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% motility  13 samples | 25-50% motility  11 samples | 50-75% motility  24 samples | 75-100% motility  60 samples |
| M251\*  M252\*  M259  M260  M272\*  M273\*  M274  M275\*  M282  M283  M285  M1018  M1025 | M223  M255  M256  M262  M280  M286  M1013  M1027  M1031  M1034  M1035 | M200  M203  M204  M205  M209  M253  M254  M269  M277  M281  M284  M1004  M1005  M1012  M1020  M1026  M1029  M1030  M1039  M1040  M1044  M1046  M1047  M1053 | M201  M202  M206  M207  M208  M210  M211  M212  M213  M214  M215  M216  M217  M218  M219  M220  M221  M222  M250  M257  M258  M268  M270  M271  M276  M278  M279  M1001  M1002  M1003  M1006  M1007  M1008  M1009  M1010  M1011  M1014  M1015  M1016  M1017  M1019  M1021  M1022  M1023  M1024  M1028  M1032  M1033  M1036  M1037  M1038  M1041  M1042  M1043  M1045  M1048  M1049  M1050  M1051  M1052 |



[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).