Validation of IMP dehydrogenase inhibitors in a mouse model of cryptosporidiosis

Running Title: *In vivo* antiparasitic activity of *Cp*IMPDH inhibitors

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Supporting Material

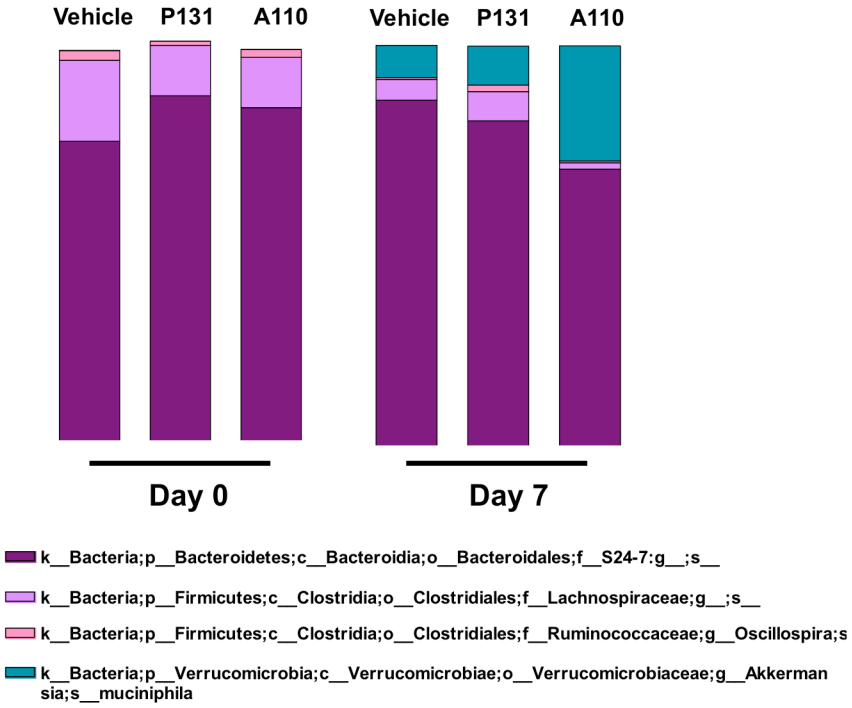

Figure S1. Effects of A110 and P131 at species level. **A110** and **P131** on fecal microbiota at the species level. The cumulative relative distribution of species in different treatments is listed.

Table S1. In vivo activity of CpIMPDH inhibitors.

Infection protocol A: IL-12 knockout mice were infected with 1000 oocysts on day 1. Mice were treated daily by oral gavage beginning 4 hours after infection. Feces were collected and counted on day 7 unless otherwise noted. Vehicle = 5% DMSO/corn oil unless otherwise noted.

Infection protocol B: IL-12 knockout mice were infected with 10,000 oocysts on day 1. Mice were treated three times daily by oral gavage beginning 4 hours after infection. Feces were collected and counted on day 4.
Vehicle = 5% DMSO/corn oil unless otherwise notec
p values calculated by Mann Whitney nonparametric test using Prizm software

Panel 2A		Oocysts per 100 ul in each mouse					
Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
A110	250	1402	50	10722	7	A	vehicle = 20% PEG
		832	177	3647			
		2015	2029	17048			
		686	1545	5395			
		715	1663	22314			
		1948	1845	8806			
		14582	1122	10519			
		7654	1450	7893			
		2866	1094	7387			
		12528		3118			
		p =	**0.18**	**0.023**			

Panel 2B		Oocysts per 100 ul in each mouse					
Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
A110	100	551	488	588	7	A	
		2227	87	5723			
		1834	510	4012			
		342		7388			
		582		1737			
		10044		165			
		508		2503			
		1088		5067			
		p =	**0.085**	**0.23**			
P75	250	as above	as above	791			
				1027			
				1040			
				1141			
				1956			
				1752			
				2072			
				1965			
		p =		**0.43**			
P83	250	as above	as above	999			
				998			
				1504			
				1846			
				859			
				2915			
				1809			
				1346			
		p =		**0.43**			

Panel 2C			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P25	250	1486	0	1875	6	A	Vehicle = 10% PEG
			2245	0	385			
			19553	428	1334			
			1802	0	9673			
			23201	150	427			
			10184	0	14584			
			1970	0	9441			
			5020	0	12149			
			19076	0	36201			
					1814			
			p =	**<0.0001**	**0.44**			
	P32	250	as above	as above	9374	6		Vehicle = 10% PEG
					15620			
					6757			
					31614			
					17015			
					16684			
					17552			
					40947			
					11170			
					13859			
			p =		**0.13**			

Panel 2D			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P82	250	5039	142	1897	7	A	
			10474	179	4255			
			6889	176	2230			
			4406		2537			
			1546		2068			
			1696		1356			
			5476		1615			
			4923					
			p =	**0.012**	**0.054**			
				vs Prm	**0.017**			
	P96	250	as above	as above	879			
					408			
					1454			
					1946			
					884			
					1174			
					2242			
					1823			
			p =		**0.0047**			significantly less than vehicle
				vs Prm	**0.012**			signficantly more than Prm

Panel 2E			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P96	250	993	420	1089	7	A	
			1991	128	3639			
			21366	84	929			
			1775	43	2443			
			4716	190	1424			
			2823	35	1203			
			2294		1172			
			1462		780			
			1076		294			
			2305		624			
			p =	**0.0002**	**0.043**			significantly less than vehicle
				vs Prm	**0.0005**			signficantly more than Prm
	P97	250	as above	as above	3600			
					2151			
					636			
					295			
					2591			
					663			
					4492			
					1225			
					1069			
					1679			
			p =		**0.24**			

Panel 2F	Compound	Dose (mg/kg)	Oocysts per 100 ul in each mouse Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P96	250	1255	52	1067	7	A	
			4139	98	206			
			3072	200	4884			
			967	63	210			
			7922	86	736			
			9041		11461			
			567		6054			
					1782			
					297			
					3690			
			p =	**0.0025**	**0.47**			
	A119	250			2122			
					3191			Several mice lost weight on day 4
					3206			and looked ruffled day 7.
					5107			These animals were not sampled.
					18006			
					15259			
					2887			
			p =		**0.31**			

Panel 2G			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P131	250	1778	67	1466	7	A	
			1190	328	575			
			1807	596	373			
			5568	580	517			
			3048		16990			
			2360		1060			
			2203		1776			
			2948		411			
			2672		1346			
			1927		88			
			p =	**0.002**	**0.0039**			significantly less than vehicle
				vs Prm	**0.22**			no signficant difference with Prm

Panel 5A			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P131	3 x 83	360	123	47	4	B	
			737	22	0			
			3288	22	15			
			143	90	24			
			459	132	0			
			239	33	0			
			269		14			
			230		63			
			194		23			
			61					
			p =	**0.0017**	**<0.0001**			significantly less than vehicle
				vs Prm	**0.045**			signficantly less than Prm

Panel 5B			Oocysts per 100 ul in each mouse					
	Compound	Dose (mg/kg)	Veh	Prm ctrl	Cmpd	DPI	Protocol	Notes
	P131	3 x 83	1690	52	0	4	B	
			917	82	0			
			1411	65	0			
			9900	45	0			
			2393	289	0			
			1814		22			
			804		0			
			1056		78			
			735		35			
			2179		103			
			973					
			p =	**0.0005**	**<0.0001**			significantly less than vehicle
				vs Prm	**0.024**			signficantly less than Prm

Table S2. Compound-dependent parameters in UPLC-MS analysis. UPLC conditions for analyzing the test compounds were: system, Waters Acquity™ with diode array detector (DAD); column, BEH C_{18} column (50 × 2.1mm I.D., 1.7 μm, Waters, Milford, MA, USA); mobile phase A (MPA), 0.1% formic acid in water; mobile phase B (MPB), 100% acetonitrile; gradient for positive scan method, 0- 0.5 min, 5 % MPB, 0.5-1.0 min, 5-50 % MPB, 1.0-2.0 min, 50-95 % MPB, 2.0-2.5 min, 95 % MPB, 2.5 – 2.6 min, 95-5 % MPB, 2.6 - 3.0 min, 5% MPB; gradient for negative scan method, 0- 0.5 min, 5 % MPB, 0.5-3.0 min, 5-50 % MPB, 3.0-4.0 min, 50-95 % MPB, 4.0-4.5 min, 95 % MPB, 4.5 – 4.6 min, 95-5 % MPB, 4.6 - 5.0 min, 5% MPB; flow rate, 0.55 ml/min; column temperature, 60 °C; injection volume, 10μL. Formononetin was used as the internal standard.

The MS analysis was performed on an API 3200 Qtrap triple quadrupole mass spectrometer (Applied Biosystem/ MDS SCIEX, Foster City, CA, USA) equipped with a TurboIonSpray™ source. The concentrations of tested compounds were determined by using MRM (Multiple Reaction Monitoring) scan type in positive mode. The instrument dependent parameters for mass spectrum were set as follows: ionspray voltage, 5.5 kV; ion source temperature, 400 °C; nebulizer gas (gas 1), nitrogen, 40 psi; turbo gas (gas 2), nitrogen 40 psi; curtain gas, nitrogen 30 psi. Unit mass resolution was set in both mass-resolving quadruples Q1 and Q3. Compound-dependent parameters are listed.

Compound	Scan Mode	Q1(*m/z*)	Q3(*m/z*)	Dwell time (ms)	DP (V)	CEP(V)	CE(V)	CXP(V)
P131	Positive	434	158	100	48	22	37	3
A110		368.3	162.2	100	44	20	22	4
A119		368.1	195.2	100	20	30	20	5
P96		405.4	190.2	100	70	22	30	6
Formononetin (IS)		269	197	100	67	17	49	3
P25	Negative	372	169	100	-51	-24	-17	-4
P32		387	169	100	-58	-24	-23	-4
P82		412	217	100	-50	-21	-18	-5
P83		426	194	100	-53	-26	-20	-8
Formononetin (IS)		267	152	100	-40	-30	-23	-3