



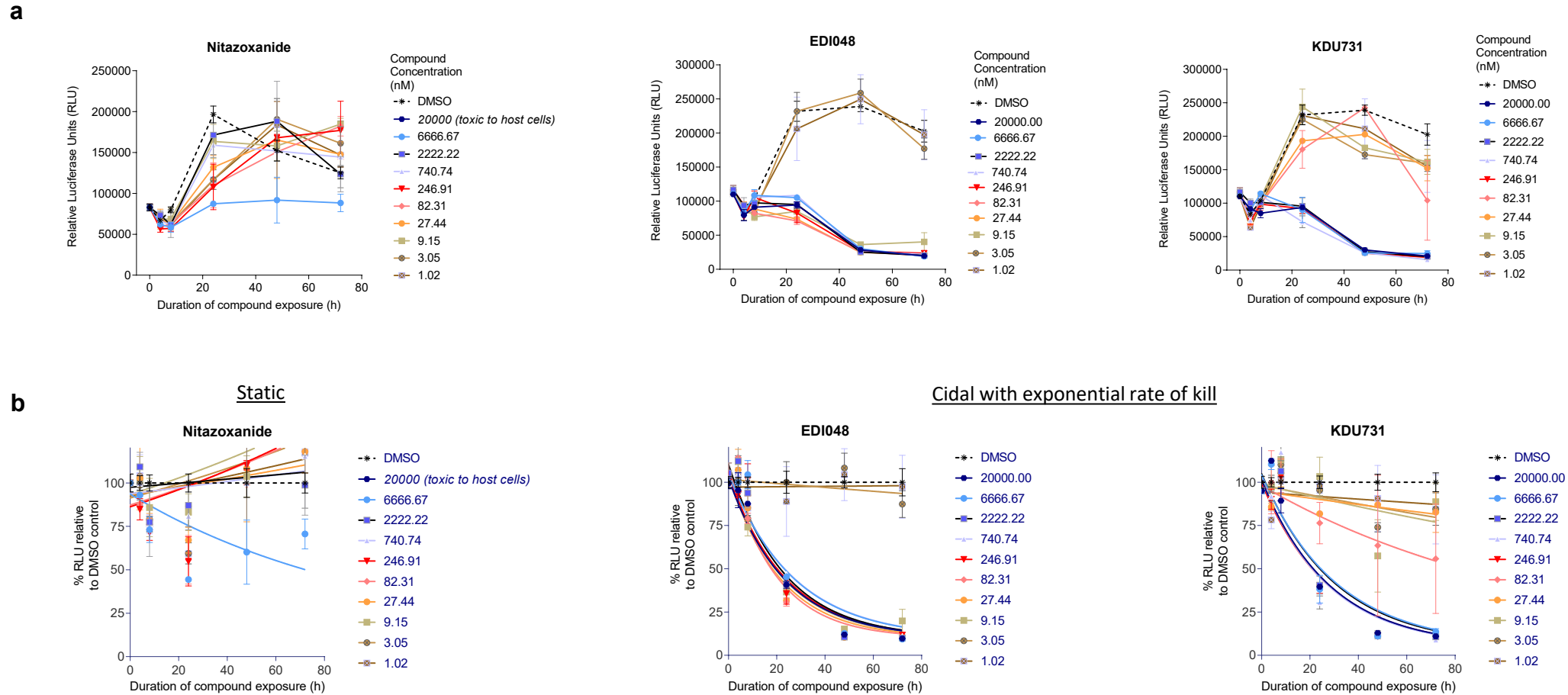
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# ***Cryptosporidium* PI(4)K inhibitor EDI048 is a gut-restricted parasitocidal agent to treat paediatric enteric cryptosporidiosis**

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**Supplementary Figure 1: EDI048 and KDU731 are parasiticidal with an exponential rate of kill while nitazoxanide is parasitistatic. a**, Raw data with relative luminescence units (RLU) demonstrating parasitistatic nature of nitazoxanide and parasiticidal activity of EDI048 and KDU731. **b**, One-phase exponential decay curve fit for nitazoxanide, KDU731 and EDI048 using time-kill data normalized to percentage of the data for the DMSO control for each time point. For nitazoxanide: at 6.7  $\mu\text{M}$ ,  $r^2 = 0.37$ ,  $k = 0.01020$  and  $T_{1/2} = 67.97$  h. For EDI048: at 20  $\mu\text{M}$ ,  $r^2 = 0.94$ ,  $k = 0.04297$ , and  $T_{1/2} = 16.13$  h; and at 9.15 nM,  $r^2 = 0.91$ ,  $k = 0.04410$ ,  $T_{1/2} = 15.72$  h. For KDU731: at 20  $\mu\text{M}$ ,  $r^2 = 0.91$ ,  $k = 0.03567$ ,  $T_{1/2} = 19.43$  h; and at 0.247  $\mu\text{M}$ ,  $r^2 = 0.91$ ,  $k = 0.03561$ , and  $T_{1/2} = 19.47$  h. EDI048 is a potent candidate and showed a steep dose response curve in the cidality assay. A higher resolution experiment with 2-fold dilution between 9-27 nM would be needed to reveal the expected dose response behavior of EDI048. All data are mean and SD of at least 2 technical replicates and representative of 2 independent experiments.

**Supplementary Table 1** | Effect of EDI048, Compounds **6** and **7** on radio-ligand binding to a panel of human recombinant receptors and pharmacologically relevant proteases/kinases

Binding assay*	IC <sub>50</sub> (μM)			Kinase assay	IC <sub>50</sub> (μM)		
	EDI048	6	7		EDI048	6	7
Acetylcholinesterase	>30	>30	>30	ABL1	>30	>30	>30
Adenosine 3 receptor	>30	-	-	AKT1	>30	>30	>30
Adrenergic α1A receptor	>30	>30	>30	ALK	>30	>30	>30
Adrenergic α2A receptor	>30	>30	>30	ATM	0.445	3.78	10.2
Androgen receptor	>30	>30	>30	AURKA	>30	15	10
Benzodiazepine receptor *	>30	>30	>30	AXL	>30	>30	>30
Cyclooxygenase 1 #	>30	>30	>30	CAMK1	>30	>30	>30
Cyclooxygenase 2	>30	>30	>30	CAMK2D	>30	>30	4.25
Dopamine D1 receptor	>30	>30	>30	CDK2	>30	>30	21.8
Dopamine D3 receptor	>30	>30	>30	CDK4	>30	>30	>30
Dopamine transporter	>30	>30	>30	CSNK1D	>30	>30	>30
Estrogen receptor	>30	>30	>30	DMPK	>30	>30	>30
Histamine H3 receptor	>30	>30	>30	EGFR	>30	>30	>30
Monoamine oxidase A	>30			EIF2AK4	>30	>30	>30
Muscarinic M1 receptor	>30	>30	>30	EPHA4	>30	>30	>30
Muscarinic M2 receptor	>30	>30	>30	ERBB2	>30	>30	>30
Norepinephrine transporter	>30	>30	>30	FGFR2	>30	>30	>30
Opioid mu receptor	>30	>30	>30	FGFR3	>30	>30	>30
Progesterone receptor	>30	>30	>30	FGFR4	>30	>30	>30
Prostanoid TP	>30	>30	>30	Fit-1	>30	>30	>30
Phosphodiesterase 3	>30	>30	>30	GRK1	>30	>30	>30
Phosphodiesterase 4D	2.22	>30	12.2	GSK3B	19.1	25	19.2
Serotonin 5-HT 1A receptor	>30	>30	>30	IGF1R	>30	>30	>30
Serotonin 5-HT2b receptor	>30	>30	>30	INSR	>30	>30	>30
Serotonin transporter	29.8	>30	>30	IRK1	>30	>30	>30
Thrombin	>30	>30	>30	JAK1	>30	>30	>30
				JAK2	>30	>30	>30
				JAK3	>30	>30	16.5
				KDR	>30	>30	>30
				KIT	>30	>30	>30
				LCK	>30	>30	>30
				MAP2K3	>30	>30	>30
				MAP3K2	>30	>30	>30
				MAPK14	>30	>30	>30
				MAPK1	>30	>30	>30
				MAPKAPK2	>30	>30	>30
				MAPKAPK5	>30	>30	>30
				MET	>30	>30	>30
				MKMK2	>30	>30	3.43
				NEK11	>30	>30	>30
				PDPK1	>30	>30	>30

				PIM1	3.72	>30	0.0937
				PKN2	>30	>30	>30
				PRKAA2	>30	>30	4.7
				PRKACA	>30	>30	>30
				PRKCA	>30	>30	>30
				PRKCQ	>30	>30	>30
				RAF1	>30	>30	>30
				RET	>30	>30	>30
				ROCK2	>30	>30	>30
				RPS6KB1	>30	>30	>30
				SLK	>30	>30	11.5
				SYK	>30	>30	>30
				TGFBR1	>30	>30	>30
				TRIB2	3.48	11.9	4.61
				TYK2	>30	>30	23.5
				WEE1	>30	>30	>30
				WNK1	>30	>30	>30
				ZAP70	>30	>30	>30

\*all human except ♦rat #sheep

**Supplementary Table 2** | Calf clinical illness fecal consistency rubric

<b>Score</b>	<b>Fecal consistency</b>
<b>1</b>	<b>Normal</b> ; Feces retain form. The feces may be pasty but do no flow across a surface
<b>2</b>	<b>Mild to moderate diarrhea</b> ; Form is a puddle, not a patty. Sufficient water content to slowly flow across or down a surface leaving some material adherent to the surface.
<b>3</b>	<b>Severe diarrhea</b> ; Watery feces or feces with sufficient water content to easily flow across or down a surface leaving little to no adherent material. Calf may have very watery feces followed by some solid material and still have severe diarrhea.

