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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	NIS elements Advanced Research (AR) version 5.02.01 CLARIOstar 5.40 R2 ChemoMetec NucleoView version 1.2.0.0 Applied biosystems 7500-FAST platform
Data analysis	NIS elements Advanced Research (AR) version 5.02.01 and NIS elements AR Analysis version 5.20.02. GraphPad Prism versions 8.1.2, 9.1.2, and 9.5.1. Microsoft Excel version 2305 Phoenix WinNonlinTM version 8.3 Bioanalysis: Applied Biosystems software Analyst, version 1.6.2 DAVID Helios version 3.01.00.360 ImageJ platform, National Institutes of Health, version 1.53t Coot software version 0.9.8.92 (ccp4) Molecular Operating Environment (MOE), Chemical Computing Group, version 2022.02 ChemBioDraw Professional, PerkinElmer, version 22.0.0.22

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The atomic model of X-ray crystallography data has been deposited in the Protein Data Bank with the accession code PDB ID 8VOF. Source data are provided with this paper. All compounds and reagents can be obtained through a materials transfer agreement from Novartis by contacting the corresponding authors and expect a response in a few weeks.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	PK studies: Sample size was determined on the basis of the minimum number of animals, technical and biological replicates required for good data distribution and statistics. For all these studies n=3 animals/dose group was used. Rat toxicology studies: The number of animals in the toxicity protocols is considered to be the minimum necessary for statistical, regulatory and scientific reasons. For rat tox studies, 10 animals per sex per group was considered the minimum number that would account for the expected variability among these animals. Dog toxicology studies: The number of animals in the toxicity protocols is considered to be the minimum necessary for statistical, regulatory and scientific reasons. For dog tox studies, 3 animals per sex per group was considered the minimum number that would account for the expected variability among these animals. Mouse efficacy studies: Sample size was determined on the basis of the minimum number of animals, technical and biological replicates required for good data distribution and statistics. For all these studies n=3 animals/dose group was used. Calf efficacy studies: Sample size was calculated assuming that 85% of treated calves stop shedding oocysts by the end of the study observation period as compared to 15% of control calves. Assuming a type I error risk of 5% and a type II error risk of 80%, seven calves were needed in each group of the Cryptosporidium-infected group.
Data exclusions	No data were excluded from analysis
Replication	For all in vitro cryptosporidium assays, data were compiled from 2 to 4 biological replicates with at least 2 technical replicates per experiment. For invitro assays, we confirm that all attempts at replication were successful.

Calf efficacy could not be replicated based on ethics guidelines, however, 7 wild neonatal calves were randomly recruited for each group to obtain statistical significance.
In all other invivo studies (mouse efficacy, PK studies in mouse, rat and dogs and toxicology studies in rat and dog), based on 3R principles, animal studies were not replicated due to ethics guidelines. However, a minimum number of animals, technical and biological replicates were used to acquire good data distribution and statistics.

Randomization

For all animal studies, animals were randomly assigned to different groups with arbitrary labels for compound dosing.
For plate-based in vitro assays, compounds were added across the plate in a random manner.
For microscopy, image acquisition was automated.

Blinding

To avoid any bias, microscopy image acquisition and analysis were all automated.

Calves: The negative control calf was sham-challenged to maintain blinding of study personnel and study personnel were blinded during drug dosing, clinical scoring and qPCR analysis of fecal samples.

PK study: Thought animals were selected randomly for each group but animal dosing was not blinded, as compound needs to be formulated in vehicle at different concentration. However, for the sample collection and analysis, analysts were blinded or followed unbiased approach using automated platform

Mouse efficacy study: Animal dosing was not blinded, as compound needs to be formulated in vehicle at different concentration, however, for the sample collection and analysis, analysts were blinded or followed unbiased approach using automated platform.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Click-IT EdU Imaging Kit (Invitrogen'M; cat# 10340) was used for EdU staining as per the manufacturer's instructions. Fluorescein-labeled Vicia villosa lectin (Vector Laboratories, Catalog# FL-1231) was used at 1.33 µg/mL, Hoechst 33258 (AnaSpec, Catalog# AS-83219) was used at 0.09 mM.

Validation

Click-iT® EdU assay kit (Invitrogen'"; cat# 10340) has been validated on mammalian cells (Salic A, et al. 2008 PNAS). The kit was further validated for mammalian cells and Cryptosporidium parvum by Jumani RS et al. 2019 Nat Commun, and negative (including no EdU) and positive controls were used while running each assay. Assays involving Vicia villosa lectin (VVL) (Vector Laboratories) included relevant controls for assays including active compound control ,and VVL has been validated for immunofluorescence staining of Cryptosporidium parasites by Bessoff K, et al. 2014 AAC, Jumani RS, et. al. 2018 AAC, Jumani RS, et al. 2019 Nat Commun among others.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HCT-8 [HRT-18] were purchased from ATCC (Catalog# CCL-244). HepG2 were also purchased from ATCC with Catalog# CRL-10741.
C. parvum Iowa isolate oocysts were purchased from Bunch Grass Farm (Deary, ID).
C. hominis TU502 isolate purchased from Dr. Saul Tzipori (Tufts University Cummings School of Veterinary Medicine, North Grafton, MA).
C. parvum Iowa nanoluciferase expressing oocysts were a kind gift from Dr. Boris Striepen from the University of Pennsylvania and routinely passaged in interferon gamma knockout (IFNγ KO) or NOD SCIOgamma (NSG) mouse models.
SF9 insect cell line from Spodoptera frugiperda was obtained from UC Berkeley Biosciences Divisional Servicer.

Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	HepG2 and HCT-8 cells were tested and were negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	Misidentified cell lines were not used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Mouse efficacy studies: Female C57BL/6 IFN-γ knockout mice (B6.129S7-Ifngtm1Ts/J, Jackson Laboratories) aged 6-8 weeks; C. parvum nluc oocyst passaging and isolation in mouse was performed in 9-11 weeks old NOD SCIO gamma (NSG, NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice. NSG mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA); In vivo PK studies: C57BL/6 male mice (8-10 weeks old), Wister rats (8-10 weeks old) and Beagle dogs (12-14weeks old)[Rat toxicology studies: Wistar male and female rats, ~7 to 8 weeks at receipt and ~9 to 10 weeks at initiation of dosing; Dog toxicology studies: Beagle male and female dogs; Pre-pubertal/pubertal (approximately 10-11 months old at dose initiation).
Wild animals	Fifteen Holstein-Friesian breed bull and heifer calves (Bos taurus taurus) were purchased from a local commercial dairy and enrolled into the study at birth. Study personnel attended the births to ensure that calves were delivered aseptically and that exposure to pathogens was limited. All calves enrolled were randomized to treatment with EDI048 (n = 7), positive infection control (n = 7), and negative infection control (n = 1) at birth. The perineum of the dam was thoroughly cleaned with povidone–iodine scrub, and calves were delivered onto single-use plastic sheets to prevent exposure to environmental pathogens. Calves with abnormal physical examination findings and those weighing less than 30 kg at birth were excluded. Enrolled calves received 4L ≥ 50g IgG/L Land O'Lakes® colostrum replacer (Purina Mills, Gray Summit, MO) and a 3mL subcutaneous injection of vitamin E and selenium (BoSe, Merck, Whitehouse Station, NJ). Calves were then transported from the commercial dairy farm to Cornell University (College of Veterinary Medicine, Ithaca, New York) in a dedicated trailer bedded with sterile straw. At Cornell University, calves were housed in individual box stalls in a Biosafety Level 2 facility. Shatter-proof mirrors were provided for enrichment. Within the first 48 h of birth, blood samples were collected and evaluated for adequate passive transfer of colostral immunity. Calves were offered a commercial 20% protein/20% fat non-medicated milk replacer (Land O'Lakes) every 12 h via nipple bucket. At each feeding, calves were fed an average of 7.6 g of dry matter per kilogram of birth weight for the duration of the study. Water was provided ad libitum. At the end of the study, calves treated with the experimental compound were humanely euthanized using American Veterinary Medical Association approved methods to prevent accidental introduction into the food chain, as per federal regulations. Calves that did not receive experimental compound were offered for adoption.
Reporting on sex	For mouse efficacy studies only, female mice were used and for PK studies only male animals were used for the study. For tox studies, both male and female were equally assigned for each group and no statistically significant sex differences were found.
Field-collected samples	This study did not involved samples collected from the field.
Ethics oversight	All mouse efficacy studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Novartis Institute for Biomedical Research Inc., Emeryville, CA, USA (animal use protocol no. 2017-055). All calves used in this study were cared for in compliance with the Virginia Tech Institutional Animal Care and Use Committee. Mice PK was performed according to the IACUC regulations of Charles River Laboratories, Worcester, MA, US (# 2100230). Rat PK was performed according to the IACUC regulations of Novartis Institute for BioMedical Research, Cambridge, MA, US (# 2100231). Dog PK was performed according to the IACUC regulations of Charles River Laboratories, Worcester, MA, US (# 2100232). Calf PK was carried in compliance with the Virginia Tech Institutional Animal Care and Use Committee (# 00271) Rat Tox study: All procedures involving animals were reviewed and approved by the institutional animal care and use committees of Covance Laboratories Inc., Somerset, NJ, USA (# 1970604) Dog Tox study: All procedures involving animals were reviewed and approved by the institutional animal care and use committees of Covance Laboratories Inc., Somerset, NJ, USA (# 1970605)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA