A validated high-throughput methodology for assaying rat lungworm (*Angiostrongylus cantonensis*) motility when challenged with a large suite of potentially anthelmintic natural products from Hawaiian fungi

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SUPPLEMENTARY MATERIAL

**General instrumentation, equipment and solvents**

1D NMR (1H, 13C) data were recorded on a Bruker AM-400 spectrometer (Bruker BioSpin AG, Faellanden, Switzerland), deuterated dimethyl sulfoxide (Sigma USA) was sued to dissolve the samples.

An Agilent 6530 Accurate-Mass Q-TOF LC-MS spectrometer (Agilent Technologies, Waldbronn, Germany) was used to record high-resolution mass spectra.

Preparative RP-HPLC was carried out on an Ultimate 3000 chromatographic system (Agilent Technologies, Waldbronn, Germany) with a Phenomenex preparative column (Phenyl-hexyl, 5 μ, 100 × 21.2 mm).

Semipreparative RP-HPLC was carried out on an Ultimate 3000 chromatographic system (Agilent Technologies, Waldbronn, Germany) with a Phenomenex semipreparative column (C18, 5 μ, 250 × 10 mm), connected to a Dionex Ultimate 3000 DAD detector (Agilent Technologies, Waldbronn, Germany) (detected at 210, 254, 320 and 365 nm) and a Dionex Ultimate 3000 automated fraction collector. All solvents were HPLC grade. Diaion HP-20 (Alfa Aesar, Japan) was used to run the open-column chromatography. All organic solvents used were purchased from Fisher Scientific, USA or Sigma-Aldrich, USA and in house double distilled mili-Q water (resistivity 18 MΩ.cm) MiliporeSigma, Fisher Scientific, USA.

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| **Table S1.** Samples screened for motility inhibition using the wMicroTracker, with sample type and whether screened as two replicate triplets (triplet/triplet), a single triplet, a triplet compared with a single well (triplet/singlet) or a singlet alone (i.e. high-throughput), and names of pure compounds. Sample numbers are internal designations in S. Cao’s laboratory. |
| Sample ID | Sample type | Pure compound name  | Configuration |
| M200 | Pure compound | Lamellicolic anhydride | triplet/triplet |
| M201 | Pure compound | Butyrolactone I | triplet/triplet |
| M202 | Fraction |  | triplet/triplet |
| M203 | Fraction |  | triplet/triplet |
| M204 | Fraction |  | triplet/triplet |
| M205 | Fraction |  | triplet/triplet |
| M206 | Fraction |  | triplet/triplet |
| M207 | Fraction |  | triplet/triplet |
| M208 | Crude |  | triplet/triplet |
| M209 | Crude |  | triplet/triplet |
| M210 | Crude |  | triplet/triplet |
| M211 | Crude |  | triplet/triplet |
| M212 | Crude |  | triplet/triplet  |
| M213 | Crude |  | triplet/triplet |
| M214 | Crude |  | triplet/triplet |
| M215 | Crude |  | triplet/triplet |
| M216 | Crude |  | triplet/triplet |
| M217 | Crude |  | triplet/triplet |
| M218 | Crude |  | triplet/triplet |
| M219 | Crude |  | triplet/triplet  |
| M220 | Crude |  | triplet/triplet |
| M221 | Crude |  | triplet/triplet  |
| M222 | Crude |  | triplet/triplet |
| M223 | Crude |  | triplet/triplet |
| M250 | Fraction |  | singlet |
| M251 | Fraction |  | singlet and triplet/singlet |
| M252 | Fraction |  | singlet |
| M253 | Sub-fractions |  | singlet |
| M254 | Sub-fraction |  | singlet |
| M255 | Sub-fraction |  | singlet and triplet/singlet |
| M256 | Sub-fraction |  | singlet |
| M257 | Sub-fraction |  | singlet |
| M258 | Sub-fraction |  | singlet |
| M259 | Sub-fraction |  | singlet |
| M260 | Sub-fraction |  | singlet |
| M262 | Sub-fraction |  | triplet/singlet |
| M268 | Sub-fraction |  | singlet |
| M269 | Fraction |  | singlet |
| M270 | Fraction |  | singlet |
| M271 | Sub-fraction |  | singlet |
| M272 | Sub-fraction |  | singlet |
| M273 | Sub-fraction |  | singlet |
| M274 | Sub-fraction |  | singlet |
| M275 | Sub-fraction |  | singlet |
| M276 | Sub-fraction |  | singlet |
| M277 | Sub-fraction |  | singlet |
| M278 | Sub-fraction |  | singlet |
| M279 | Sub-fraction |  | singlet |
| M280 | Sub-fraction |  | triplet |
| M281 | Sub-fraction |  | triplet |
| M282 | Sub-fraction |  | triplet and triplet/singlet |
| M283 | Sub-fraction |  | triplet |
| M284 | Sub-fraction |  | triplet  |
| M285 | Sub-fraction |  | triplet |
| M286 | Fraction |  | triplet  |
| M1001 | Pure compound | Salazinic acid  | singlet |
| M1002 | Pure compound | Asperterone | singlet |
| M1003 | Pure compound | Butyrolactone IV | singlet |
| M1004 | Pure compound | 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde | singlet |
| M1005 | Pure compound | Butyrolactone II | singlet |
| M1006 | Pure compound | Terrin | singlet |
| M1007 | Pure compound | Terremide C | singlet |
| M1008 | Pure compound | Butyrolactone V1 | singlet |
| M1009 | Pure compound | 3-isopentyl-4-hydroxyphenylacetic acid methyl ester | singlet |
| M1010 | Pure compound | Butyrolactone III | singlet |
| M1011 | Pure compound | Ergosterol | singlet |
| M1012 | Pure compound | Nocarsin A | singlet |
| M1013 | Pure compound | 4-Hydroxybenzylacetamide | singlet |
| M1014 | Pure compound | L,L-Phenylalanine anhydride | singlet |
| M1015 | Pure compound | rostratin B | singlet |
| M1016 | Pure compound | Epicoccin B | singlet |
| M1017 | Pure compound | rostratin C | singlet |
| M1018 | Pure compound | Emethacin B | singlet |
| M1019 | Pure compound | rostratin D | singlet |
| M1020 | Pure compound | Eutypellazine S | singlet |
| M1021 | Pure compound | Epicoccin A | singlet |
| M1022 | Pure compound | Exserohilone | singlet |
| M1023 | Pure compound | Epicoccin C | singlet |
| M1024 | Pure compound | 7'-Demethoxyrostratin C | singlet |
| M1025 | Pure compound | Epicoccin E | singlet |
| M1026 | Pure compound | Diphenylalazine A | singlet |
| M1027 | Pure compound | Methyl dichloroasterrate | singlet |
| M1028 | Pure compound | epicoccolide B | singlet |
| M1029 | Pure compound | dihydrogeodin | singlet |
| M1030 | Pure compound | Asperchalasine H | singlet |
| M1031 | Pure compound | preechinulin | singlet |
| M1032 | Pure compound | Neoechinulin B | singlet |
| M1033 | Pure compound | Neoechinulin A | singlet |
| M1034 | Pure compound | Flavoglaucin | singlet |
| M1035 | Pure compound | 2-(E-1-heptenyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)benzaldehyde | singlet |
| M1036 | Pure compound | 2-(3E,5E-hepadienyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)benzaldehyde | singlet |
| M1037 | Pure compound | Sulochrin | singlet |
| M1038 | Pure compound | O-deacetyl- tryptoquivaline A | singlet |
| M1039 | Pure compound | Deoxytryptoquivaline | singlet |
| M1040 | Pure compound | Tryptoquivalines X | singlet |
| M1041 | Pure compound | Tryptoquivalines W | singlet |
| M1042 | Pure compound | Epifiscalin E | singlet |
| M1043 | Pure compound | Tryptoquivaline A | singlet |
| M1044 | Pure compound | 6-Deacetyl-1,2-dihydrohelvolic acid | singlet |
| M1045 | Pure compound | Helvolic acid | singlet |
| M1046 | Pure compound | FM701\_90\_MW299 | singlet |
| M1047 | Pure compound | FM701\_90\_MW431 | singlet |
| M1048 | Pure compound | FM701\_90\_MW402 | singlet |
| M1049 | Pure compound | Kaneoheoic acid C | singlet |
| M1050 | Pure compound | Kaneoheoic acid D | singlet |
| M1051 | Pure compound | Kaneoheoic acid B | singlet |
| M1052 | Pure compound | Kaneoheoic acid E | singlet |
| M1053 | Pure compound | Kaneoheoic acid F | singlet |

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| **Table S2.** Samples of high interest (*A. cantonensis* L3 motility reduced to 0-25%), moderate interest (motility reduced to 25-50%), low interest (motility reduced to 50-75) and no interest (motility reduced only to 75-100%). Asterisks indicate samples of top priority interest. [Note that M209 was a triplet/triplet comparison; the value for one triplet fell just into the 50-75% range, while the value for the other was in the 25-50% range. We categorized it as low interest.] |
| High Interest | Moderate Interest | Low Interest | No Interest |
| 0-25% motility13 samples | 25-50% motility11 samples | 50-75% motility24 samples | 75-100% motility60 samples |
| M251\*M252\*M259M260M272\*M273\*M274M275\*M282M283M285M1018M1025 | M223M255M256M262M280M286M1013M1027M1031M1034 M1035 | M200M203M204M205M209M253M254 M269 M277M281M284M1004M1005M1012M1020M1026M1029M1030M1039M1040M1044 M1046M1047M1053 | M201M202M206M207M208M210M211M212M213M214M215M216M217M218M219M220M221M222M250M257M258M268M270M271M276M278M279M1001M1002M1003M1006M1007M1008M1009M1010M1011M1014M1015M1016M1017M1019M1021M1022M1023M1024M1028M1032M1033M1036M1037M1038M1041M1042M1043M1045M1048M1049M1050M1051M1052 |

[M+H]+

**Fig. S1.** LC-MS (low resolution) spectrum of lamellicolic anhydride (M200).

**Fig. S2.** 1HNMR spectrum of lamellicolic anhydride (M200) (DMSO-*d*6, 400 MHz).

**Fig. S3.** 13CNMR spectrum of lamellicolic anhydride (M200) (DMSO-*d*6, 100 MHz).