

Deciphering chemical rules for drug penetration into *Strongyloides*

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SUPPLEMENTARY MATERIAL

Figure S1. Chemical structures of the dyes. Positively and negatively charged groups have been highlighted with pale blue and orange circles, respectively.

*Figure S2: Healthy parthenogenetic adult females of *Strongyloides venezuelensis* (A) and treated with 10 μ M albendazole for 48 hours.*

Table S1. Normalized numbers of parthenogenetic females and L3s in the in vivo experiments. EPGF = Eggs per gram in feces.

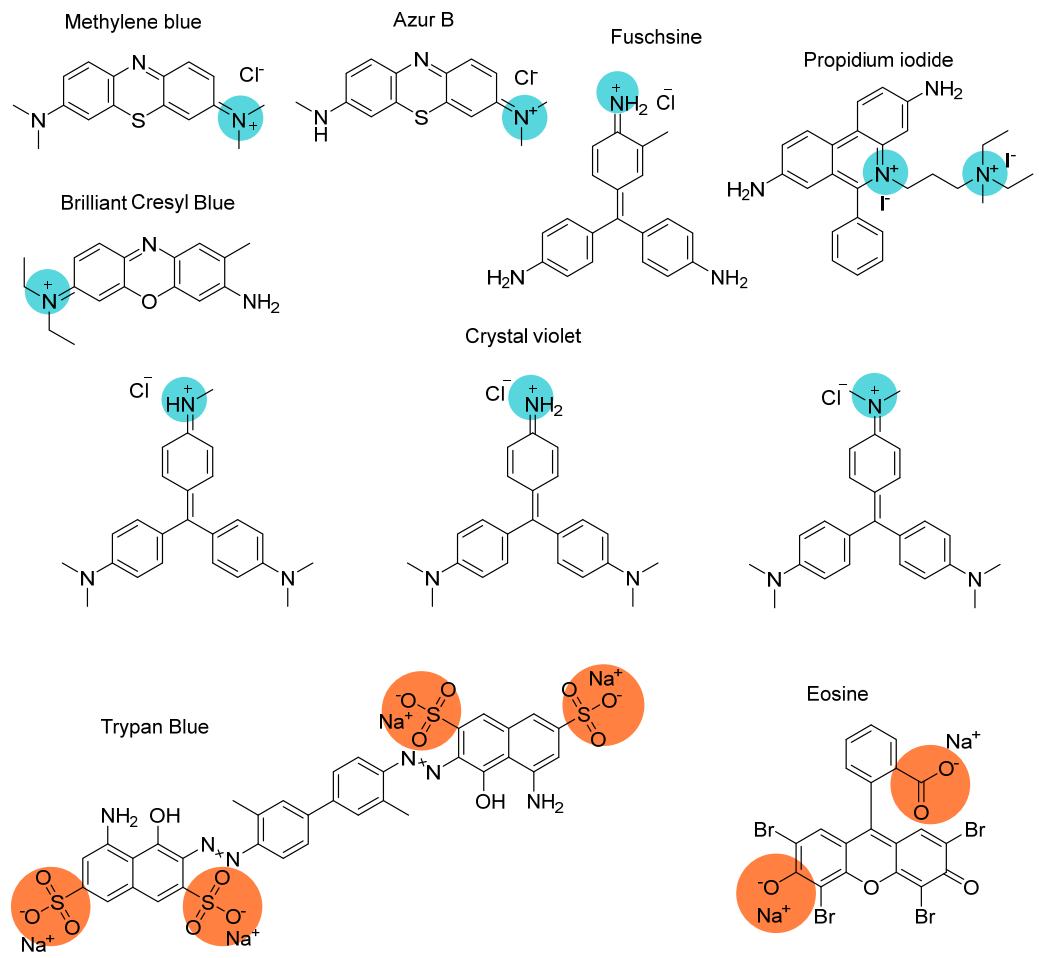
Table S2. Properties calculated with SwissADME. The meaning of the parameters can be found at <http://www.swissadme.ch/index.php>

Table S3. Properties calculated with the entry rules page for the compounds (<http://www.entry-way.org/pages/about>). Active compounds against L3s are indicated in yellow and a plus sign, and those against adult females with a cream plus. Rb = rotatable bonds. Glob = globularity. Pbf = average distance to the plane of best fit. Func_group = functional groups. Charge = total charge. Cells are colored by values to ease the comparisons.

Table S4. Activities against different population densities (1-3 adults, 10-15 adults and 50-70 adults) at 72 hours with 10 μ M treatments.

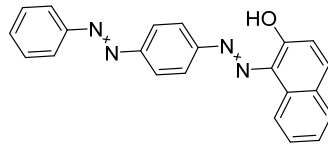
Video S1. Adult female treated with fluorescein acetate under clear field and fluorescence microscope.

17 *Figure S1.*

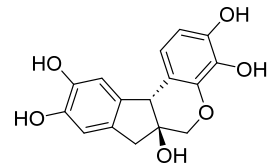


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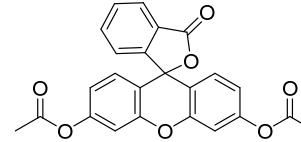
Sudan III



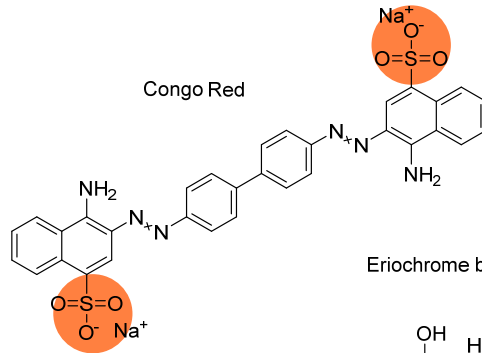
Haematoxylin



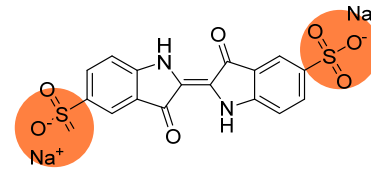
Fluorescein diacetate



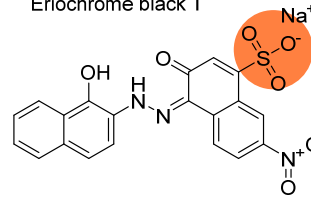
Congo Red



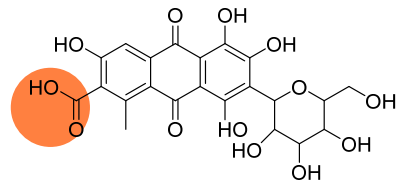
Indigo carmine



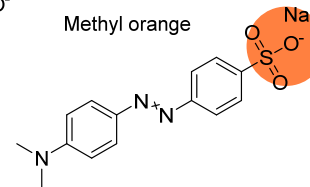
Eriochrome black T



Laca carmine



Methyl orange



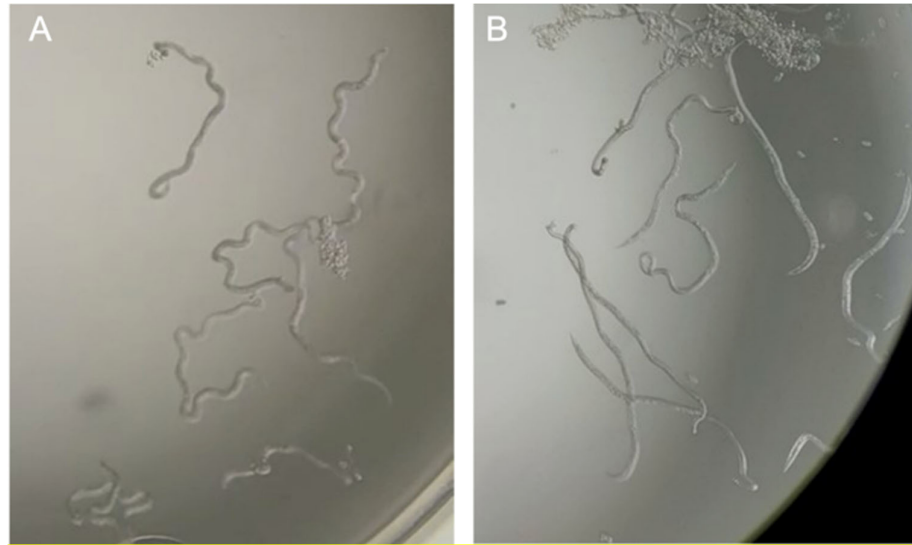


Figure S2: Healthy parthenogenetic adult females of *Strongyloides venezuelensis* (A) and treated with 10 μ M albendazole for 48 hours (B). The serpent shape is lost to a more elongated one. The same shape is observed for adults treated with mebendazole (10 μ M) (data not shown).

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26 *Table S1*

Group		Mean Female	EPGF day 4	EPGF day 5	EPGF day 6	EPGF day 7	EPGF day 8
Infection	G1	340	0	23141	137471	71954	40190
Ivermectin	G2	29	160	20534	9559	8544	3290
MB Gavage	G3	318	0	17652	91673	104635	33039
MB Water	G4	388	0	19131	80617	74079	26410
CV Water	G5	182	0	6861	116364	83275	7189

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30 *Table S2 Properties calculated with SwissADME*

Molecule	Canonical SM Formula	MW	#Heavy atom	aromatic heavy	fraction csp ²	rotatable bon	bond accept	#H-bond don	MR	TPSA	iLOGP	XLOGP3	WLOGP	MLOGP
Methylene Blue	CN(c1ccc2c(c(c16H18ClN3S	319,85	21	14	0,25	1	1	0	93,3	47,38	-4,21	3,04	-0,5	2,5
Brilliant Cresyl Blue	CC[N+](=c1cc17H20N3O+	282,36	21	14	0,29	2	2	1	86,61	55,06	-0,87	1,86	2,64	1,65
Crystal Violet	C[N+](=C1C= C25H30ClN3	407,98	29	12	0,24	4	0	0	128,45	9,49	-1,03	0,74	1,46	4,1
Fuschine	Nc1ccc(cc1)C C20H20ClN3	337,85	24	12	0,05	2	0	3	105,72	77,63	0	4,49	-0,61	3,04
Propidium iodide	CC[N+](CCC[C27H34I2N4	668,39	33	20	0,3	7	0	2	163,35	55,92	-5,5	6,67	-0,99	0,73
Trypan Blue	Cc1cc(ccc1N= C34H24N6Na	960,81	62	32	0,06	9	18	4	202,94	404,26	-56,22	4,35	9,64	2,72
Eosine	[O-]C(=O)c1c C20H6Br4Na	691,85	31	20	0	2	5	0	120,49	93,4	-23,51	5,63	6,12	4,12
Sudan III	Oc1ccc2c(c1) C22H16N4O	352,39	27	22	0	4	5	1	107,21	69,67	4,02	5,91	7,38	4,15
Haematoxylin	Oc1cc2C[C@ C16H14O6	302,28	22	12	0,25	0	6	5	77,22	110,38	1,69	1,19	1,32	0,49
Fluorescein diacetate	CC(=O)Oc1cc C24H16O7	416,38	31	18	0,12	4	7	0	107,7	88,13	3,28	3,64	4	3,49
Congo Red	Nc1c(N=Nc2c C32H22N6Na	696,66	48	32	0	7	10	2	172,95	232,64	-24,06	5,73	9,64	4,12
Indigo Carmine	O=C1/C(=C/2 C16H8N2Na2	466,35	30	12	0	2	8	2	97,34	189,36	-18,14	1,22	1,64	-0,18
Methyl Orange	CN(c1ccc(cc1 C14H14N3Na	327,33	22	12	0,14	4	5	0	79,27	93,54	-9,96	3,33	4,15	2,31
Laca Carmine	OCC1OC(C(C C22H20O13	492,39	35	12	0,32	3	13	9	112,39	242,51	-1,31	0,53	-1,52	-3,21
Ivermectin a	COC1CC(OC2 C48H74O14	875,09	62	0	0,81	8	14	3	230,77	170,06	5,93	6,34	5,6	1,25
Ivermectin b	COC1CC(OC(C47H72O14	861,07	61	0	0,81	7	14	3	225,96	170,06	6,62	5,99	5,21	1,09
Edelfosine	CCCCCCCCC C27H58NO6P	523,73	35	0	1	27	6	0	146,91	86,86	1,76	7,64	7,56	-0,43
Albendazole	CCCScc1ccc2c C12H15N3O2	265,33	18	9	0,33	6	3	2	73,22	92,31	1,69	2,81	3,05	2,03
Mebendazole	COC(=O)Nc1 C16H13N3O3	295,29	22	15	0,06	5	4	2	81,75	84,08	1,47	3,01	2,78	1,68
Compound 20	Cc1ccc2c(c1) C14H11N3OS	269,32	19	15	0,07	3	3	1	76,6	83,12	1,92	2,84	3,06	1,94
Compound 22	CN1CCN(CC1 C19H21N5OS	367,47	26	15	0,32	4	4	1	112,86	89,6	2,79	3,11	2,05	2,19

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Molecule	Silicos-IT Log	Consensus L	ESOL Log S	ESOL Solubil	ESOL Solubil	ESOL Class	Ali Log S	Ali Solubility	Ali Solubility	Ali Class	Silicos-IT Log	Silicos-IT Sol	Silicos-IT Sol	Silicos-IT clas
Methylene Blue	3,24	0,81	-4,17	2,18E-02	6,83E-05	Moderately s	-3,7	6,37E-02	1,99E-04	Soluble	-6	3,21E-04	1,00E-06	Moderately s
Brilliant Cresyl Blue	2,87	1,63	-3,12	2,12E-01	7,52E-04	Soluble	-2,64	6,50E-01	2,30E-03	Soluble	-6,67	6,02E-05	2,13E-07	Poorly solub
Crystal Violet	3,11	1,68	-2,88	5,40E-01	1,32E-03	Soluble	-0,52	1,24E+02	3,03E-01	Very soluble	-6,65	9,09E-05	2,23E-07	Poorly solub
Fuschine	3,41	2,07	-5	3,37E-03	9,97E-06	Moderately s	-5,84	4,88E-04	1,44E-06	Moderately s	-5,58	8,78E-04	2,60E-06	Moderately s
Propidium iodide	1,99	0,58	-8,17	4,49E-06	6,72E-09	Poorly solub	-7,65	1,51E-05	2,25E-08	Poorly solub	-9,5	2,10E-07	3,14E-10	Poorly solub
Trypan Blue	1,28	-7,65	-8,33	4,54E-06	4,73E-09	Poorly solub	-12,55	2,68E-10	2,79E-13	Insoluble	-9,08	7,97E-07	8,30E-10	Poorly solub
Eosine	6,63	-0,2	-8,02	6,58E-06	9,51E-09	Poorly solub	-7,35	3,06E-05	4,42E-08	Poorly solub	-9,64	1,57E-07	2,28E-10	Poorly solub
Sudan III	5,95	5,48	-6,09	2,88E-04	8,18E-07	Poorly solub	-7,15	2,51E-05	7,13E-08	Poorly solub	-8,68	7,29E-07	2,07E-09	Poorly solub
Haematoxylin	1,37	1,21	-2,87	4,10E-01	1,36E-03	Soluble	-3,1	2,38E-01	7,87E-04	Soluble	-2,63	7,10E-01	2,35E-03	Soluble
Fluorescein diacetate	4,29	3,74	-4,88	5,48E-03	1,32E-05	Moderately s	-5,18	2,76E-03	6,62E-06	Moderately s	-7,39	1,68E-05	4,04E-08	Poorly solub
Congo Red	4,29	-0,05	-7,8	1,10E-05	1,58E-08	Poorly solub	-10,38	2,89E-08	4,14E-11	Insoluble	-11,1	5,52E-09	7,93E-12	Insoluble
Indigo Carmine	-0,5	-3,19	-3,66	1,01E-01	2,17E-04	Soluble	-4,79	7,50E-03	1,61E-05	Moderately s	-4,47	1,58E-02	3,38E-05	Moderately s
Methyl Orange	1,63	0,29	-4,11	2,56E-02	7,82E-05	Moderately s	-4,97	3,50E-03	1,07E-05	Moderately s	-4,46	1,14E-02	3,48E-05	Moderately s
Laca Carmine	-0,55	-1,21	-3,28	2,57E-01	5,22E-04	Soluble	-5,19	3,15E-03	6,40E-06	Moderately s	-0,62	1,19E+02	2,42E-01	Soluble
Ivermectin a	2,72	4,37	-8,73	1,62E-06	1,85E-09	Poorly solub	-9,7	1,74E-07	1,99E-10	Poorly solub	-3,89	1,13E-01	1,29E-04	Soluble
Ivermectin b	2,3	4,24	-8,49	2,78E-06	3,23E-09	Poorly solub	-9,34	3,95E-07	4,59E-10	Poorly solub	-3,51	2,67E-01	3,10E-04	Soluble
Edelfosine	4,63	4,23	-6,12	3,99E-04	7,62E-07	Poorly solub	-9,3	2,61E-07	4,97E-10	Poorly solub	-8,62	1,26E-06	2,41E-09	Poorly solub
Albendazole	2,35	2,39	-3,23	1,56E-01	5,90E-04	Soluble	-4,41	1,04E-02	3,93E-05	Moderately s	-4,35	1,19E-02	4,48E-05	Moderately s
Mebendazole	2,55	2,3	-3,74	5,35E-02	1,81E-04	Soluble	-4,44	1,07E-02	3,63E-05	Moderately s	-5,5	9,42E-04	3,19E-06	Moderately s
Compound 20	3,68	2,69	-3,69	5,56E-02	2,06E-04	Soluble	-4,24	1,54E-02	5,70E-05	Moderately s	-5,5	8,46E-04	3,14E-06	Moderately s
Compound 22	3,2	2,67	-4,24	2,11E-02	5,75E-05	Moderately s	-4,66	8,04E-03	2,19E-05	Moderately s	-5,62	8,84E-04	2,40E-06	Moderately s

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Molecule	log Kp (cm/s)	BB permeant	gp substrate	P1A2 inhibit	P2C19 inhibit	P2C9 inhibit	P2D6 inhibit	P3A4 inhibit	inski	#violatio	ose	#violatio	ber	#violatio	gan	#violatio	iegge	#violatio	availability	Sc ²⁺	AINS	#alerts	Brenk	#alerts	keness	#violatio	netic	Accessit
Methylene Blue	-6,09	No	Yes	No	No	No	No	No	No	0	1	0	0	0	0	0,55	0	2	0								3,44	
Brilliant Cresyl Blue	-6,7	Yes	Yes	Yes	No	No	Yes	Yes	0	0	0	0	0	0	0	0,55	0	3	0								3,52	
Crystal Violet	-8,26	Yes	Yes	No	No	No	No	No	0	0	0	0	0	0	0	0,55	1	2	1								4,11	
Fuschine	-5,17	No	No	Yes	No	No	Yes	No	0	1	0	0	0	0	0	0,55	0	2	1								3,47	
Propidium iodide	-5,64	No	Yes	Yes	No	No	No	No	1	3	0	0	0	2	0	0,55	0	5	2								3,39	
Trypan Blue	-9,07	No	Yes	No	No	No	No	No	2	4	1	2	3	0	0,17	1	3	3									5,19	
Eosine	-6,52	No	Yes	No	No	No	No	No	1	2	0	1	2	0	0,55	0	1	2									3,55	
Sudan III	-4,25	No	Yes	No	Yes	No	No	No	0	1	0	1	1	1	0,55	1	1	2									3,04	
Haematoxylin	-7,3	No	Yes	Yes	No	No	No	No	0	0	0	0	0	0	0,55	1	1	0									3,74	
Fluorescein diacetate	-6,26	No	No	Yes	Yes	Yes	No	Yes	0	0	0	0	0	0	0,55	0	1	2									3,81	
Congo Red	-6,48	No	Yes	No	No	No	No	No	2	3	1	2	3	0	0,17	1	3	2									4,22	
Indigo Carmine	-8,28	No	Yes	No	No	No	No	No	0	0	1	1	1	1	0,55	1	2	1									3,02	
Methyl Orange	-5,93	No	Yes	No	No	No	No	No	0	0	0	0	0	0	0,55	2	2	0									2,95	
Laca Carmine	-8,93	No	No	No	No	No	No	No	2	2	1	1	3	0	0,11	2	2	1									5,04	
Ivermectin a	-7,14	No	Yes	No	No	No	No	No	2	4	1	1	4	0	0,17	0	1	3									10	
Ivermectin b	-7,3	No	Yes	No	No	No	No	No	2	3	1	1	4	0	0,17	0	1	2									10	
Edelfosine	-4,07	No	Yes	No	No	No	No	Yes	1	4	1	1	2	0	0,55	0	2	3									6,43	
Albendazole	-5,92	No	No	Yes	No	No	No	No	0	0	0	0	0	0	0,55	0	0	0									2,41	
Mebendazole	-5,96	No	No	Yes	No	No	No	No	0	0	0	0	0	0	0,55	0	0	0									2,26	
Compound 20	-5,93	No	No	Yes	Yes	Yes	No	Yes	0	0	0	0	0	0	0,55	0	0	0									2,43	
Compound 22	-6,33	No	Yes	Yes	No	Yes	Yes	Yes	0	0	0	0	0	0	0,55	0	0	1									3,09	

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49 Table S3.

name	L3	Ad	formula	molwt	rb	glob	pbf	func_group	Charge
Methylene Blue	+	+	C16H18ClN3S	320	1	0,01	0,27	No Amine	1
Brilliant Cresyl Blue	+	+	C17H20N3O+	282	2	0,03	0,56	No Amine	1
Crystal Violet	+		C25H30ClN3	408	4	0,12	0,94	No Amine	1
Fuchsin	+		C20H20ClN3	338	2	0,13	0,73	No Amine	1
Propidium iodide	+		C27H34I2N4	668	7	0,11	0,95	No Amine	2
Trypan Blue	+		err	err	err	err	err	err	1
Eosine			C20H6Br4Na2O5	692	2	0,18	0,66	No Amine	-4
Sudan III			C22H16N4O	352	4	0,13	1,17	No Amine	-2
Haematoxylin			C16H14O6	302	0	0,17	0,98	No Amine	0
Fluorescein diacetate			C24H16O7	416	4	0,12	1,03	No Amine	0
Congo Red			C32H22N6Na2O6S2	697	7	0,01	0,67	No Amine	-2
Indigo Carmine			C16H8N2Na2O8S2	466	2	0,01	0,20	No Amine	-1
Methyl Orange			C14H14N3NaO3S	327	4	0,03	0,63	No Amine	-1
Carmine			C22H20O13	492	3	0,05	0,70	No Amine	-1
Ivermectin a			C48H74O14	875	8	0,11	1,54	No Amine	0
Ivermectin b			C47H72O14	861	7	0,08	1,40	No Amine	0
Edelfosine			C27H58NO6P	524	27	0,03	1,06	No Amine	0
Albendazole			C12H15N3O2S	265	5	0,07	0,74	No Amine	0
Mebendazole			C16H13N3O3	295	4	0,03	0,65	No Amine	0
Compound 20			C14H11N3OS	269	2	0,01	0,35	No Amine	0
Compound 22	+	+	C19H21N5OS	367	3	0,01	0,47	Tertiary Amine	1

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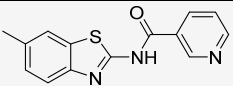
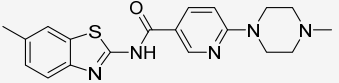
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Table S4. Activities against different population densities (1-3 adults, 10-15 adults and 50-70 adults) at 72 hours with 10 μ M treatments. Culture control was 1% DMSO as basal. Healthy: Normal and vivid movement and shape, no differences against control; Slightly affected: slower movement or shape different from the control; Affected: abnormal movements and shape clearly different from the control and clearly incompatible with normal viability, no deaths; Dead: immobile larvae. Increase in potencies can be observed when decreasing the number of adults per well

Number	Compound	Activity against adults 10 μ M 72h 1-3 adults	Activity against adults 10 μ M 72h 10-15 adults	Activity against adults 10 μ M 72h 50-70 adults
1	Methylene blue	Dead	Dead	Dead
2	Brilliant cresyl blue	Dead	Affected / Some deaths	Affected
3	Crystal violet	Slightly affected	Slightly affected	Slightly affected
4	Fuch sine	Healthy	Healthy	Healthy
5	Propidium iodide	Affected / Some deaths	Affected / Some deaths	Affected / Some deaths
16	Ivermectin	Affected	Affected	Slightly affected
18	Albendazole	Affected	Slightly affected	Slightly affected
19	Mebendazole	Affected	Slightly affected	Slightly affected
20		Healthy	Healthy	Healthy
22		Affected	Affected	Slightly affected

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62 *Video S1*



63 Video 1S.mp4

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