Supporting Information

Optimization of benzoxazole-based inhibitors of *Cryptosporidium parvum* inosine 5'-monophosphate dehydrogenase

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Table S1: Statistics for data collection and refinement

DATA COLLECTION	
X-ray wavelength (Å)	0.97923
Temperature (°K)	100
Space group	$P2_1$
Unit cell (Å, °)	$a = 89.19$, $b = 92.02$, $c = 92.14$, $\alpha = 90$, $\beta = 103.85$, $\gamma = 90$
Resolution (Å)	2.10
Total no. of reflections	83034
No. of unique reflections	83034
‹I/σ(I)›	8.2
Completeness of data (%)	99.2
R_{merge}[a] (%)	11.9
REFINEMENT	
Resolution range (Å)	35.5-2.1
Reflections used (working/free)	78768/4141
R_{work} /R_{free}[b] (%)	16.2/21.0
Total number of non-hydrogen atoms in asymmetric unit	10776
R.m.s. deviations from ideal geometry	
Bond length (Å)	0.007
Bond angles (deg)	1.104
Mean B value (Å^2)	36.9
PDB accession code	4IXH

[a] $R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(\text{hkl}) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i|\langle I_i(hkl)\rangle|$, expressed as %, where I_i(hkl) is the intensity for the ith measurement of an equivalent reflection with indices h, k, and l.

[b] $R = \Sigma_{hkl}\ ||F_{obs}| - |F_{calc}||/\ \Sigma_{hkl}|F_{obs}|$, expressed as %, where F_{obs} and F_{calc} are observed and calculated factors, respectively. R_{free} is calculated analogously for the test reflections, which were randomly selected and excluded from refinement.

Determination of microsomal stability in mouse liver microsomes. The test agent (1 – 3 μM) was incubated in duplicate with microsomes at 37 ºC. The reaction contained microsomal protein in 100 mM potassium phosphate, 2 mM NADPH, 3 mM $MgCl_2$ and pH 7.4. A control was run for each test agent omitting NADPH to detect NADPH-free degradation. Aliquots were removed at various time points from each experimental and control reaction and mixed with an equal volume of ice-cold Stop Solution (0.3% acetic acid in acetonitrile containing haloperidol, diclofenac, or other internal standard). Stopped reactions were incubated at least ten minutes at -20 ºC, and an additional volume of water was added. The samples were centrifuged to remove precipitated protein, and the supernatants were analyzed by LC/MS/MS to quantitate the remaining parent. Data were converted to % remaining by dividing by the time zero concentration value. Data were fit to a first-order decay model to determine half-life.

Determination of cytotoxicity in mammalian cell lines. HeLa, HEK293, COS and CHO cells were diluted to a concentration of $5x10^4$ cells/mL in growth medium. Cell suspensions (100 μL) were added to 100 μL of DMEM and varying concentrations of compounds in a 96 well flat-bottom plate (Corning). Bortezomib was used as positive control for cytotoxicity. Additional controls included "no compound" and "medium only" wells. Each concentration was tested in triplicate. Plates were incubated at 37 °C with 5% CO_2 for 3 d. Media (100 μL) was removed, and the remaining sample was treated with 10 uL of 10X alamarBlue® solution (Invitrogen) and processed as recommended by the manufacturer.

Table S2: Cytotoxicity evaluation against mammalian cell lines. LD_{50} values in μM. ND = not determined.

Compound	HeLa	HEK293	COS	CHO
15a	>50	>50	>50	>50
40a	>50	>50	>50	>50
41	>50	12.5	>50	>50
44	>50	>50	>50	>50
46	>50	ND	>50	>50
57	>50	>50	>50	>50