

Supplementary material

Review

Promising Drug Repurposing Candidates Targeting Free-Living Amoebae: A Systematic and Critical Review of Laboratory-Based Evidence

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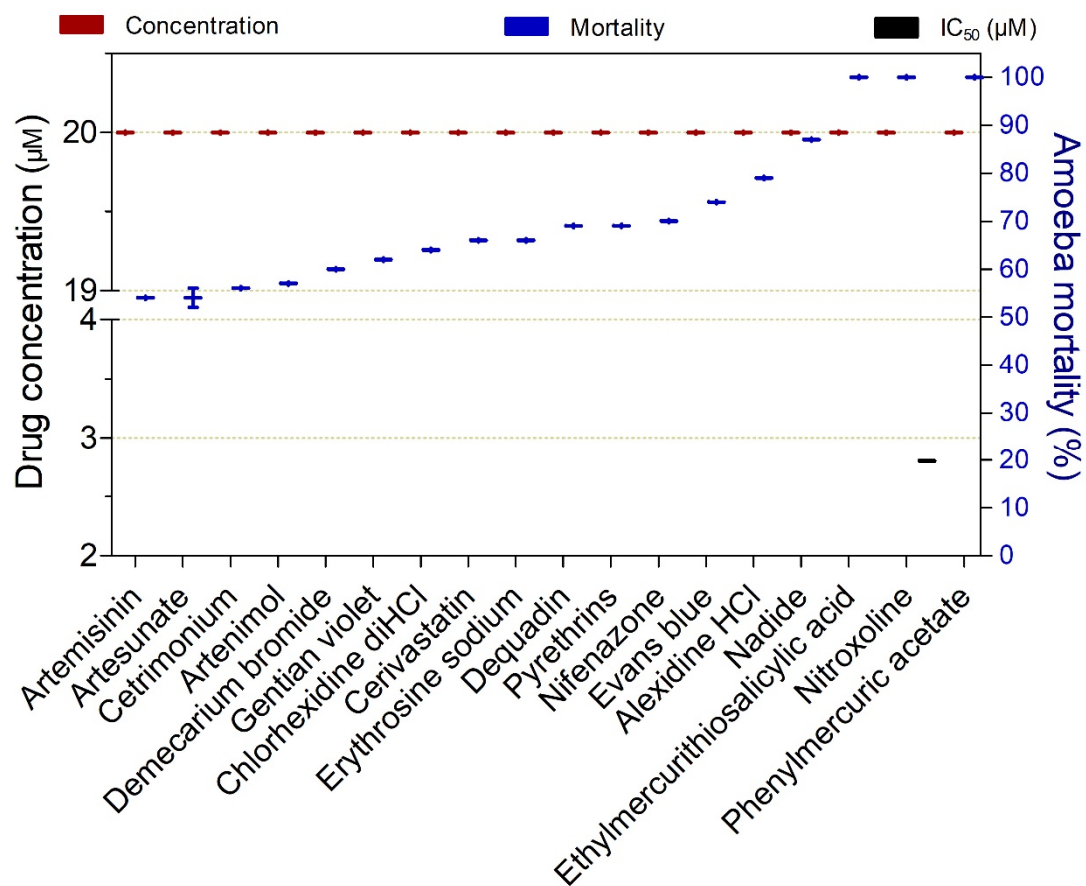


Figure S1. Drugs exhibiting $\geq 50\%$ trophocidal activity against *Balamuthia mandrillaris* at average concentrations of 20 μM (72 h exposure time).

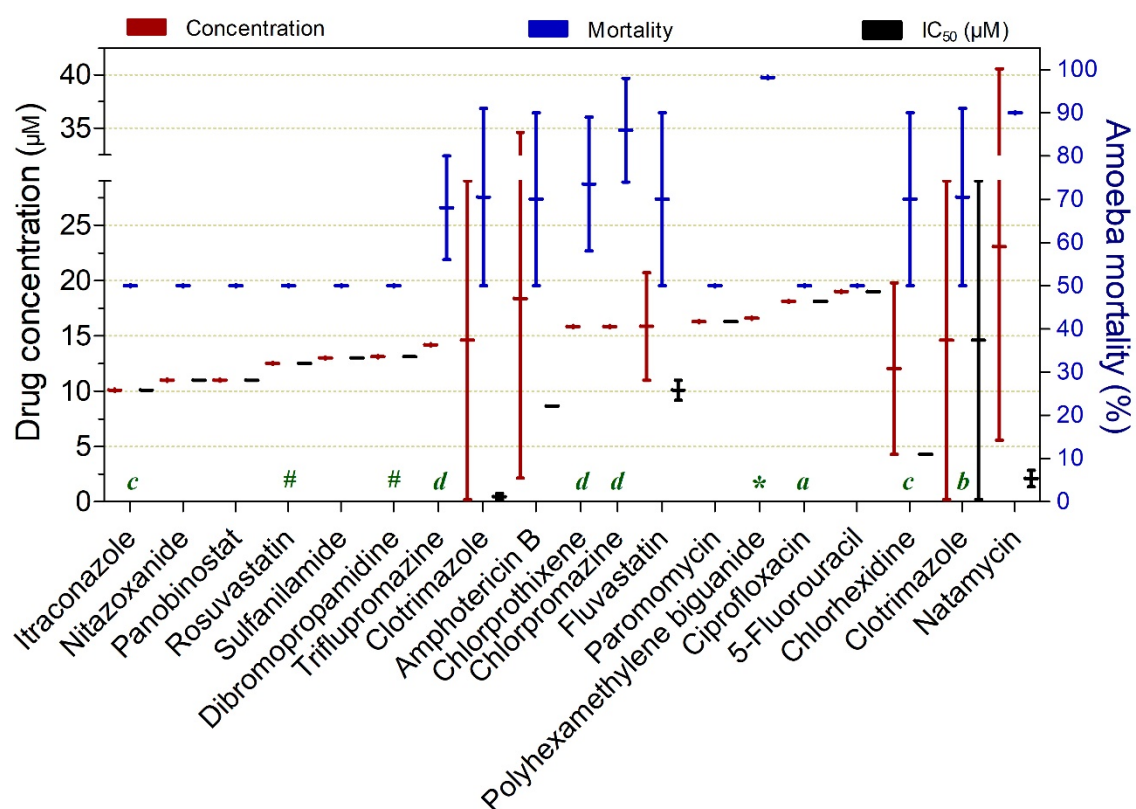


Figure S2. Drugs exhibiting $\geq 50\%$ trophocidal activity against *Acanthamoeba* spp. at mean concentrations ranging from 10.1 to 23.06 μM . Exposure time of 72 h, except (*) – 4 h, (**) – 12 h, (#) – 48 h, (a) – 24 h, (b) – 60 h, (c) – 96 h, (d) – 120 h.

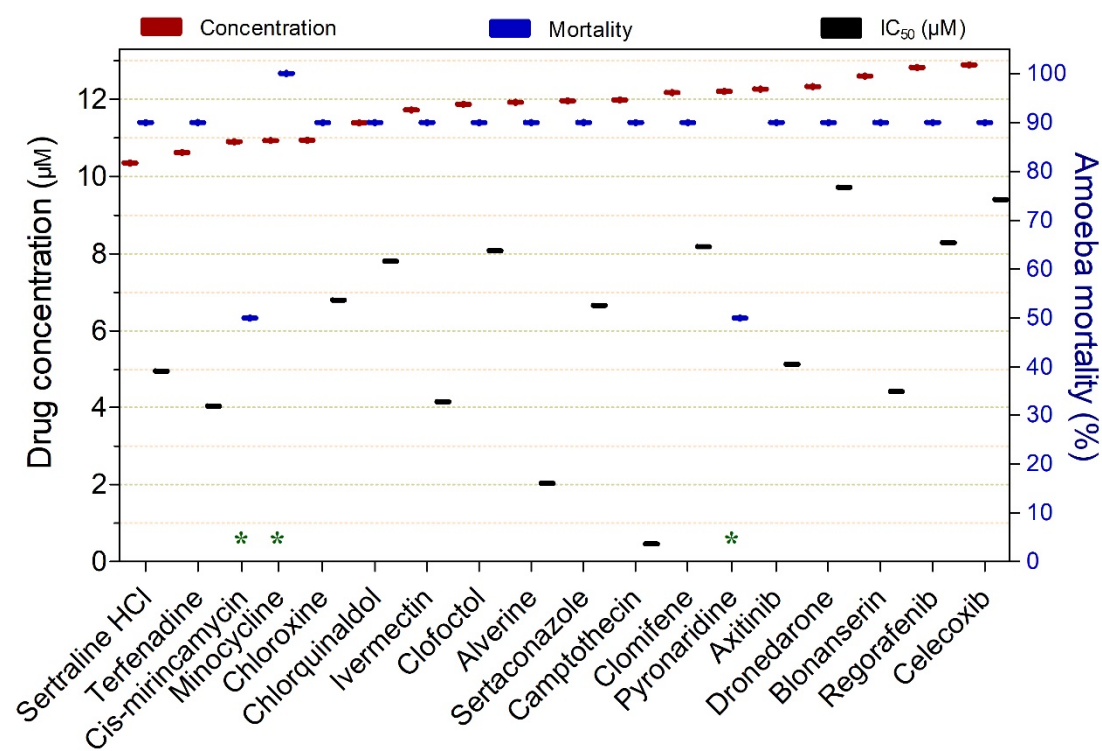


Figure S3. Drugs exhibiting $\geq 50\%$ trophocidal activity against *Naegleria* spp. at mean concentrations ranging from 10 to 12.89 μM and an exposure time of 120 h, except (*) – 72 h.

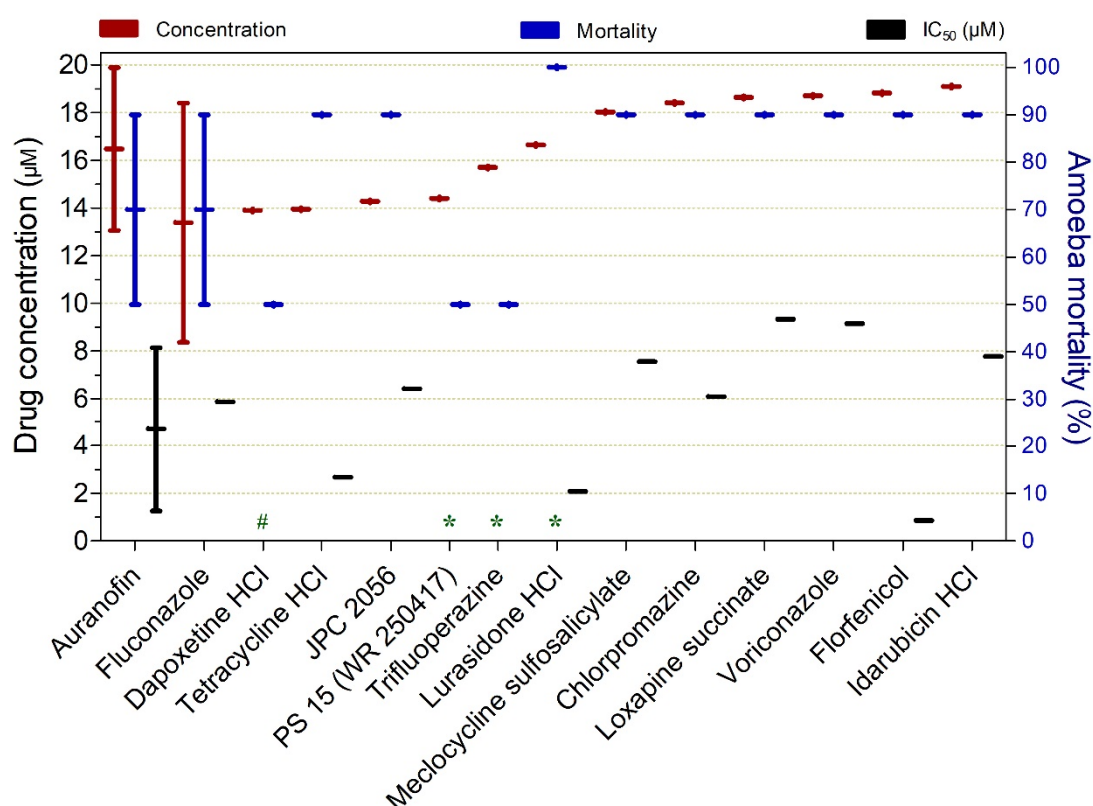


Figure S4. Drugs that exert a trophocidal effect $\geq 50\%$ against *Naegleria* spp. at average concentrations ranging from 13 to 19.09 μM and exposure time of 120 hours, except (#) – 48 h, (*) – 72 h.

Table S1 Comparative performance of drugs tested in rat models of Granulomatous Amebic Encephalitis caused by *Acanthamoeba* spp. All compounds were administered via intraperitoneally, except those indicated by (*) administered through a subcutaneous injection

Drug	Studies (n)	Dose (mg/kg)	Treatment time (days)	Start of treatment dpi	Cure rate (%)
Rifampicin *	1	100	2	2 **	100
Sulphadiazine	1	200	10	1	100
Rifampicin *	1	100	5	2	88
5-Fluorocytosine *	1	6	21 (2x/day)	1 **	0
Sulphamethizole	1	200	10	1	0
Sulphmethoxazole	1	200	10	1	0
Trimethoprim	1	40	10	1	0

(dpi) – days post-infection. (**) – Before infection (prophylactic treatment).

Table S2. Therapeutic efficacy of drugs against *Acanthamoeba* keratitis in animal models.

Drug	n	Model	Administrative route	Dose (mg/kg)	Treatment time (days)	Start of treatment dpi	Cure rate (%)	Toxicity (%)
Aprotinin + Neomycin (0.283mg/mL) + Atropine ointment (10)	1	Mouse	Eye drops	13	17 (5x/day)	2	100	-
Chlorhexidine + neomycin sulfate + polymyxin B sulfate + gramicidin	1	Mouse	Eye drops	0.02 + 4.486 + 0.5 + 0.025	7 (8x/day) + 21 (3x/day)	5	70.5 ± 4.5	-
Chlorhexidine gluconate	1	Mouse	Eye drops	0.2	28 (8x/day/1st wk → 3x/day/2 wks)	5	60.09	78
Dexamethasone sodium phosphate	1	Rabbit	Intracorneal injection	4	8	0	0	-
Fluconazole + Neomycin (0.283) + Atropine ointment (10)	1	Mouse	Eye drops	2	22 (5x/day)	2	100	-
Miltefosine	2	Hamsters/ Mouse	Eye drops	0.06512	29 ± 1 (8x/day/1st wk → 3x/day/3 wks)	5	72.5 ± 12.5	2
Miltefosine + Propamidine isethionate	1	Mouse	Eye drops	0.06512 + 0.2	28 (8x/day/1st wk → 3x/day/2 wks)	5	70.04	79
Miltefosine + Chlorhexidine	1	Mouse	Eye drops	0.06512 + 0.2	28 (8x/day/1st wk → 3x/day/2 wks)	5	70.04	77
Miltefosine + Polyhexanide	1	Mouse	Eye drops	0.06512 + 0.2	28 (8x/day/1st wk → 3x/day/2 wks)	5	80.12	69
Neomycin (0.283) + Atropine ointment (10)	1	Mouse	Eye drops	13	28 (5x/day)	2	100	-
Polyhexamethylene biguanide + Neomycin (0.283) + Atropine ointment (10)	1	Mouse	Eye drops	0.2	13 (5x/day)	2	100	-
Povidone iodine + Neomycin (0.283) + Atropine ointment (10)	1	Mouse	Eye drops	50	23 (5x/day)	2	100	
Polyhexanide	1	Mouse	Eye drops	0.2	28 (8x/day/1st wk → 3x/day/2 wks)	5	70.04	65
Propamidine isethionate	1	Mouse	Eye drops	1.0	28 (8x/day/1st wk → 3x/day/2 wks)	5	60.09	69
Voriconazol	1	Rats	Eye drops	10	21 (13x/day/3days; 7x/day/11 days and 4x/day/7days)	7	88.9	-
Voriconazol	1	Rats	Gavage	60 mg/kg	21 (2x/day)	7	33.3	-
Riboflavin + Ultraviolet A	1	Mouse	Eye drops	0.1% riboflavina + UVA, 3 mW/cm ² (dose of 5.4 J/cm ²)	3 (5 – 30 min before UV A, → 5 min in UV A)	3	0	-
Polyhexamethylene biguanide	1	Mouse *	Eye injection	0.2	1	-	-	100
Polyhexamethylene biguanide	1	Mouse *	Eye injection	0.1	1	-	-	0
Propamidine isethionate	1	Mouse *	Eye	1	1	-	-	100

			injection					
Propamidine isethionate	1	Mouse *	Eye injection	0.5	1	-	-	0

(dpi) – days post-infection. (*) – Uninfected animals.

Table S3. Drug efficacy in mouse models of primary amoebic meningoencephalitis caused by *Naegleria fowleri*. All drugs were administered intraperitoneally.

Drug	Studies (n)	Dose (mg/mL)	Treatment time (days)	Start of treatment dpi	Cure rate (%)	Toxicity
Amphotericin B + azithromycin	1	2.5 + 25	5	3	100	-
Cyclophosphamide	1	30	10	0	92	-
Amphotericin B + tetracycline	1	2.5 + 150	7	3	87.5	-
Rokitamycin	1	20	3 (3x/day)*	3	80	No
Chlorpromazine	1	20	3*	3	75	-
Azithromycin + posaconazole	1	25 + 20	3	3	70	-
Amphotericin B	1	75	14	1#	60	-
Azithromycin	1	25	5	3	55	-
Miltefosine	1	20	3*	3	55	-
Amphotericin B	1	10	8	3	40	-
Amphotericin B + rifamycin	1	2.5 + 150	10	2	40	-
Azithromycin	2	25	4 ± 1	3	35 ± 5	-
Posaconazole	1	20	3	3	33	-
Azithromycin + fluconazole	1	25 + 30	3	3	30	-
Azithromycin + ketoconazole	1	25 + 25	3	3	30	-
Clotrimazole	1	100	5	1	30	-
Fluconazole	1	30	3	3	30	-
Amphotericin B	5	2.5	7 ± 3	3 ± 0.5	30 ± 15	-
AN3057	1	50	10 (3x/day)	1	28	No
Roxithromycin	1	20	3 (3x/day)*	3	25	-
Ketoconazole	1	25	3	3	10	-
Hygromycin B	1	20	3 (3x/day)*	3	0	-
Metronidazole	1	1**	10	1#	0	-
Miltefosine	1	20	3	3	0	-
Pyrimethamine	1	20	10	2	0	Yes
Rifamycin	1	150	10	2	0	-

(AN3057) – 4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yloxy)phenyl)methanaminium chloride. (dpi) – days post-infection. (**) – g/kg. (*) – Animals were treated on days 3, 7 and 11 after infection. (#) – Before infection (prophylactic treatment).