus oxantel pamoate, and mebendazole alone against Trichuris trichiura and concomitant soil-transmitted helminth infections: a four-arm, randomised controlled trial. Lancet Infect Dis 15**:** 277-284.

Srinivasan, L., N. Mathew and K. Muthuswamy, 2009 In vitro antifilarial activity of glutathione S-transferase inhibitors. Parasitol Res 105**:** 1179-1182.

Taft, A. S., F. A. Norante and T. P. Yoshino, 2010 The identification of inhibitors of Schistosoma mansoni miracidial transformation by incorporating a medium-throughput small-molecule screen. Exp Parasitol 125**:** 84-94.

Tomosky, T. K., J. L. Bennett and E. Bueding, 1974 Tryptaminergic and dopaminergic responses of Schistosoma mansoni. J Pharmacol Exp Ther 190**:** 260-271.

Wakelin, D., 1967 Acquired immunity to Trichuris muris in the albino laboratory mouse. Parasitology 57**:** 515-524.

Weeks, J. C., W. M. Roberts, C. Leasure, B. M. Suzuki, K. J. Robinson *et al.*, 2018 Sertraline, Paroxetine, and Chlorpromazine Are Rapidly Acting Anthelmintic Drugs Capable of Clinical Repurposing. Sci Rep 8**:** 975.

Willighagen, E. L., J. W. Mayfield, J. Alvarsson, A. Berg, L. Carlsson *et al.*, 2017 The Chemistry Development Kit (CDK) v2.0: atom typing, depiction, molecular formulas, and substructure searching. J Cheminform 9**:** 33.

Wils, P., A. Warnery, V. Phung-Ba, S. Legrain and D. Scherman, 1994 High lipophilicity decreases drug transport across intestinal epithelial cells. J Pharmacol Exp Ther 269**:** 654-658.

Wishart, D. S., Y. D. Feunang, A. C. Guo, E. J. Lo, A. Marcu *et al.*, 2018 DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 46**:** D1074-D1082.

Ziniel, P. D., B. Karumudi, A. H. Barnard, E. M. Fisher, G. R. Thatcher *et al.*, 2015 The Schistosoma mansoni Cytochrome P450 (CYP3050A1) Is Essential for Worm Survival and Egg Development. PLoS Negl Trop Dis 9**:** e0004279.