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Invited Article

Screening of the 'Pathogen Box' identifies an approved pesticide with major anthelmintic activity against the barber's pole worm

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ABSTRACT

There is a substantial need to develop new medicines against parasitic diseases *via* public-private partnerships. Based on high throughput phenotypic screens of largely protozoal pathogens and bacteria, the Medicines for Malaria Venture (MMV) has recently assembled an open-access 'Pathogen Box' containing 400 well-curated chemical compounds. In the present study, we tested these compounds for activity against parasitic stages of the nematode *Haemonchus contortus* (barber's pole worm). In an optimised, whole-organism screening assay, using exsheathed third-stage (xL3) and fourth-stage (L4) larvae, we measured the inhibition of larval motility, growth and development of *H. contortus*. We also studied the effect of the 'hit' compound on mitochondrial function by measuring oxygen consumption. Among the 400 Pathogen Box compounds, we identified one chemical, called tolfenpyrad (compound identification code: MMV688934) that reproducibly inhibits xL3 motility as well as L4 motility, growth and development, with IC_{50} values ranging between 0.02 and 3 μM. An assessment of mitochondrial function showed that xL3s treated with tolfenpyrad consumed significantly less oxygen than untreated xL3s, which was consistent with specific inhibition of complex I of the respiratory electron transport chain in arthropods. Given that tolfenpyrad was developed as a pesticide and has already been tested for absorption, distribution, excretion, biotransformation, toxicity and metabolism, it shows considerable promise for hit-to-lead optimisation and/or repurposing for use against *H. contortus* and other parasitic nematodes. Future work should assess its activity against hookworms and other pathogens that cause neglected tropical diseases.

Contents

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1. Introduction

Compounded by massive global food and water shortages and climate change, parasitic illnesses, including neglected tropical diseases (NTDs; WHO, 2015), have a devastating, long-term impact on human and animal health and welfare worldwide, and thus represent a major global challenge. Together, NTDs infect more than one billion people worldwide, resulting in an estimated loss of 26 million disability-adjusted life years (Hotez et al., 2014).

Despite their adverse socioeconomic impact, there are major limitations in the diagnosis, treatment and control of NTDs. Currently, there are no commercial vaccines available against most of these diseases (Pedrique et al., 2013; Hotez et al., 2016), diagnostic methods frequently suffer from insufficient specificity and sensitivity (Utzinger et al., 2012; Assefa et al., 2014), and treatments are often not highly effective and/or are toxic (Castro et al., 2006; Witschel et al., 2012; Molina et al., 2015). In addition, often the small numbers of drugs (or drug classes) frequently used, limited use of combination drug therapies and the implementation of mass drug administration programs bear the risk of drug resistance emerging in some groups of target pathogens (Humphries et al., 2012; Witschel et al., 2012; Webster et al., 2014). Therefore, the development of new drugs is crucial to ensure effective and sustained treatment and control into the future.

In spite of some success through the discovery of, for example, monepantel (Kaminsky et al., 2008; Prichard and Geary, 2008) and derquantel (Little et al., 2011), progress in discovering new drugs against parasitic worms of animal health importance has been relatively poor. Likely reasons for limited success beyond the lack of resources include an over-confidence in the validation of molecular targets (enzymes and receptors) and in studying an inappropriate developmental stage of a pathogen. However, key gaps include a lack of readily available curated sets of compounds for targeted screening and subsequent evaluation, limited cooperation among different areas (including parasitology, drug discovery, medicinal chemistry and safety evaluation) which are essential to find starting points for drug discovery, and to bring them to tangible and translational outcomes and outputs.

In the late 1990s, an innovative collaboration model for research and development for neglected diseases emerged in the form of public-private partnerships (PPPs) that came to be known as product development partnerships (PDPs). A key example is the Medicines for Malaria Venture (MMV), created from a desire to catalyse the discovery development and delivery of new medicines against malaria. Over the last decade, almost seven million compounds have been tested in phenotypic assays against malaria, and this has resulted in a solid pipeline of new preclinical and clinical candidates. In addition, an open science initiative has made many of these structures available, and a collection of 400 key malaria phenotypic 'hits', called the 'Malaria Box', was launched in 2013. Building on this model, in December 2015, MMV took this a stage further, with an initiative to stimulate the discovery of drugs for neglected parasitic diseases. The 'Pathogen Box' (www.pathogenbox.org), contains 400 diverse drug-like molecules, and is provided at no cost to research groups.

Each of the 400 compounds in the 'Pathogen Box' has confirmed activity against one or more key pathogens that cause some of the most socioeconomically important diseases worldwide, including tuberculosis, malaria, sleeping sickness, leishmaniasis, schistosomiasis, hookworm disease, toxoplasmosis and cryptosporidiosis. In addition, all compounds have been tested for cytotoxicity, with compounds included in the library being at least 5-fold more selective for the pathogen than its mammalian host. The complete set of compounds is dispatched to laboratories around the world to boost drug discovery efforts. This initiative provided us with a unique opportunity to assess these curated compounds for nematocidal activity in a recently developed whole-organism screening assay (Preston et al., 2015, 2016). Our aim was to rapidly screen all 400 compounds against parasitic stages of the barber's pole worm, *Haemonchus contortus*, and to identify hit compounds and characterise/assess them for further evaluation as nematocidal candidates. This worm was used because it is one of the best-studied members of a large order (Strongylida) of socioeconomically important nematodes of animals, including humans, because there is extensive information available on its biology and molecular biology, and because its genome and developmental transcriptome have been characterised in detail (Gasser and von Samson-Himmelstjerna, 2016), providing a foundation for drug discovery efforts.

2. Materials and methods

2.1. Procurement of H. contortus

The Haecon-5 strain of *Haemonchus contortus*, which is partially resistant to benzimidazoles (Dr Jody Zawadzki, personal communication), was maintained in experimental sheep as described previously (Schwarz et al., 2013; Preston et al., 2015) and in accordance with the institutional animal ethics guidelines (permit no. 1413429; The University of Melbourne, Australia). L3s were produced from *H. contortus* eggs by incubating faeces from infected sheep at 27 °C for 1 week (Preston et al., 2015), sieved through nylon mesh (pore size: 20 μm) to remove debris or dead larvae and then stored at 10 °C for a maximum of 3 months. For screening and basal oxygen consumption measurements (see following subsections), L3s were exsheathed and sterilised in 0.15% v/v sodium hypochlorite (NaClO) at 37 °C for 20 min (Preston et al., 2015). Thereafter, xL3s were washed five times in sterile physiological saline by centrifugation at 600 *g* (5 min) at 22–24 °C. Then, xL3s were immediately suspended in Luria Bertani medium [LB: 10 g of tryptone (cat no. LP0042; Oxoid, England), 5 g of yeast extract (cat no. LP0042; Oxoid) and 5 g of NaCl (cat. no. K43208004210; Merck, Denmark)] in 1 l of reverse-osmosis deionised water). LB was autoclaved and supplemented with 100 IU/ml of penicillin, 100 μg/ml of streptomycin and 2.5 μg/ml of amphotericin (Fungizone, antibiotic – antimycotic; cat. no. 15240-062; Gibco, USA); this supplemented LB was designated LB*. Fourth-stage larvae (L4s) were produced from xL3s *in vitro* for 7 days at 38 °C and 10% CO_2, as described by Preston et al. (2015, 2016).