Supplementary Note 1

Independent benchmarking of cytotoxicity models

Our benchmarking of the Chemprop-based cytotoxicity models used the following independently-acquired datasets:

(1) the HepG2 and mitochondria toxicity datasets from Tox21 dataset[22]
(2) a metabolite dataset from The Human Metabolome Database[23], whose molecules are putatively non-cytotoxic as human metabolites[55].

Tox21 datasets were filtered to include only agonists and inactive compounds, as determined by Tox21 criteria; additionally, due to the possibility of both mitochondria hyperpolarizing and depolarizing agents to be associated with toxicity, the mitochondria toxicity dataset was also tested where it included antagonists and inactive compounds. This resulted in a dataset of 7,151 compounds for HepG2 cytotoxicity, 5,726 compounds for mitochondria toxicity (agonists and inactive compounds), and 6,425 compounds for mitochondria toxicity (antagonists and inactive compounds). We found that the size of the overlap between the HepG2 dataset and our training dataset of 39,312 molecules was 961 molecules; similarly, for the mitochrondria toxicity datasets, the overlap was 859 molecules and 976 molecules, respectively. Given the small fraction of overlapping molecules relative to the sizes of the datasets, we present results below for which overlapping molecules have not been removed. However, removing the overlapping molecules does not substantially change the following results.

The metabolite dataset was downloaded from The Human Metabolome Database[23]. A set of 3,126 molecules that were "PREDICTED AND QUANTIFIED", as well as "FOOD" and "ENDOGENOUS", were selected. We found 223 molecules to overlap with our training set of 39,312 compounds. The SMILES of all compounds from the datasets were supplied as inputs to the HepG2, HSkMC, or IMR-90 cytotoxicity models and compared with known toxicity values (in the case of Tox21) or the assumption that human metabolites are non-cytotoxic (in the case of human metabolites).

As shown in Supplementary Table 7, our final ensembles of 20 Chemprop models trained on the full training dataset of 39,312 compounds exhibited encouraging performance, as measured by the area under the receiver operating characteristic curve (AUROC), the area under the precision recall curve (AUPRC), and the Matthews correlation coefficient (MCC). In the case of the human metabolite set as a putative negative control, we found that our models exhibited false positive rates of ~1% to ~10% depending on the prediction score thresholds used (Supplementary Table 8). Together, these findings suggest that our Chemprop models for HepG2, HSkMC, and IMR-90 cytotoxicity exhibit encouraging performance in accurately predicting cytotoxic or putatively cytotoxic compounds.

Supplementary Note 2

Comparison of graph-based rationales with maximal common substructures

As graph neural networks make predictions based on the information contained in the atoms and bonds of each molecule, we also investigated whether compounds with similar prediction scores possessed similar molecular substructures. For these initial analyses, we focused on the maximal common substructures (MCSs) shared by hits and non-hits. As a straightforward combinatorial calculation of all possible MCSs is computationally prohibitive (2^n MCS computations for n molecules), we employed a randomized method to quickly identify substructures shared between large numbers of compounds (see *Methods* for details). We computed the MCS shared between two randomly chosen molecules, checked that this MCS contains at least a threshold number of atoms, removed all molecules that contain this MCS, then recorded the MCS and its associated molecules. This process is repeated until a prespecified fraction of molecules remain, and the process allows for variations in structural features to be conveniently coarsened and molecules to be productively grouped.

Applying this analysis on the predicted hits, we identified and shortlisted MCSs according to the number of hits having in common, as a rudimentary cut-off, at least 12 atoms (Extended Data Fig. 6 and Supplementary Data 2). We verified that different atom number thresholds, including 10 and 15, resulted in the identification of similar substructures (Extended Data Fig. 6). Intriguingly, large fractions (>40%) of all hits were associated with small numbers of MCSs (Extended Data Fig. 6). These MCSs included substructures indicative of known antibiotic classes. As expected from the active compounds in the training set (Supplementary Data 1), MCSs associated with hits contain substructures found in β-lactams and quinolones (Extended Data Fig. 6): these include variations of the cephalosporin ring (MCSs **A1, A4, A6, A12**) and the 4-quinolone bicyclic ring (MCS **A10**). Less common substructures, including MCSs **A9** and **A11**, also emerged. In contrast to the MCSs enriched in hits, sulfonamide-like substructures largely lacking heterocycles (MCSs **B1-B12**) were enriched in non-hits (Extended Data Fig. 6). While sulfonamide antibiotics including sulfamethoxazole comprise an important class of antibiotics in combination with trimethoprim, sulfonamide compounds (including sulfamethoxazole) were inactive as single-agent compounds in the training data, consistent with the model predictions. We anticipate that future work will model situations in which compounds become active only in combination with other drugs (e.g., sulfamethoxazole/trimethoprim).

The MCSs associated with hits and non-hits, viewed as molecular inputs to Chemprop in their own right, produce prediction scores that may correlate (Pearson's R>0.2) with the prediction scores of hits and non-hits, respectively (Extended Data Fig. 6 and Supplementary Table 9). Moreover, the prediction scores for all of MCSs **A1-A12** were larger than those for MCSs **B1-B12**, which were $<10^{-6}$ (Supplementary Table 9). Supplementary Table 9 further indicates that MCSs associated with hits are never associated with non-hits and MCSs associated with non-hits are never associated with hits, demonstrating as well that the calculated MCSs are largely unique to hits and non-hits. However, MCSs **A2**, **A3**, **A7**, and **A10** exhibited prediction scores <0.005, suggesting that the presence of these substructures alone is not sufficient for high prediction scores (Extended Data Fig. 6 and Supplementary Table 9). Indeed, while MCS **A10** contains a quinolone ring and many quinolone-like compounds are predicted to be active, this approach does not view MCS **A10** as specific enough as to be diagnostic of antibiotic activity.

As discussed in the main text, computing rationales allows us to identify substructures that directly determine high antibiotic prediction scores, as opposed to only identifying similar substructures shared by compounds with similar (high or low) prediction scores. We found that MCSs **A1-A12** coincide with many such rationales computed from the 3,646 hits, indicating that the MCS- and rationale- based approaches to substructure identification can result in consistent substructures (Extended Data Fig. 6). Importantly, the prediction scores associated with all rationales were >0.1, even in cases where the MCS prediction scores were <0.005 and not diagnostic of antibiotic activity (Extended Data Fig. 6). This finding indicates that identifying rationales is more predictive than identifying the MCSs of hits alone.

Supplementary Note 3

Prior knowledge of validated hits

Compounds were searched for using their SMILES strings on PubChem, Google Patents, and ChEMBL. Additionally, compounds **1** and **2** were searched for using their CAS Registry Numbers (which were unavailable for compounds **I** and **II**; Extended Data Fig. 7) on SciFinder^{n}. In this way, we determined that all four compounds were not previously studied for their antibacterial activities against the bacterial strains considered in this study. In addition, we note here that compounds **1** and **2** have been previously screened in assays, including the inhibition of human p53 and serine/threonine kinase 33, and were found to be inactive[56].

Supplementary Note 4

Structure-activity relationship analyses

Building on our study of compounds **1** and **2**, we further explored the chemical space described by the rationale shared by the two compounds (Extended Data Fig. 10). By iteratively modifying the functional groups of the rationale and procuring the resulting compounds, we tested 17 additional compounds and found that five (compounds **3-7**) exhibit at least weak (MIC ≤64 μg/mL) growth inhibitory activity against *S. aureus* RN4220, with various corresponding therapeutic windows (Extended Data Fig. 10 and Supplementary Data 2). This analysis further suggested that the presence of the benzoic acid group was associated with antibiotic activity: compounds **1-7** all contained this group, which is found in none of the 12 remaining inactive analogues (Supplementary Data 2). These results indicate that the structure-activity space of our rationale of interest is not flat, supporting the suggestion that compounds **1** and **2** hold promise for further optimization. Moreover, these preliminary structure-activity relationship analyses illustrate the ability of a rationale predicted by our deep learning approach to further guide traditional QSAR efforts and confirm that compounds **1** and **2** are members of a structural class with selective antibiotic activity.

Supplementary References

55. Sharma, A. K., Srivastava, G. N., Roy, A., and Sharma, V. K. ToxiM: A toxicity prediction tool for small molecules developed using machine learning and chemoinformatics approaches. *Front. Pharmacol.* **8,** 880 (2017).
56. Luminescence cell-based primary HTS to identify inhibitors of STK33. Accessed 20 October 2022 at https://pubchem.ncbi.nlm.nih.gov/bioassay/2330.

Supplementary Data

Supplementary Data 1. Training set of 39,312 compounds tested for antibiotic activity and cytotoxicity, in addition to 200 RDKit features used to augment the models and cytotoxicity testing results. Antibiotic activity was defined using a 20% relative mean growth cutoff in *S. aureus* RN4220. Cytotoxicity was defined using a 90% relative mean cell viability cutoff in HepG2 cells, HSkMCs, and IMR-90 cells. Data are from two biological replicates.

Supplementary Data 2. Model predictions, rationales, and procured compounds from the ensemble Chemprop model. Compound SMILES strings and corresponding prediction scores are shown for all 3,646 hits, out of 12,076,365 compounds whose antibiotic activity and cytotoxicity against human cells were predicted. Rationale and scaffold SMARTS strings, vendor catalog information for all 283 procured and tested compounds shown in Fig. 3e of the main text, and vendor catalog information for all 17 procured and tested compounds as part of the structure-activity relationship analyses shown in Extended Data Fig. 10 are also provided, in addition to the maximal common substructure SMARTS strings for the analyses described in Supplementary Note 2 and Extended Data Fig. 6.

Supplementary Data 3. Mutations arising in cells exposed to compounds. For each compound, results are shown for at least two independently passaged or suppressor mutant populations. All mutations that passed mapping filters are listed here. Black boxes highlight mutations in similar regions across sequencing replicates either present in the same gene, or present in an adjacent gene or intergenic region.

Supplementary Data 4. Training and test data for models predicting proton motive force-altering activity. Proton motive force-altering activity was defined using a 30% relative mean fluorescence change in *S. aureus* RN4220 in the presence of $DiSC_3(5)$, a proton motive force-sensitive dye. 475 antibacterial compounds from Supplementary Data 1 were tested, and all inactive antibacterial compounds were assumed to not alter proton motive force. Data are from two biological replicates.

	Antibiotics	HepG2 cytotoxicity	HSkMC cytotoxicity	IMR-90 cytotoxicity
	Area under the receiver operating characteristic curve (AUROC) on withheld test set			
Chemprop	0.921	0.683	0.835	0.829
Chemprop (no RDKit features)	0.899	0.682	0.818	0.817
Best-performing random forest	0.938	0.671	0.801	0.830
	***P*-value from DeLong's test for differences in AUROC**			
Chemprop vs. Chemprop (no RDKit features)	1.36E-4	0.561	8.16E-05	8.97E-07
Chemprop vs. best-performing random forest	0.149	0.216	8.04E-06	0.968
Chemprop (no RDKit features) vs. best-performing random forest	0.001	0.272	0.013	0.038

Supplementary Table 1. Model benchmarking.

Values were computed on the same 80%-20% train-test splits of the data. Models refer to those shown in Fig. 1 of the main text and Extended Data Figs. 2 and 3. AUROC, area under the receiver operating characteristic curve.

Compound	**Mol. weight (Da)**	**MIC (µg/mL)**						**IC_{50} (µg/mL) [Cell viability at 10 µM, relative to DMSO vehicle]**		
		S. aureus RN4220	*S. aureus* USA300 (MRSA)	*B. subtilis* 168	*E. coli* BW25113	*A. baumannii* ATCC17978	*P. aeruginosa* PAO1	HepG2	HSkMC	IMR-90
1 (BRD-K12804514)	374.6	2	4	2	>128	>128	>128	128 [0.986]	>128 [0.952]	128 [0.955]
2 (BRD-K80450985)	419.1	2	4	2	>128	>128	>128	128 [0.927]	128 [0.946]	128 [0.912]
I (Z225617188)	392.5	16	16	n.d.	n.d.	n.d.	n.d.	32 [1.021]	32 [1.026]	32 [1.013]
II (STK661606)	380.2	32	64	n.d.	n.d.	n.d.	n.d.	128 [1.025]	64-128 [1.035]	64 [1.019]
8919377*	525.6	1	16	2	>32	>32	>32	n.d.	n.d.	n.d.
7492221*	518.5	0.25	16	0.25	8	>32	>32	n.d.	n.d.	n.d.
9300332*	437.4	0.5	>32	0.25	32	32	>32	n.d.	n.d.	n.d.
8926827*	439.4	4	>32	0.5	>32	>32	>32	n.d.	n.d.	n.d.
8914392*	508.4	8	>32	32	>32	>32	>32	n.d.	n.d.	n.d.
8919389*	487.0	0.25	1	16	>32	>32	>32	n.d.	n.d.	n.d.
8919381*	515.0	1	16	2	>32	>32	>32	n.d.	n.d.	n.d.
STK784397*	528.6	<0.03	0.25	<0.03	1	0.5	8	n.d.	n.d.	n.d.
STL018675*	361.4	0.25	8	0.12	0.12	0.25	0.5	n.d.	n.d.	n.d.
STK249734*	527.6	0.25	4	0.5	0.12	16	16	n.d.	n.d.	n.d.
STK784382*	448.5	<0.03	0.5	<0.03	2	2	16	n.d.	n.d.	n.d.
9324160*	417.4	<0.03	0.5	<0.03	1	1	16	n.d.	n.d.	n.d.
BRD-K30397580†	411.3	32	32	n.d.	n.d.	n.d.	n.d.	32 [1.021]	32 [1.002]	16 [0.959]
STK223827†	388.6	4	8	n.d.	n.d.	n.d.	n.d.	64 [1.001]	64 [0.901]	32 [0.923]
BRD-A35476492†	357.2	16	16	8	>128	>128	>128	>128 [0.824]	>128 [0.785]	128 [0.899]
BRD-K09705226†	449.0	32	32	n.d.	n.d.	n.d.	n.d.	32 [0.959]	32 [1.010]	32 [0.994]
BRD-K74229244†	403.4	8	8	n.d.	n.d.	n.d.	n.d.	32 [0.945]	32 [0.996]	32 [0.955]
BRD-K71366765†	403.4	8	8	n.d.	n.d.	n.d.	n.d.	64 [0.944]	64 [0.995]	64 [1.012]
BRD-K11266665†	403.4	4	4	n.d.	n.d.	n.d.	n.d.	32 [0.942]	32 [0.910]	32 [0.911]
BRD-K97660061†	403.4	4	4	n.d.	n.d.	n.d.	n.d.	32 [0.955]	32 [0.981]	32 [0.954]
BRD-K81544562†	403.4	8	8	n.d.	n.d.	n.d.	n.d.	64 [0.941]	64 [0.973]	64 [1.014]

STK539876†	334.7	16	>128	n.d.	n.d.	n.d.	n.d.	128 [0.967]	128 [0.945]	128 [0.996]
BRD-K23677682†	401.4	8	8	n.d.	n.d.	n.d.	n.d.	128 [0.937]	128 [0.943]	128 [0.944]
BRD-K95863777†	417.4	4	4	n.d.	n.d.	n.d.	n.d.	32 [0.903]	32 [0.958]	32 [0.933]
BRD-A73398991†	409.3	16	32	8	>128	>128	>128	>128 [1.053]	>128 [0.940]	128 [0.925]
BRD-K84278600†	417.4	16	16	n.d.	n.d.	n.d.	n.d.	>128 [0.959]	>128 [0.945]	>128 [0.955]
BRD-K76701247†	417.4	4	4	n.d.	n.d.	n.d.	n.d.	32 [0.960]	32 [0.960]	32 [0.933]
STL090984†	400.4	32	32	n.d.	n.d.	n.d.	n.d.	>128 [0.923]	>128 [0.910]	>128 [0.907]
BRD-A42002693†	446.9	32	32	n.d.	n.d.	n.d.	n.d.	32 [0.912]	32 [0.906]	32 [0.930]
Ampicillin	349.4	0.06	1	0.03	4	8	>32	>128	>128	>128
Ciprofloxacin	331.3	0.16	8	0.1	0.03	0.2	0.1	>128	>128	>128

Supplementary Table 2. Additional MIC measurements for validated compounds.

* indicates a bona fide quinolone or β-lactam. † indicates a structurally novel, validated hit with no computed rationale, or one not associated with any of rationale groups **G1-G5** (Fig. 3d of the main text and Supplementary Data 2). n.d., not determined. Values shown are representative of two biological replicates.

#	SMILES	Mol weight (Da)	Num. heavy atoms	Num. aro-matic heavy atoms	Num. rotat-able bond	Num. H-bond accept-or	Num. H-bond donor	Mol. refra-ctivity	TPSA	Log *P*	Lipinski violation	Ghose criteria violation	PAINS	Brenk alert
1	OC(=O)c1ccc(cc1NC(=O)COc1ccc(cc1Cl)Cl)Cl	374.6	23	12	6	4	2	88.76	75.63	3.8	None	None	None	None
2	OC(=O)c1cc(ccc1NC(=O)COc1ccc(cc1Cl)Cl)Br	419.1	23	12	6	4	2	91.45	75.63	3.81	None	None	None	None
I	OC(C1=C(CSC2OC(C3CCCCC3)=NN=2)C2=C(C=CC=C2F)S1)=O	392.5	26	14	5	6	1	99.69	129.76	4.59	None	None	None	None
II	OC(=O)[C@H]1CN(C(=O)C1)c1ccc(cc1)OCc1ccc(cc1Cl)Cl	380.2	25	12	5	4	1	98.86	66.84	3.25	None	None	None	None
OM	**N/A**	**813**	**N/A**	**N/A**	**N/A**	**16.3**	**7.1**	**N/A**	**243**	**2.1**	**N/A**	**N/A**	**N/A**	**N/A**

Supplementary Table 3. *In silico* predicted properties of four validated hits associated with rationale groups G1-G5.

Log *P* values were estimated as the average of predictions from five physics-based, topological, and atomistic models using SwissADME[50]. TPSA values were calculated using the ESOL method using SwissADME[50]. All other properties were calculated using OpenBabel as part of SwissADME[50]. TPSA, topological polar surface area (Å²). For comparison, the last row shows average values of properties computed for known Gram-positive antibiotics by O'Shea and Moser (**OM**; ref. 33).

		MIC (μg/mL)		
Strain	**Resistance/Markers**	**1**	**2**	**Vancomycin**
Bacterial strains				
Gram-positive bacteria				
Staphylococcus aureus RN4220	Methicillin-susceptible (MSSA)	2	2	1
Staphylococcus aureus **1**-30P	RN4220 passaged in compound **1** for 30 days	2	2	1
Staphylococcus aureus **2**-30P	RN4220 passaged in compound **2** for 30 days	2	2	1
Staphylococcus aureus **1**-5SM	Suppressor mutant of RN4220 in compound **1** for 5 days	8	8	1
Staphylococcus aureus **2**-5SM	Suppressor mutant of RN4220 in compound **2** for 5 days	8	8	1
Staphylococcus aureus USA300	Methicillin-resistant (MRSA), SCC*mec*IV	4	4	1
Staphylococcus aureus CDC 215	Vancomycin-intermediate, *aadD, blaZ, erm(A), mecA, spc*	8	4	4
Staphylococcus aureus CDC 216	Vancomycin-intermediate, *aph(3')-III, mecA, mph(C), msr(A)*	16	16	4
Staphylococcus aureus CDC 217	Vancomycin-intermediate, *blaZ, dfrG, mecA*	4	2	4
Staphylococcus aureus CDC 218	Vancomycin-intermediate, *aph(3')-III, erm(A), mecA, spc, tet(K)*	2	2	8
Staphylococcus aureus CDC 219	Vancomycin-intermediate, *aac(6')-aph(2''), aadD, erm(A), mecA, spc, tet(M)*	8	8	8
Staphylococcus aureus CDC 220	Vancomycin-intermediate, *aadD, blaZ, erm(A), mecA, spc*	4	2	4
Staphylococcus aureus CDC 221	Vancomycin-intermediate, *aac(6')-aph(2''), mecA, tet(M)*	2	2	4
Staphylococcus aureus CDC 222	Vancomycin-intermediate, *blaZ*	2	2	4
Staphylococcus aureus CDC 223	Vancomycin-intermediate, *mecA*	2	2	4
Staphylococcus aureus CDC 224	Vancomycin-intermediate, *aph(3')-III, erm(A), mecA, spc, tet(K)*	8	8	4
Staphylococcus aureus CDC 225	Vancomycin-intermediate, *aph(3')-III, blaZ, mecA, mph(C), msr(A)*	2	2	4
Staphylococcus aureus CDC 226	Vancomycin-intermediate, *aph(3')-III, blaZ, mecA, mph(C), msr(A)*	4	4	4
Staphylococcus aureus CDC 561	AG/TC-resistant, *aph-STPH, fosB, mecA*	4	4	2
Staphylococcus aureus CDC 562	AG/TC-resistant, *aph-STPH, DHA-1, erm(A), mecA, spc, tet(38), tet(M)*	16	16	2
Staphylococcus aureus CDC 563	AG/TC-resistant, *aac(6')-aph(2''), aadD, aph-STPH, fosB, mecA*	4	4	1
Staphylococcus aureus CDC 564	AG/TC-resistant, *aac(6')-aph(2''), aadD, aph(3')-III, aph-STPH, DHA-1, erm(A), mecA, mph(C), sat4A, spc*	2	4	2
Staphylococcus aureus CDC 565	AG/TC-resistant, *aph(3')-III, aph-STPH, dfrG, erm(A), fosB, mecA, sat-4A, spc, tet(K), tet(M), Z*	4	8	2
Staphylococcus aureus CDC 566	AG/TC-resistant, *aac(6')-aph(2''), aph-STPH, fosB, mecA*	2	2	0.5
Staphylococcus aureus CDC 567	AG/TC-resistant, *aac(6')-aph(2''), aph(3')-III, aph-STPH, erm(C), fosB, mecA, sat-4A*	2	2	1

		MIC (μg/mL)		
Strain	**Resistance/Markers**	**1**	**2**	**Vancomycin**
Bacterial strains				
Gram-positive bacteria				
Staphylococcus aureus CDC 568	AG/TC-resistant, *aph(3')-III, aph-STPH, DHA-1, erm(A), sat-4A, spc, tet(38), tet(K)*	4	2	0.5
Staphylococcus aureus CDC 569	AG/TC-resistant, *tet(M)*	8	16	0.5
Staphylococcus aureus CDC 570	AG/TC-resistant, *aac(6')-aph(2''), aadD, aph(3')-III, aph-STPH, DHA-1, erm(A), mecA, mph(C), spc, tet(K)*	4	8	1
Enterococcus avium CDC 571	AG/TC-resistant VRE, *aac(6')-aph(2''), erm(B), tet(O), VanA, VanH-A, VanR-Pt2, VanS-A, VanX-A, VanZ-A*	2	2	16
Enterococcus faecium CDC 572	AG/TC-resistant VRE, *aph(3')-III, dfrG,* VanA, VanH-A, VanR-Pt2, VanS-A, VanX-A, VanY-A, VanZ-A	4	2	32
Staphylococcus aureus CDC 701	Oxazolidinone-resistant, *mecA*	16	16	1
Staphylococcus aureus CDC 702	Oxazolidinone-resistant, *mecA*	8	8	2
Staphylococcus aureus CDC 703	Oxazolidinone-resistant, *mecA*	2	2	0.5
Staphylococcus aureus CDC 704	Oxazolidinone-resistant, *mecA*	4	4	1
Staphylococcus aureus CDC 705	Oxazolidinone-resistant, *mecA*	2	4	1
Staphylococcus aureus CDC 706	Oxazolidinone-resistant, *mecA*	2	2	1
Staphylococcus aureus CDC 707	Oxazolidinone-resistant, *mecA*	4	8	1
Staphylococcus aureus CDC 708	Oxazolidinone-resistant, *mecA*	2	2	2
Staphylococcus aureus CDC 709	Oxazolidinone-resistant, *mecA*	2	4	1
Staphylococcus aureus CDC 710	Oxazolidinone-resistant, *mecA*	2	2	1
Staphylococcus aureus CDC 711	Oxazolidinone-resistant, *mecA*	4	8	2
Staphylococcus aureus CDC 712	Oxazolidinone-resistant, *mecA*	8	8	1
Enterococcus casseliflavus CDC 788	VRE	2	2	4
Enterococcus gallinarum CDC 796	VRE, VanC	2	2	8
Enterococcus casseliflavus CDC 797	VRE	2	2	2
Enterococcus casseliflavus CDC 798	VRE	2	2	2
Bacillus subtilis 168		2	2	0.25
Gram-negative bacteria				
Escherichia coli BW25113		>128	>128	>128
Escherichia coli RFM795	*lptD4213*	2	2	≤2
Escherichia coli JW5503-KanS	*ΔtolC832::FRT*	2	2	>128
Acinetobacter baumannii ATCC17978		>128	>128	>128
Pseudomonas aeruginosa PAO1		>128	>128	>128
Human cell types (IC_{50})				
HepG2		128	128	>128
HSkMC		>128	128	>128
IMR-90		128	128	>128

Supplementary Table 4. MICs of compound 1, compound 2 and vancomycin against various bacterial isolates, strains, and human cell types.

Values shown are representative of two biological replicates.

Hyperparameter	Range	Value used
Chemprop model (antibiotics)		
Number of message-passing steps	[2, 5]	5
Neural network hidden size	[900, 2000]	1600
Number of feed-forward layers	[1, 3]	3
Dropout probability	[0.05, 0.4]	0.35
Chemprop model (HepG2 cytotoxicity)		
Number of message-passing steps	[4, 5]	5
Neural network hidden size	[1500, 1700]	1600
Number of feed-forward layers	[2, 3]	3
Dropout probability	[0.3, 0.4]	0.35
Chemprop model (HSkMC cytotoxicity)		
Number of message-passing steps	[4, 5]	5
Neural network hidden size	[1500, 1700]	1600
Number of feed-forward layers	[1, 3]	3
Dropout probability	[0.3, 0.4]	0.35
Chemprop model (IMR-90 cytotoxicity)		
Number of message-passing steps	[4, 5]	5
Neural network hidden size	[1600, 1700]	1600
Number of feed-forward layers	[2, 3]	3
Dropout probability	[0.3, 0.4]	0.35
Random forest model (antibiotics)		
Maximum depth	[5, 40]	15
Number of trees	[20, 100]	100
Number of features	[20, 180]	20
Random forest model (HepG2 cytotoxicity)		
Maximum depth	[5, 40]	40
Number of trees	[20, 100]	40
Number of features	[20, 180]	180
Random forest model (HSkMC cytotoxicity)		
Maximum depth	[5, 40]	15
Number of trees	[20, 100]	100
Number of features	[20, 180]	20
Random forest model (IMR-90 cytotoxicity)		
Maximum depth	[5, 10]	10

Number of trees	[20, 40]	40
Number of features	[20, 60]	40
Chemprop model (leave-one-out for quinolones)		
Number of message-passing steps	N/A	5
Neural network hidden size	N/A	1600
Number of feed-forward layers	N/A	3
Dropout probability	N/A	0.35
Chemprop model (leave-one-out for β-lactams)		
Number of message-passing steps	N/A	5
Neural network hidden size	N/A	1600
Number of feed-forward layers	N/A	3
Dropout probability	N/A	0.35
Chemprop model (proton motive force alteration)		
Number of message-passing steps	N/A	5
Neural network hidden size	N/A	1400
Number of feed-forward layers	N/A	2
Dropout probability	N/A	0.25

Supplementary Table 5. Hyperparameters used for deep learning.

For details, see *Methods*.

Strain	Genotype	Source
Staphylococcus aureus RN4220 (MSSA)	Restriction-minus, modification-plus derivative of NCTC 8325, *rsbU-, agr-*	Michael Gilmore Lab
Staphylococcus aureus FPR3757 (MRSA USA300)	USA300, SCC*mec*IV, *pvl+*	ATCC BAA-1556
Bacillus subtilis 168	*trpC2 ind- tyr+*	ATCC 23857
Escherichia coli BW25113	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	Lab stock
A. baumannii ATCC17978	Cerebrospinal fluid isolate, reference strain	ATCC 17978
P. aeruginosa PAO1	Derivative of Australian PAO isolate, reference strain	Lab stock

Supplementary Table 6. Common bacterial strains used in this study.

	HepG2 Chemprop model	HSkMC Chemprop model	IMR-90 Chemprop model
HepG2 toxicity	AUROC: 0.786 AUPRC: 0.343 MCC: 0.291	AUROC: 0.791 AUPRC: 0.343 MCC: 0.286	AUROC: 0.761 AUPRC: 0.310 MCC: 0.254
Mitochondria toxicity (agonists and inactive compounds)	AUROC: 0.835 AUPRC: 0.290 MCC: 0.310	AUROC: 0.831 AUPRC: 0.280 MCC: 0.312	AUROC: 0.809 AUPRC: 0.279 MCC: 0.277
Mitochondria toxicity (antagonists and inactive compounds)	AUROC: 0.805 AUPRC: 0.404 MCC: 0.217	AUROC: 0.805 AUPRC: 0.421 MCC: 0.191	AUROC: 0.705 AUPRC: 0.295 MCC: 0.154

Supplementary Table 7. Performance metrics of Chemprop cytotoxicity models on Tox21 datasets. 7,151 compounds were contained in the filtered HepG2 toxicity dataset, 5,726 compounds were contained in the filtered mitochondria toxicity dataset containing agonists and inactive compounds, and 6,425 compounds were contained in the filtered mitochondria toxicity dataset containing antagonists and inactive compounds.

	PS > 0.1	PS > 0.2	PS > 0.3	PS > 0.4	PS > 0.5
Fails HepG2 Chemprop model	429 (13.7%)	125 (4.0%)	40 (1.3%)	14 (0.4%)	4 (0.1%)
Fails HSkMC Chemprop model	562 (18.0%)	144 (4.6%)	46 (1.5%)	21 (0.7%)	5 (0.2%)
Fails IMR-90 Chemprop model	850 (27.2%)	365 (11.7)	127 (4.1%)	24 (0.8%)	4 (0.1%)
Fails all models	346 (11.1%)	80 (2.6%)	17 (0.5%)	4 (0.1%)	0 (0.0%)

Supplementary Table 8. Performance metrics of Chemprop cytotoxicity models on a human metabolite dataset. 3,126 metabolites were considered. PS, prediction score.

MCS	MCS PS	Hits (3646)		Non-hits (3355)		MCS	MCS PS	Hits (3646)		Non-hits (3355)	
		N	Average hit PS	*N*	Average non-hit PS			*N*	Average hit PS	*N*	Average non-hit PS
A1	0.294	748	0.472	0	N/A	**B1**	8.570E-09	0	N/A	172	4.482E-07
A2	0.001	145	0.227	0	N/A	**B2**	2.320E-08	0	N/A	121	4.412E-07
A3	0.001	118	0.332	0	N/A	**B3**	1.380E-07	0	N/A	88	5.866E-07
A4	0.007	109	0.482	0	N/A	**B4**	6.000E-08	0	N/A	85	5.222E-07
A5	0.009	94	0.226	0	N/A	**B5**	5.820E-08	0	N/A	84	4.694E-07
A6	0.419	78	0.264	0	N/A	**B6**	1.580E-07	0	N/A	60	4.436E-07
A7	0.002	74	0.240	0	N/A	**B7**	1.040E-07	0	N/A	55	4.747E-07
A8	0.014	68	0.221	0	N/A	**B8**	3.220E-08	0	N/A	45	5.102E-07
A9	0.005	55	0.223	0	N/A	**B9**	1.610E-07	0	N/A	32	6.080E-07
A10	0.003	39	0.223	0	N/A	**B10**	5.990E-07	0	N/A	29	6.315E-07
A11	0.007	38	0.254	0	N/A	**B11**	7.360E-07	0	N/A	23	5.781E-07
A12	0.207	34	0.254	0	N/A	**B12**	1.280E-07	0	N/A	22	4.466E-07
Pearson's *R* between MCS PS and average molecule PS for hits: 0.261, *p*-value 0.413 Pearson's *R* between MCS PS and average molecule PS for non-hits: 0.639, *p*-value 0.0254 Pearson's *R* between MCS PS and average molecule PS for hits and non-hits combined: 0.448, *p*-value 0.0280											

Supplementary Table 9. Average prediction scores of hits and non-hits associated with MCSs. Here, *N* denotes the numbers of associated hits or non-hits. PS, prediction score. MCS PS refers to the PS of the isolated MCS viewed as a molecular input to the ensemble Chemprop model, and the average molecule PS refers to the average PS of all hits or non-hits associated with the MCS of interest.