

# Supplementary Materials

## High-throughput phenotypic assay to screen for anthelmintic activity on *Haemonchus contortus*

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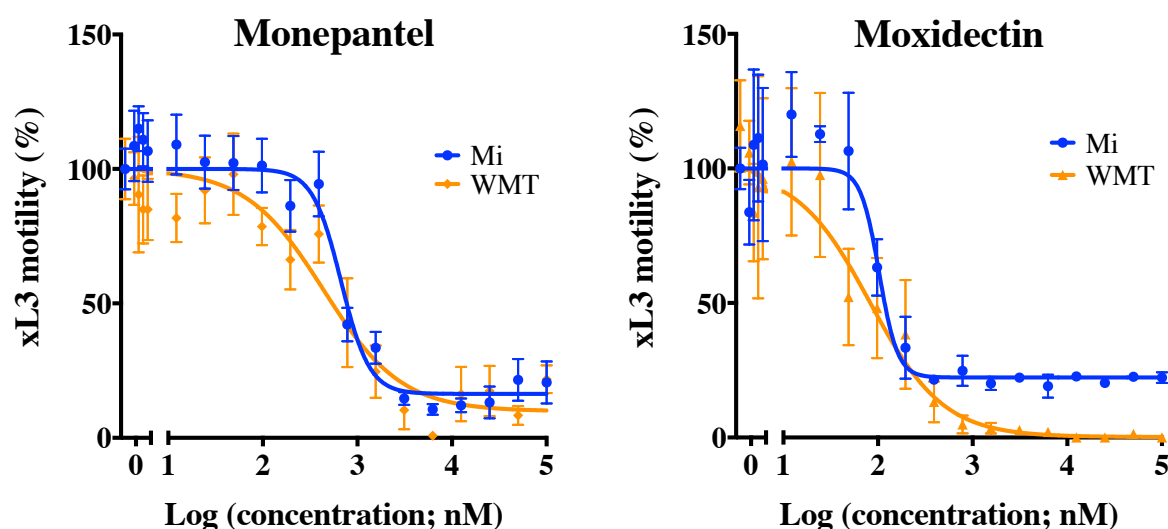
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**Figure. S1. Anthelmintic activity of positive-control compounds in vitro against exsheathed third-stage larvae (xL3s) of *Haemonchus contortus*.** Larval motility values (WMT and Mi) were recorded using the WMicroTracker ONE instrument and the original screening assay [30], respectively, at 90 h after exposure to each of the compounds (monepantel and moxidectin). Data points are triplicates in three independent experiments on different days; the mean  $\pm$  standard error of the mean (SEM).

**Table S1. The 42 ‘hit’ compounds identified in the primary screen with in vitro-activity against exsheathed third-stage larvae (xL3s) of *Haemonchus contortus*.** Information about compounds (code, chemical name, SMILES and structure) and the percentage of motility reduction in xL3s at 90 h (primary screen) and the larval phenotypes (L4) detected at 168 h. A subset (\*) of these 42 compounds was further assessed in dose-response assay (Fig. 5) and phenotypes recorded (cf. Table S2).

| Compound code | Motility reduction (%) in xL3 at 90 h | Abnormal morphological phenotype detected at 168 h |
|---------------|---------------------------------------|--|
| UoM-7625      | 76.6                                  | Not detected in the primary screen (nd)            |
| UoM-2588      | 75.3                                  | nd   |
| UoM-9701      | 76.7                                  | nd   |
| UoM-8811*     | 81.4                                  | ‘Evisceration’ ( <i>Evi</i> )*                     |
| UoM-5416      | 71.3                                  | nd   |
| UoM-5470      | 70.5                                  | nd   |
| UoM-8574      | 83.5                                  | nd   |
| UoM-4502      | 71.0                                  | nd   |
| UoM-6793      | 72.9                                  | ‘Coiled’ ( <i>Coi</i> )                            |
| UoM-2615      | 87.9                                  | nd   |
| UoM-6741      | 78.0                                  | nd   |
| UoM-4965      | 89.3                                  | nd   |
| UoM-0920      | 74.5                                  | <i>Evi</i>   |
| UoM-5126      | 73.4                                  | nd   |
| UoM-6404      | 88.8                                  | nd   |
| UoM-2807      | 81.4                                  | nd   |
| UoM-4068      | 74.4                                  | nd   |
| UoM-0377      | 73.7                                  | nd   |
| UoM-6038      | 70.5                                  | nd   |
| UoM-2808      | 82.9                                  | nd   |
| UoM-8496      | 83.5                                  | nd   |
| UoM-6088      | 78.6                                  | <i>Evi</i>   |
| UoM-4350      | 76.2                                  | nd   |
| UoM-9644      | 85.5                                  | nd   |
| UoM-9012      | 71.9                                  | nd   |
| UoM-7026      | 70.8                                  | <i>Evi</i>   |
| UoM-7300      | 72.8                                  | nd   |
| UoM-7024*     | 72.3                                  | nd*  |
| UoM-2533      | 72.0                                  | nd   |
| UoM-2866      | 77.4                                  | nd   |
| UoM-5961      | 90.5                                  | nd   |
| UoM-7347      | 85.8                                  | nd   |
| UoM-9466      | 79.3                                  | nd   |
| UoM-4380      | 79.3                                  | nd   |
| UoM-4423      | 79.2                                  | nd   |
| UoM-5812      | 70.8                                  | <i>Evi</i>   |
| UoM-8035*     | 90.5                                  | nd*  |
| UoM-3235      | 77.1                                  | ‘Curved’ ( <i>Cur</i> )                            |
| UoM-7382      | 72.8                                  | nd   |
| UoM-4124      | 74.0                                  | nd   |
| UoM-4865      | 76.5                                  | nd   |
| UoM-0366      | 71.1                                  | nd   |

\* When subsequently tested individually in the dose-response assay (sub-section 3.4), compounds UoM-8811, UoM-7024 and UoM-8035 (highlighted in grey) markedly inhibited larval development, achieving IC<sub>50</sub> values of ~ 4 µM, 25 µM and 41 µM, respectively (Fig. 5), with each of them inducing abnormal phenotypes (*Cur* and *Evi*) detected by detailed microscopic examination at 100-times magnification.

**Table S2. Distinct phenotypes of exsheathed third-stage larvae (xL3s) or fourth-stage larvae (L4s) of *Haemonchus contortus* observed in this study.**

| Morphology*   | Phenotype       | Abbreviation | Description   |
|---|-----------------|--------------|---|
| <i>Normal</i>   |                 |              |   |
|                | Wild-type (xL3) | WT           | Larvae with ‘normal’ morphology and motility.   |
| Mouth<br>↓<br> | Wild-type (L4)  | WT           | Larvae with ‘normal’ morphology and motility. A buccal capsule (mouth) and pharynx present (cf. [45]).                            |
| <i>Abnormal</i>   |                 |              |   |
|                | Coiled          | <i>Coi</i>   | Tightly coiled and not motile.  |
|              | Curved          | <i>Cur</i>   | U-shape and not motile.   |
|              | Evisceration    | <i>Evi</i>   | Evisceration through the excretory pore of the worm (anterior); not motile. Exhibited by L3s or premature L4s (sheathed); lethal. |

\* The lengths of wild-type xL3s and L4s cultured *in vitro* in LB\* medium (containing 0.4 % DMSO) for 90 h and 168 h are ~ 600-650 µm and 700-750 µm, respectively.