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# Screening the Medicines for Malaria Venture (MMV) Pandemic Response Box chemical library on *Caenorhabditis elegans* identifies re-profiled candidate anthelmintic drug leads

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The 3 major classes of soil transmitted helminths (whipworm, hookworm and *Ascaris*) affect 1.5 billion people worldwide mostly in poor countries, where they have adverse effects on child development, nutrition, and the work capacity of adults. Although there are drugs effective on *Ascaris*, notably the benzimidazoles, those same drugs show poor efficacy particularly against whipworm (*Trichuris trichiura*) and to a certain extent hookworm. Parasitic nematodes also infect farm livestock and companion animals. Resistance to currently deployed human and veterinary anthelmintic drugs is a growing problem. Therefore, new chemical anthelmintic lead compounds are urgently needed. One of the fastest routes to a novel therapeutic lead is to screen libraries of drugs which are either already approved for human use or have already been part of clinical trials. We have pursued this approach to anthelmintic lead discovery using an invertebrate automated phenotyping platform (INVAPP) for screening chemicals and the well-established nematode genetic model organism *Caenorhabditis elegans*. The 400 compound Medicines for Malaria Venture (MMV) Pandemic Response Box library was screened with each compound tested initially at  $1.0 \times 10^{-4}$  M. We identified 6 compounds (MMV1593515 (vorapaxar), MMV102270 (diphyllin), MMV1581032 (ABX464), MMV1580796 (rubitecan), MMV1580505 and MMV1593531) active in both an L1-L4 growth/motility assay and in an L4 motility assay. For vorapaxar, an  $EC_{50}$  of  $5.7 \times 10^{-7}$  M was observed, a value comparable to those of some commercial anthelmintics. Although not a parasite, the ease with which high-throughput screens can be pursued on

the free-living nematode *C. elegans* makes this a useful approach to identify chemical leads and complements the often lower-throughput experiments on parasitic nematode models.

#### KEYWORDS

Medicines for Malaria Venture (MMV) Pandemic Response Box, phenotypic chemical library screen, anthelmintic drug candidates, drug re-purposing, invertebrate automated phenotyping platform (INVAPP), *Caenorhabditis elegans*

## Introduction

The major human gastrointestinal tract parasites collectively known as soil transmitted helminths (STHs) are the whipworm (*Trichuris trichiura*), the roundworm (*Ascaris lumbricoides*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*). Over one billion people are estimated to be infected with at least one STH (1). STH infection is a significant but neglected cause of morbidity (2, 3). According to Hotez and Kamath (2) more than 50 million school-aged children and 7 million child-bearing-age women in Sub-Saharan Africa are infected with one or more STH (2). Due to the remoteness and inaccessibility of the worst-affected places, the worldwide burden of STHs has most likely been underestimated (4).

Currently, preventive chemotherapy, based on single-dose, mass drug administration (MDA), is used to treat STHs in endemic regions, with the goal of minimising morbidity in pre-school and school-aged children with moderate to heavy infections. Such treatments, which use the benzimidazole drugs albendazole and mebendazole remain effective against ascariasis but less so against hookworm and whipworm. (3–7). Therefore, treatment for the STHs is an example of an unmet clinical need that necessitates the development of novel therapeutics. Recent experience from the animal health field showed that resistance to the anthelmintic monepantel (8) appeared within two years of its introduction (9). Therefore, current MDA programmes may lead to similar selection for resistance, and the efficacy of, for example, benzimidazole drugs against *T. trichiura* has fallen in recent years (10, 11).

Despite the long history of anthelmintic medication development, the majority of currently used anthelmintics were discovered by phenotypic screening of chemical candidates. Medicines that act on ligand-gated ion channels (12) and drugs that target the cytoskeletal protein tubulin are among the products of this method (13). In the search for new anthelmintic drug candidates, several laboratories have developed high-throughput screening methods (14). In our laboratory an invertebrate automated phenotyping platform (INVAPP) and the algorithm Paragon (15) have been developed. This system has been used to identify two novel chemical classes of anthelmintics, the 2, 4-dihydrobenz[e][1,4]

oxazepin-2(3H)-ones (11, 16) and the diaminothieno[3,2-d]pyrimidines (17). We have therefore adopted the INVAPP/Paragon system to explore a new chemical library.

*C. elegans* is a nematode genetic model organism (18) that is frequently employed in anthelmintic discovery research (8, 14, 19, 20). The Pandemic Response Box is a chemical library supplied by Medicines for Malaria Venture (MMV) and the Drugs for Neglected Diseases Initiative (DNDi). It includes 400 drug-like compounds that are either already on the market or in various stages of research for uses other than as anthelmintics. The library was selected by experts from Academia and Industry with the aim of pursuing an Open Source approach to discovering new chemical leads that could impact on neglected, pandemic-scale diseases with unmet clinical needs (21). The library contains 201 antibacterial compounds, 153 antivirals, and 46 antifungals. Compounds of interest from this library may therefore offer a fast-track route into clinical trials in the search for new anthelmintics. We have used INVAPP to assess the activities of all 400 drugs on *C. elegans* L1–L4 stages using a growth/motility assay and on the L4 stage utilising a motility assay in order to understand the potential of library compounds for re-purposing as candidate anthelmintic drug leads.

## Methods

### The Pandemic Response Box library

The Pandemic Response Box library was supplied by the Medicines for Malaria Venture. All stock compounds were supplied as  $10^{-2}$  M stocks in DMSO.

### *C. elegans* – maintenance and preparation of L1 and L4 stages

The *C. elegans* wild type N2 strain was maintained at 20°C on nematode growth medium (NGM) agar seeded with the *Escherichia coli* strain OP50. To prepare worm populations for screening, first a mixed population was obtained. 5 to 7 NGM

plates were seeded with 250  $\mu\text{L}$  of the *E. coli* strain OP50 and incubated at 37°C for 3 to 5 days to form a lawn. A 2.5cm square section of the agar plate rich in worms was transferred to each NGM plate and maintained at 20°C for 5 days.

Such *C. elegans* cultures contain worms of different ages. To eliminate variation caused by age differences, worms need to be synchronised. To obtain a synchronised L1 population we used filtration. Each NGM plate was washed with 50 mL of S-basal medium into a falcon tube and centrifuged at 3000 x g at 20°C for 4 min. The pellet was then re-washed (3x) and re-centrifuged, using the same procedure, to clear any remaining bacteria. Worms were then filtered (100  $\mu\text{m}$  filter) to remove any adult and late-stage larvae. Finally, they were passed through a 40  $\mu\text{m}$  sieve (3x) to obtain a synchronous L1 larval population.

To obtain a synchronised L4 larval population, a population enriched in L4s was first obtained by incubating the NGM plates, prepared as described, for between 8-10 days after transferring *C. elegans*. Plates with the highest number of L4 larvae were selected and washed with S-basal medium. Worms were then filtered (100  $\mu\text{m}$  filter) to capture any L4 stage larvae and remove earlier stages. The filter was rinsed with S-basal media into a falcon tube and centrifuged at 3000 x g at 20°C for 4 min. The pellet was then re-washed (2x) using the same procedure to remove any residual bacteria.

## The INVAPP/Paragon system for Automated Phenotyping of nematodes

The INVAPP/Paragon system used in these experiments has already been described in detail (15, 22). Two hundred frame movies were captured at 25 frames  $\text{s}^{-1}$  for 8 s using  $\mu\text{Manager}$  (23). Movies were analysed using MATLAB scripts (available at <https://github.com/fpartridge/invapp-paragon>) and the variance determined through time for each pixel. The distribution of these pixel variances was then considered, and pixels whose variance was above the threshold (those greater than one standard deviation away from the mean variance) were considered 'motile'. Motile pixels within each well were counted to obtain a movement score.

## *C. elegans* – growth/motility assay

Screening experiments were conducted in 96-well plate liquid cultures. Synchronised L1s were diluted to approximately 15-25 worms per 50  $\mu\text{L}$  in S complete buffer with 1% w/v HB101 *E. coli*. Assay plates were prepared with 99  $\mu\text{L}$  of L1 suspension and 1  $\mu\text{L}$  of each compound per well. As a control 16 wells in each plate were prepared with 1  $\mu\text{L}$  DMSO solution (1% v/v final concentration). Plates were incubated at 25°C and motility was recorded using the INVAPP/Paragon system 3 days later. By this time control worms developed to L4 or adult stage. Movies were recorded and the median growth/motility score measured using INVAPP as described above.

This assay measures both growth and motility in a combined score (15). A schematic of this assay is shown in Figure 1A.

## *C. elegans* - motility assay

Synchronised L4 were diluted to approximately 15-25 worms per 50  $\mu\text{L}$  in S basal buffer. No bacterial food is used in this assay. Assay plates were prepared with 99  $\mu\text{L}$  of L4 suspension and 1  $\mu\text{L}$  of each compound per well. As a control 16 wells in each plate were prepared with 1  $\mu\text{L}$  DMSO solution (1% v/v final concentration). Plates were incubated at 25°C before the motility of *C. elegans* was recorded using the INVAPP/Paragon system after 24 h. Due to the absence of food, worms do not grow or develop in this assay, which therefore exclusively measures changes in motility and is recorded as a motility score. A schematic of this assay is shown in Figure 3A.

## Primary screen of MMV Pandemic Response Box library at $1.0 \times 10^{-4}$ M using growth/motility assay

The 400 drugs of the MMV Pandemic Response Box library were screened at  $1.0 \times 10^{-4}$  M on wild type *C. elegans* in a growth/motility assay primary screen (L1 to L4 or adult development). 1% v/v DMSO was used as a negative control. Three sets of identical assay plates were prepared for each experiment and the entire screen was repeated on 3 different days (n=3).

## Secondary screen to confirm candidate lead compounds and additionally test activity in a motility assay

The 18 potential candidate lead compounds identified in the primary screen of the MMV Pandemic Response Box library were re-tested on *C. elegans* in the growth/motility assay, at  $7.5 \times 10^{-5}$  M (0.75% v/v DMSO) and  $5.0 \times 10^{-5}$  M (0.5% v/v DMSO). In addition, *C. elegans* L4 animals were screened at  $1.0 \times 10^{-4}$  M in the motility assay. In all cases, screens were undertaken on three separate occasions (n=3), each time with 4 assay repeats. Levamisole at the same concentration was the positive control and 1% v/v DMSO the negative control.

## Concentration-response curves

The concentration-response relationship for selected compounds was determined by testing activity in the *C. elegans* growth/motility assay. Compounds were tested at each of 12 concentrations from  $5.0 \times 10^{-5}$  M to  $2.0 \times 10^{-8}$  M (10 to 12 replicates tested on three occasions so n=3).  $\text{EC}_{50}$  values were estimated by fitting curves using a four-parameter log-logistic function in Graphpad Prism 9.3.

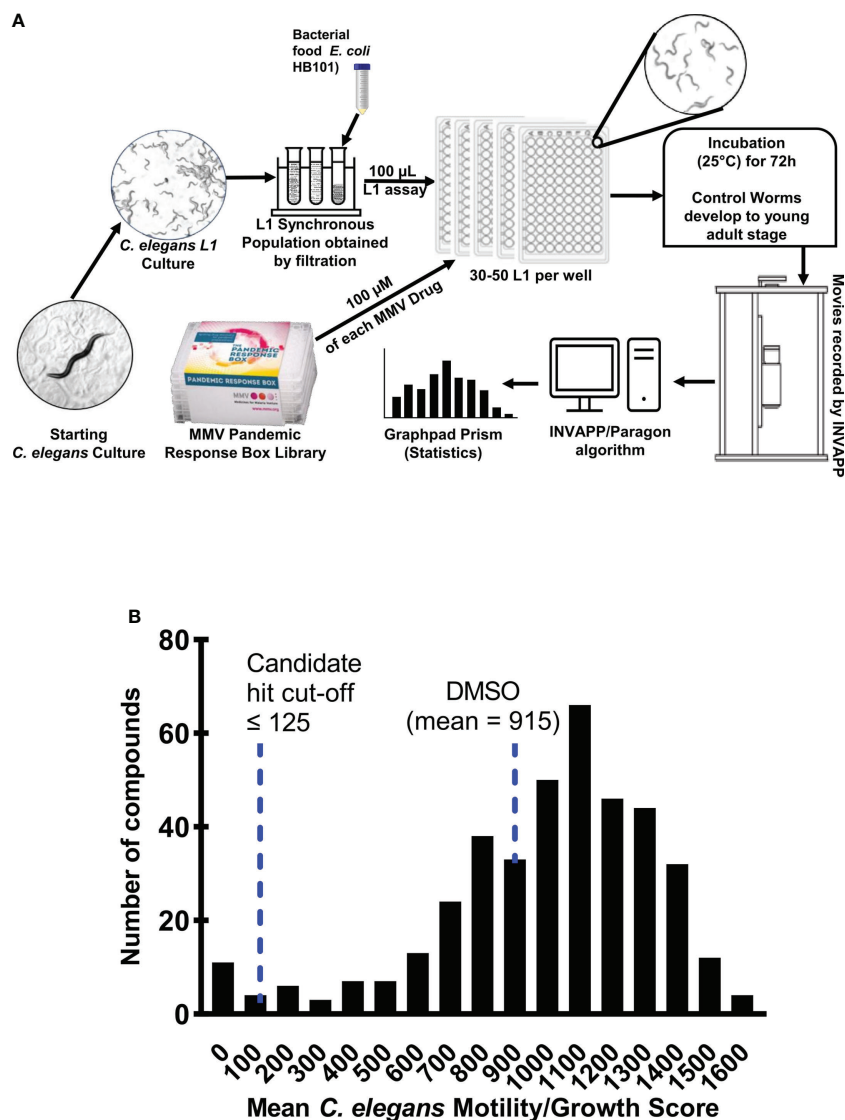


FIGURE 1

(A) Cartoon illustration of preparation and screening of *C. elegans* growth/motility assay. By the time that the movies are recorded, surviving *C. elegans* worms are expected to have developed to the (larger) L4 or adult stages. Therefore, their movement recorded by INVAPP/Paragon is a Growth/Motility score. (B) Frequency distribution of the mean *C. elegans* growth/motility scores for each of the Pandemic Response Box compounds tested at  $1.0 \times 10^{-4}$  M concentration, along with the DMSO-only control. Each bin is a range of scores from 50 below the axis value to 50 above the axis value. For example, the 200 bin counts the number of compounds scoring between 150 and 250. The screen was performed on three separate occasions ( $n = 3$ ). The mean of the three *C. elegans* growth/motility scores were plotted. A cut-off score for candidate hits of 125 was selected.

## Results

### Identification of candidate lead compounds by screening the 400 compound MMV Pandemic Response Box Library in the *C. elegans* growth/motility assay

Primarily the Pandemic Response Box library was screened using the *C. elegans* growth/motility assay. The 400 compounds

were screened at  $1.0 \times 10^{-4}$  M and the growth/motility score recorded (15). A histogram showing the distribution of the mean *C. elegans* growth/motility scores for each compound, along with the DMSO-only control is shown in Figure 1B.

The primary screen was used to prioritise the most active compounds for confirmatory rescreening. We chose the 14 compounds with the lowest mean growth/motility score (3.5% of the library) for rescreening (each compound had a mean growth/motility score below 125). In addition, we selected a further 4 compounds for

rescreening with scores close to this cut-off value. The full data for all three repeats of the primary screen using the *C. elegans* growth/motility assay at  $1.0 \times 10^{-4}$  M are presented in the [Table S1](#).

## Confirmation of 18 active anthelmintic compounds in a secondary screen

To confirm the activity of the lead compounds, we conducted a secondary screen. The 18 candidates were re-tested in the same *C. elegans* growth/motility assay at two lower concentrations ( $7.5 \times 10^{-5}$  M and  $5.0 \times 10^{-5}$  M). The results for the  $7.5 \times 10^{-5}$  M secondary screen are shown in [Figure 2A](#). A one-way ANOVA showed there was a significant effect of compound treatment ( $P \leq 0.0001$ ). The effectiveness of each compound compared to the DMSO-only control was determined using Dunnett's multiple comparison test. All 18 candidate lead compounds significantly reduced the *C. elegans* growth/motility score at  $7.5 \times 10^{-5}$  M ([Figure 2A](#), [Table S3](#)).

The results of the *C. elegans* growth/motility screen at  $5.0 \times 10^{-5}$  M are shown in [Figure 2B](#). A one-way ANOVA showed when there was a significant effect of compound treatment ( $P \leq 0.0001$ ). The effectiveness of each compound was determined using Dunnett's multiple comparison test with reference to the DMSO-only control. 13 Pandemic Response Box compounds significantly reduced the *C. elegans* growth/motility score at  $5.0 \times 10^{-5}$  M, as indicated by red asterisks in [Figure 2B](#). Six compounds were highly effective ( $P$  values  $\leq 0.0001$ ). Data for the secondary screen of the *C. elegans* growth/motility assay at  $5.0 \times 10^{-5}$  M are presented in [Table S2](#).

## Identification of compounds that act to block motility

Many existing anthelmintics act by reducing motility, for example by acting on ion channels that function in the nervous system and/or at neuromuscular junctions (24–26). We therefore wanted to determine which of the candidate lead compounds impair motility. The 18 candidate lead compounds were tested on *C. elegans* L4 stage worms in a pure motility assay at  $1.0 \times 10^{-4}$  M. This allowed us to verify whether, in addition to showing activity in the *C. elegans* growth/motility assay over 72 h, the candidate hit compounds were also effective in an assay using *C. elegans* L4 stage worms without bacterial food, over 24 h. The method is shown in [Figure 3A](#) and the results are presented in [Figure 3B](#). A one-way ANOVA for this dataset showed there was a significant effect of compound treatment ( $P \leq 0.0001$ ). The effectiveness of each compound was then determined using Dunnett's multiple comparison test compared to the DMSO-only control. In addition to the positive control drug levamisole, 6 compounds that significantly reduced the *C. elegans* L4 motility score were identified and indicated with red asterisks ( $P$  values: \*  $P \leq 0.05$ , \*\*  $P$

$\leq 0.01$ ). Data for the *C. elegans* L4 motility assay screen is presented in the [Table S4](#).

## Summary of the most active compounds in the secondary screens

Thus, six of the hit compounds from the Pandemic Response Box library were significantly active on both the *C. elegans* growth/motility assay at  $1.0 \times 10^{-4}$  M,  $5.0 \times 10^{-5}$  M and  $7.5 \times 10^{-5}$  M as well as the L4 motility assay at  $1.0 \times 10^{-4}$  M. These compounds were MMV1593515 (vorapaxar), MMV102270 (diphyllin), MMV1581032 (ABX464), MMV1580796 (rubitecan), MMV1580505 and MMV1593531. Four are previously described antivirals and two have antibacterial activity (21). Vorapaxar is a human approved drug while diphyllin, ABX464 and rubitecan are in various human clinical trials ([Table 1](#)).

## Determination of the relative potency of the active anthelmintic compounds

We wanted to ensure that the anthelmintic compounds we identified showed concentration-dependent activity and to estimate their relative potency to inform future work. We used the efficacy of the best candidate lead compounds in the secondary screens to prioritize which to investigate. Of the six compounds significantly reducing L1 growth/motility at both  $5.0 \times 10^{-5}$  M and  $7.5 \times 10^{-5}$  M, as well as significantly reducing L4 motility, four (ABX464, diphyllin, rubitecan and vorapaxar) were readily available as solid material. Concentration-response curves for these four compounds are shown in [Figure 4](#). It was encouraging to see  $EC_{50}$  values in the low micromolar – ABX464 ( $EC_{50} = 2.3 \times 10^{-6}$  M), diphyllin ( $EC_{50} = 3.9 \times 10^{-6}$  M) and rubitecan ( $EC_{50} = 1.2 \times 10^{-5}$  M) and even sub-micromolar range – vorapaxar ( $EC_{50} = 5.7 \times 10^{-7}$  M).

In mammalian studies, MMV1593515 (vorapaxar) the human approved drug is a protease activated receptor-1 (PAR-1) inhibitor (33, 34), MMV102270 (diphyllin) is a vacuolar type  $H^+$ -ATPase (V-ATPase) inhibitor (35), MMV1581032 (ABX464) is a microRNA stimulant (36) and MMV1580796 (rubitecan) is a DNA topoisomerase inhibitor (37). Thus the 4 candidate compounds of interest have diverse mechanisms of action but all showed anthelmintic activity. MMV1593515 (vorapaxar), MMV1581032 (ABX464) and MMV1580796 (rubitecan) are antivirals and MMV102270 (diphyllin) is an antibacterial drug. Their mechanisms of action in nematodes remain to be determined.

## Discussion

The 400 compound MMV Pandemic Response Box library has proved useful in identifying from library screens compounds



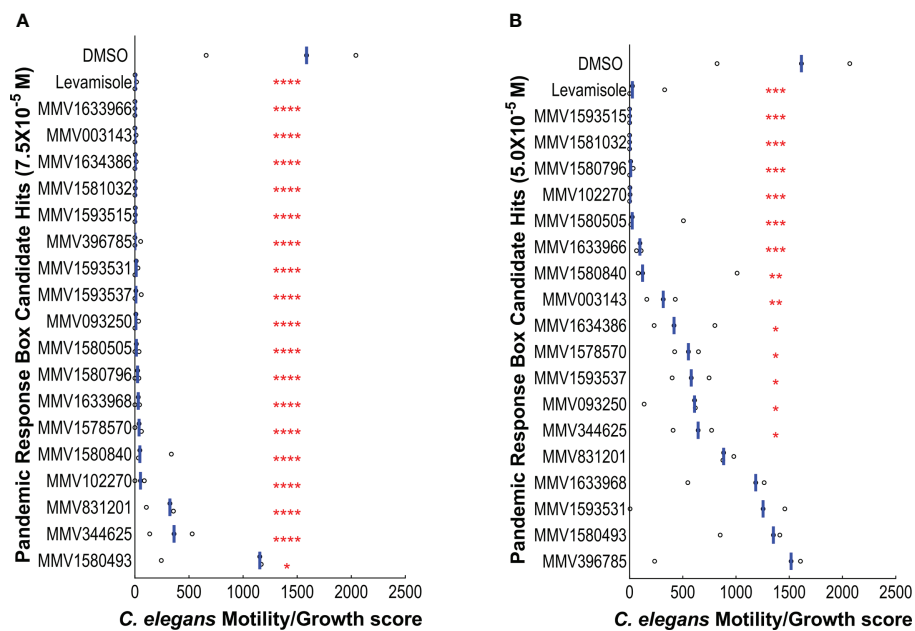


FIGURE 2

Retesting the 18 lead candidates as well as levamisole and DMSO as controls in the *C. elegans* growth/motility assay at (A)  $7.5 \times 10^{-5}$  M and (B)  $5.0 \times 10^{-5}$  M. Each point shows one well. The blue bar indicates the mean movement index for each treatment. The screen was performed with four replicates and on three occasions ( $n = 3$ ). A one-way ANOVA for the  $7.5 \times 10^{-5}$  M concentration dataset showed there was a significant effect of compound treatment  $P < 0.0001$ . A *post-hoc* Dunnett's test compared each compound to the DMSO-only control (P values\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$ ).

of interest for re-purposing as antiparasitics for helminth control, and the control of schistosome and protozoan parasites. A recent study reported a screen of the MMV Pandemic Response Box against *C. elegans* young adults in a motility screening assay. This assay is similar to our L4 motility assay that is reported in Figure 3. They also screened against *Haemonchus contortus* exsheathed third-stage larvae (xL3s) in motility and development screening assays (28).

Shanley et al. identified two compounds, MMV1581032 (ABX464) and MMV1593515 (vorapaxar), that inhibited *C. elegans* motility. These compounds are among our four most active compounds, and we confirm their relatively low  $EC_{50}$  values. In addition, Shanley et al. (28) showed that MMV1581032 (ABX464), as well as another drug, MMV1593539, have anthelmintic activity against *H. contortus*. As in their studies, we also found that MMV1593539 is not active against *C. elegans*. The accord between our findings and those of Shanley et al. (28) confirms the utility of our INVAPP screening approach.

However, in this study we also identify an additional 16 active anthelmintic compounds. This difference is likely due to our use of a *C. elegans* assay that measures growth/motility as worms develop from the L1 stage. This might reflect either a greater sensitivity of the L1 stages, or the choice of a higher initial screening concentration. Differential sensitivity of different life stages of nematodes and the importance of this in the choice of screening assays in order to avoid false negatives has been well described

elsewhere (1). There are likely more biological processes (and hence drug targets) that could be targeted by anthelmintic assays in our growth/motility assay compared to a pure motility assay, which may account for the greater diversity of compounds detected with anthelmintic properties. The known inactivity of the approved anthelmintic drug mebendazole on L4 motility in *C. elegans*, compared to its high activity in the L1 growth/motility assay (15) demonstrates that viable anthelmintics will be missed by pure motility screens. Our study demonstrates the utility of INVAPP/Paragon and is complementary to that of Shanley et al. (28).

MMV1581032 (ABX464) is a first-in-class, clinical-stage, oral small molecule immunomodulator (32). ABX464 is reported to bind to the RNA cap-binding complex, which modulates both viral and cellular RNA biogenesis (36). ABX464 was originally developed for its antiviral potential but it was redeployed for chronic inflammatory diseases due to potent anti-inflammatory effects in preclinical testing. It has shown safety and tolerability in clinical trials, which makes it a good candidate for repurposing as an anthelmintic.

Diphyllin occurs naturally in *Cleistanthus collinus*, which is known as an antiparasitic medicinal plant in Asia and India (38). Diphyllin proved deadly for the promastigote and amastigote stages of the protozoan parasite *Leishmania infantum* (39), while the structurally related compound justicidin  $\beta$  suppressed the growth of the parasites causing sleeping sickness *Trypanosoma brucei rhodesiense* and *T. cruzi* (40).

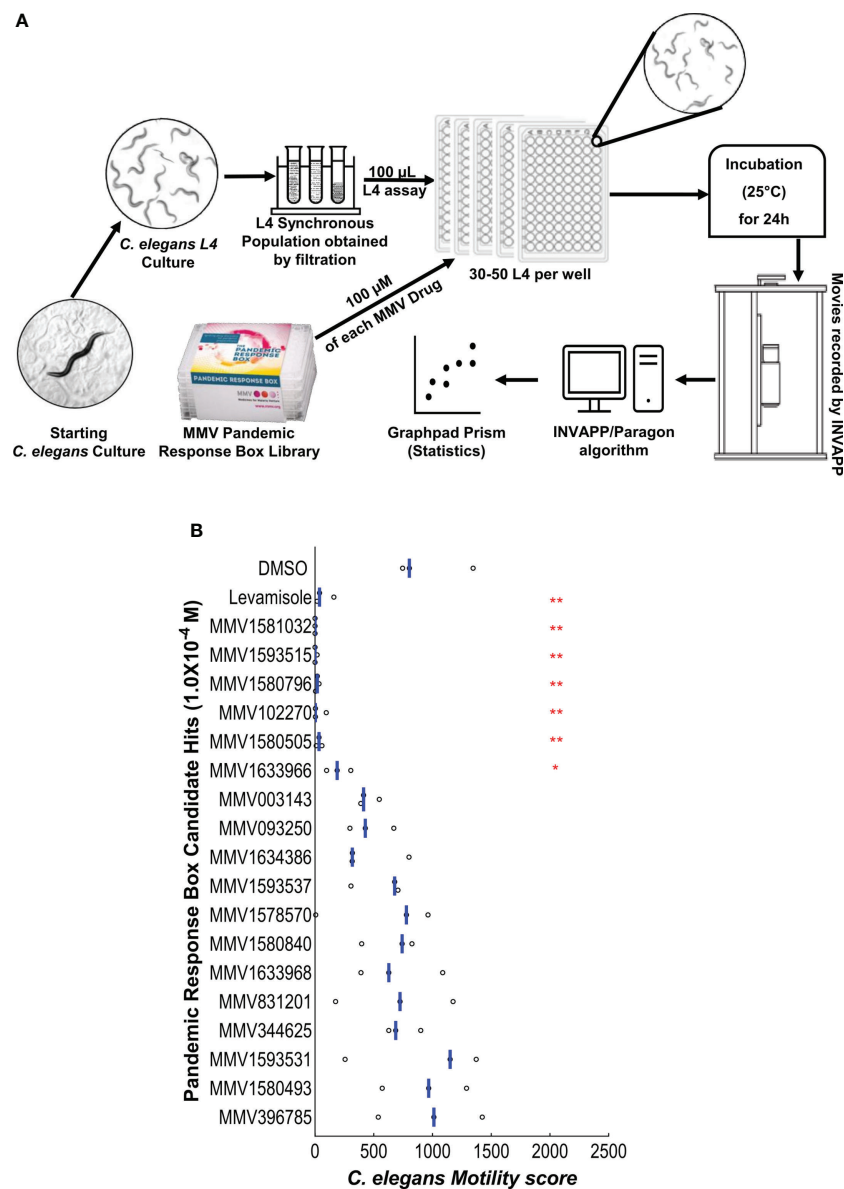


FIGURE 3

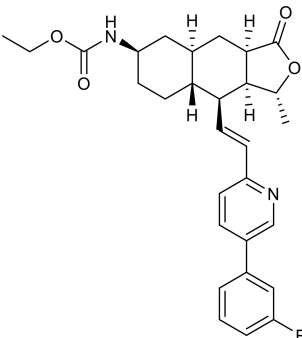
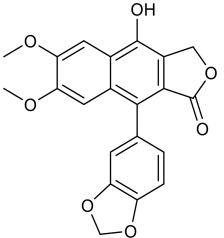
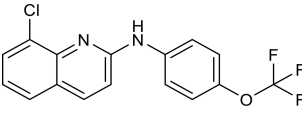
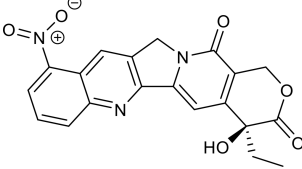
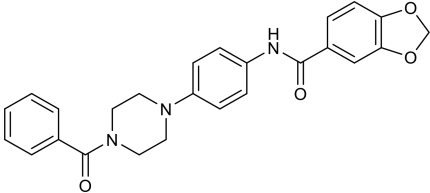
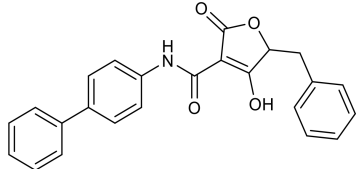
(A) Cartoon illustration of preparation and screening *C. elegans* motility assay. When the movies are recorded, surviving *C. elegans* worms have not developed further due to the lack of bacterial food. Therefore, their movement recorded by INVAPP/Paragon is a pure motility score.

(B) Retesting the candidates in the *C. elegans* L4 motility assay at  $1.0 \times 10^{-4}$  M. Each point shows one well. The blue bar indicates the mean movement index for each treatment. The screen was performed with four replicates and on three occasions ( $n = 3$ ). A one-way ANOVA for the  $1.0 \times 10^{-4}$  M concentration dataset showed there was a significant effect of compound treatment ( $P \leq 0.0001$ ). A *post-hoc* Dunnett's test compared each compound to the DMSO-only control ( $P$  values: \* $P \leq 0.05$ , \*\* $P \leq 0.01$ ).

The MMV Pandemic Response Box library has also been screened against *Schistosoma mansoni* with the aim of discovering an effective drug for the neglected tropical disease schistosomiasis (41). The study identified 26 compounds active against newly transformed schistosomula, of which 17 were active against adult *S. mansoni*. Three compounds with anti-schistosomal activity (MMV396785 [alexidine], MMV1634386 [oteseconazole] and MMV1578570) showed activity against *C. elegans* growth/

motility in this study. All three of these compounds showed efficacy against adult *S. mansoni* at  $10 \mu\text{M}$  but not at  $1 \mu\text{M}$ . Of particular interest alexidine was one of only two compounds which was lethal to adult *S. mansoni* at  $10 \mu\text{M}$  but was not tested *in vivo*. In addition, oteseconazole showed good efficacy *in vitro* but did not alter worm burdens *in vivo* when dosed by a single oral gavage of  $200 \text{ mg/kg}$  7 weeks post infection. These compounds therefore have potential for development as a broad-spectrum anthelmintics.

TABLE 1 List of Pandemic Response Box hit compounds from the *C. elegans* growth/motility screen at  $1.0 \times 10^{-4}$  M,  $5.0 \times 10^{-5}$  M and  $7.5 \times 10^{-5}$  M and *C. elegans* L4 motility screen at  $1.0 \times 10^{-4}$  M.

MMV ID	NAME molecular weight	STRUCTURE	Drug properties
MMV1593515	Vorapaxar (MW: 492.6)		<ul style="list-style-type: none"> <li>• Human approved drug, Protease Activated Receptor-1 (PAR-1) Inhibitor. It reduces cardiovascular thrombosis.</li> <li>• In <i>C. elegans</i>, the human muscarinic receptor ortholog, GAR-3, has been implicated in the regulation of muscle contraction (27) thus, it is proposed that GAR-3 antagonism by MMV1593515 is responsible for motility reduction in <i>C. elegans</i>, although a mode of action study would be needed to test this hypothesis (28)</li> <li>• Antiviral activity (21, 29)</li> </ul>
MMV102270	Diphyllin (MW: 380.4)		<ul style="list-style-type: none"> <li>• Vacuolar type H<sup>+</sup>-ATPase (V-ATPase) inhibitor.</li> <li>• Naturally occurs in <i>Cleistanthus collinus</i>, an antiparasitic medicinal plant in Asia and India.</li> <li>• Antibacterial activity (; 21, 30, 31)</li> </ul>
MMV1581032	ABX464 (MW: 338.7)		<ul style="list-style-type: none"> <li>• MicroRNA stimulant.</li> <li>• ABX464 is under investigation in several clinical trial including for Crohn's Disease, Rheumatoid Arthritis, HIV</li> <li>• Antiviral activity (21, 32)</li> </ul>
MMV1580796	Rubitecan (MW: 393.3)		<ul style="list-style-type: none"> <li>• DNA topoisomerase inhibitor.</li> <li>• Investigated for anti-tumour activity</li> <li>• Antiviral activity (21)</li> </ul>
MMV1580505	None (MW: 429.5)		<ul style="list-style-type: none"> <li>• Antiviral activity (21)</li> </ul>
MMV1593531	None (MW: 385.4)		<ul style="list-style-type: none"> <li>• Antibacterial activity (21)</li> </ul>

Some of our confirmed active compounds have also shown activity against other infectious diseases. MMV1593537 has antifungal activity against *Cryptococcus neoformans*, *Cryptococcus deuterogattii*, and the emerging global threat *Candida auris* (42). MMV1578570, MMV396785 (alexidine), MMV1634386 and MMV1580796 (rubitecan) are active against the pathogenic amoebae *Balamuthia mandrillaris*,

*Naegleria fowleri*, and *Acanthamoeba castellanii* (43). Rubitecan is one of our four most active anthelmintic compounds. It prevents DNA from unwinding during replication *via* DNA topoisomerase 1, therefore interfering with tumour growth (44). Rubitecan is a derivative of a compound extracted from the *Camptotheca acuminata* tree with potent antitumor and antiviral properties.



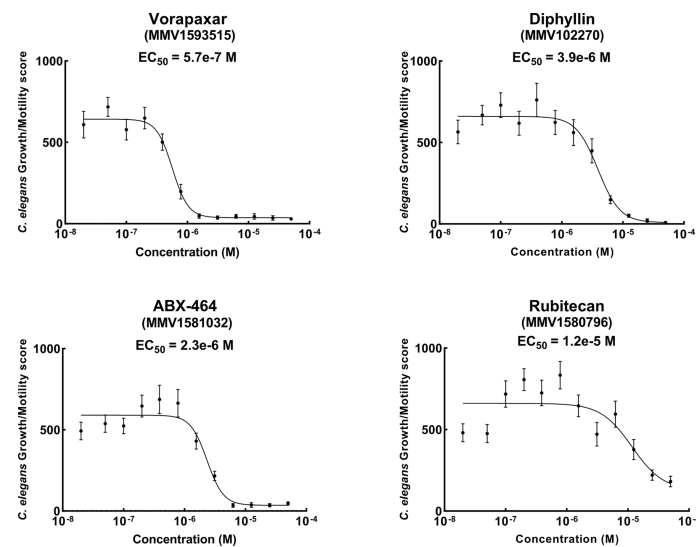


FIGURE 4

EC<sub>50</sub> values are determined from concentration-response curves for the 4 compounds consistently showing activity in the growth/motility assay. Each point shows the mean and standard error (error bars) for each concentration tested. Compounds were tested between 10 to 12 replicates and 12 concentrations ranging from  $5.0 \times 10^{-5}$  M to  $2.0 \times 10^{-8}$  M (tested on three occasions so  $n = 3$ ). EC<sub>50</sub> curves were fitted using a four-parameter log-logistic function in Graphpad Prism 9.3.

## Conclusions

This study identified 18 candidate lead compounds which impair nematode growth or motility, and the four most active include one drug (vorapaxar) with a sub-micromolar EC<sub>50</sub>, comparable to some current commercial anthelmintic drugs. It is of interest that comparisons between the growth/motility assay and the pure motility assay show that some candidate leads could be missed in assays monitoring only motility. Vorapaxar emerged as a lead compound of particular interest as it is approved for human use. Some vorapaxar analogues have been reported (45, 46) and in future it would be interesting to explore these compounds to see how the activity of such closely related structures compare with that of vorapaxar itself. The original targets of these re-purposed drugs are not necessarily those responsible for their anthelmintic activity. Important future work to identify their nematode molecular targets will be facilitated by studies on *C. elegans*, via genetic screens (47, 48). Another line of future research of interest would be to explore whether newly identified candidate lead compounds such as vorapaxar can circumvent benzimidazole resistance in *C. elegans* lines expressing resistance mutations in tubulin genes (49, 50). Finally, where that is possible, it will also be useful to explore on parasitic worms the actions of chemical leads derived from model organism studies of the type described here.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

## Author contributions

MN, FP, DS, KE, AR contributed to conception and design of the study. MN performed the experiments, and the statistical analysis was performed by MN and FP. MN, FP, and DS wrote the first draft of the manuscript. All authors MN, FP, DS, CB, AR, RF and KE contributed to manuscript draft revision, read, and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fitd.2022.1017900/full#supplementary-material>

### SUPPLEMENTARY TABLE 1

Result of Primary screen of MMV Pandemic Response Box library at  $1.0 \times 10^{-4}$  M using growth/motility assay and DMSO-only as negative control. Table S1 contains data for three separate screen batches undertaken at three separate occasions each with 3 x identical

replicates for each compound. INVAPP score indicates the rate of movement for each well of 96 well plates containing growth/motility assay. The table also contains the position of each compound at the experiments. Median rate for each replicate also included in the table with MMV name of each drug, Trival name, Disease area and Smile structure of each compound. The cut off to identify active compounds also presented.

### SUPPLEMENTARY TABLE 2

Result of Secondary screen of the 18 hit compounds identified in the primary screen of the MMV Pandemic Response Box library. They were re-tested on *C. elegans* L1s growth/motility assay at  $5.0 \times 10^{-5}$  M and (0.5% v/v DMSO) as negative control. Screens were undertaken at 3 separate occasions with 4 replicates each (n=3) with similar concentration of levamisole as a positive control. INVAPP score indicates the rate of movement for each well of 96 well plates containing growth/motility assay. Table also contains the position of each compound at the experiments, median rate for each replicate with MMV name of each compound.

### SUPPLEMENTARY TABLE 3

Result of Secondary screen of the 18 hit compounds identified in the primary screen of the MMV Pandemic Response Box library. They were re-tested on *C. elegans* L1s growth/motility assay at  $7.5 \times 10^{-5}$  M and (0.75% v/v DMSO) as negative control. Screens were undertaken at 3 separate occasions with 4 replicates each (n=3) with similar concentration of levamisole as a positive control. INVAPP score indicates the rate of movement for each well of 96 well plates containing growth/motility assay. Table also contains the position of each compound at the experiments, median rate for each replicate with MMV name of each compound.

### SUPPLEMENTARY TABLE 4

Result of Secondary screen of the 18 hit compounds identified in the primary screen of the MMV Pandemic Response Box library. They were re-tested on *C. elegans* L4 motility assay at  $1.0 \times 10^{-4}$  M and DMSO-only as negative control. Screens were undertaken at 3 separate occasions with 4 replicates each (n=3) with similar concentration of levamisole as a positive control. INVAPP score indicates the rate of movement for each well of 96 well plates containing motility assay. Table also contains the position of each compound at the experiments, median rate for each replicate with MMV name of each compound.

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