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Corresponding author(s): Paul J. Hergenrother

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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.

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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	ImageJ (Fiji) was used to measure the pixels/area of bacteria from confocal imaging. Autodock Vina was used for docking studies with lolamicin and LolCDE PDB 7MDX. CHARMM-GUI was used with with membrane builder module and membrane mixer plugin of VMD for simulations. Molecular dynamic simulations performed in NAMD.
Data analysis	Custom code: Source code for data analysis used in microbiome study is available on Github (https://github.com/HPCBio/hergenrother-16S-mouse-2022Sept) and Zenodo (HTML: https://zenodo.org/doi/10.5281/zenodo.10980655; DOI: 10.5281/zenodo.10980656) Commercial software used for data analysis: Phoenix WinNonlin Version 8.1 was used for data analysis for PK studies GraphPad Prism 10.2.0. was used for data analysis for MIC panels, compound accumulation in bacteria, MTD, efficacy studies, resistance mutants studies, time-kill kinetics, competition studies, plotting confocal measurements, and Clostridioides difficile challenge model. Excel (Microsoft 365) was used for MIC determination and mammalian cell toxicity (percent cell death). OriginPro 2020 (OriginLab, Northampton, MA) was used for data analysis in determining mammalian cell toxicity (IC50). MNova was used for NMR analysis.

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

Source code for data analysis can be found at Github (https://github.com/HPCBio/hergenrother-16S-mouse-2022Sept) and Zenodo (HTML: https://zenodo.org/doi/10.5281/zenodo.10980655; DOI: 10.5281/zenodo.10980656). Raw sequencing data from microbiome experiments can be found at https://www.ncbi.nlm.nih.gov/sra/PRJNA1101557. The authors declare that all other data supporting the findings of this study are available within the paper and the Supplementary Information.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	No statistical methods were used to predetermine samples size for MTD. Sample size was selected based on literature precedence and sample sizes needed to achieve statistically significant results. For bacterial burden and mortality studies, sample size was calculated based on 80% power to detect a difference in the means between the compound-treated and vehicle-treated groups, based on a t-test with a two-sided 0.05 level of significance and with expected outcome difference of 50% between these two groups.
Data exclusions	No data exclusions.
Replication	We have three or more biological replicates as indicated in the manuscript for MIC determination, mammalian cell toxicity (percent cell death and IC50), compound accumulation studies, fitness of resistance mutants, competition studies, confocal microscopy, and spontaneous frequency of resistance in bacteria. For MTD and PK studies three mice were used per group. For survival studies, 15 mice were used for each group. For bacterial burden studies, 8 mice were used per group. For microbiome studies and C. difficile challenge studies, 6 mice per group were used. All replicates were reported and were successful.
Randomization	Mice were randomly assigned to treatment or vehicle groups.
Blinding	Investigators were not blinded as it was not relevant to treatment outcomes or data analysis for MTD, bacterial burden, survival, microbiome, or C. difficile challenge experiments.

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

Methods

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HFF-1 and A-549 cells were obtained from ATCC.
Authentication	Cell lines were authenticated externally by commercial vendor and were inspected visually in-house.
Mycoplasma contamination	Cell lines were not verified to be free of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified species used.

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	For MTD and PK studies, 10- to 12-week-old female C57BL/6 mice purchased from Charles River were used. For efficacy studies and Clostridioides difficile challenge, six- to eight-week-old male CD-1 mice purchased from Charles River were used. For microbiome study, four-week-old male CD-1 mice purchased from Charles River Laboratories were used. These mice were housed in positively ventilated microisolator cages equipped with automatic recirculating water, situated in a room featuring laminar, high-efficiency particle accumulation-filtered air. The animals were provided with autoclaved food, water, and bedding". The animal room operates on an alternating 12-hours light/dark cycle, with ambient temperature set to 68 C (range 67 C - 71 C) and humidity at 28% (range 17% - 59%)
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex-based analysis was not performed. This study was not intended to test for sex differences.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The protocols were approved by the IACUC at the University of Illinois at Urbana-Champaign. (MTD and PK protocol number: 19181; efficacy studies, microbiome study, and Clostridioides challenge protocol number: 20256).

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