Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 484–487

Antimicrobial screening of a historical collection of over 140 000 small molecules

Victor V. Semenov, Mikhail M. Raihstat, Leonid D. Konyushkin, Roman V. Semenov, Mark A. T. Blaskovich, Johannes Zuegg, Alysha G. Elliott, Karl A. Hansford and Matthew A. Cooper

Intellectual property

In order to make this service as attractive as possible to the chemical community, the compound provider owns/retains all IP during the free screening stages, with CO-ADD making no claim to IP. All results and data generated by screening the compound(s) or material shall are the property of the participant, with the expectation, as mentioned earlier, that they will be made publically accessible in the CO-ADD database (and other databases such as ChEMBL) after an embargoed period of time. The ChEMBL database[S1] curates and stores standardized bioactivity, molecule, target and drug data extracted from multiple sources, including the primary medicinal chemistry literature. It brings together chemical, bioactivity and genomic data to aid the translation of genomic information into effective new drugs.[S2] Contributors can publish their results with no CO-ADD authorship, though an acknowledgment is requested. CO-ADD will work with collaborators to conduct additional characterization or support if requested, in which case authorship is requested Terms and Conditions for the provision of compounds & material to CO-ADD are presented,[S3] with a simple online agreement available if the contributing institution is amenable.

Screening Practical Aspects

All compounds must be soluble in DMSO or water and are preferably shipped as dry material (only 1–2 mg needed) in appropriate containers, such as 1–2 ml Eppendorf tubes. For larger collections, CO-ADD will send out bar-coded tubes (or 96 well-plates if DMSO stock solutions are provided) to the academic chemist groups. Chemists must provide the molecular weight and chemical formula of the substance and weigh an exact amount (1–2 mg) of pure compound. This quantity is sufficient to conduct primary screening, hit confirmation andpreliminary hit validation testing (see below). If samples are provided in DMSO, a volume of 100 μl at 10 mg ml^{-1} or 10 mM is required. Note that CO-ADD will only test isolated pure compounds for which structures are known: natural product extracts and fractions are not accepted

due to the large amount of work subsequently required to confirm what the active ingredient is, and whether it is novel. Furthermore, there are a large number of groups still conducting natural product prospecting, so CO-ADD feels this area of antibiotic discovery is adequately covered.

Step 1: Primary Screening

CO-ADD tests compound libraries against five key ESKAPE pathogens: gram-positive – *Staphylococcus aureus* (MRSA, ATCC 43300), gram-negative – *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ESBL,[†] ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), as well as the fungi *Cryptococcus neoformans* (H99, ATCC 208821) and *Candida albicans* (ATCC 90028). Fungal resistance is a growing concern, and there are even fewer antifungal therapies in the drug development pipeline than antibacterials.[S2,S5] The screening is at a single concentration (32 μg ml^{-1}) and provides the initial activity data to select compounds for additional detailed screening.

Step 2: Hit Confirmation

Active compounds from the primary screening will be tested in dose-response antimicrobial assays to confirm their antimicrobial activity and against mammalian HEK-293 cells to assess their cytotoxicity and for hemolytic activity against human red blood cells. This Hit confirmation process evaluates the basic therapeutic window of a compound, comparing their potency against pathogenic microbes to their cell toxicity, identifying the best potential compounds for further development.

Step 3: Hit Validation

The next step of the CO-ADD process is to test promising hit compounds against a broader panel of microbes with MDR and extensively-resistant (XDR) bacterial strains and clinical isolates, including with different co-factors (such as serum or lung surfactant). The Hit Validation process can include initial ADME screening to establish that the molecule is drug-like, including microsome and plasma stability and protein binding, as well as additional microbiological investigations, like time-kill, resistance frequency and membrane depolarization investigation. This stage will, however, be done as part of a separate research collaboration arrangement, with the research group providing additional material and structural analogues and CO-ADD providing the extended microbiological investigations. This Hit Validation will evaluate the basic *drug* qualities of actives, any structure-activity relationship, and early mode of action and resistance, selecting hit or hit clusters with potential for optimization to treat infection caused by drug-resistance bacteria.

[†] Extended spectrum beta-lactamase producing.

Some of the unique chemical diversity within the library of compounds assayed by CO-ADD is reflected by the analysis of metal-containing compounds. Nearly 1000 were identified, and, illustrating the power of data analysis from a large set of compounds tested under standardized conditions, they were found to have a 'hit rate' over ten-fold higher than standard 'organic' compounds.[S4].

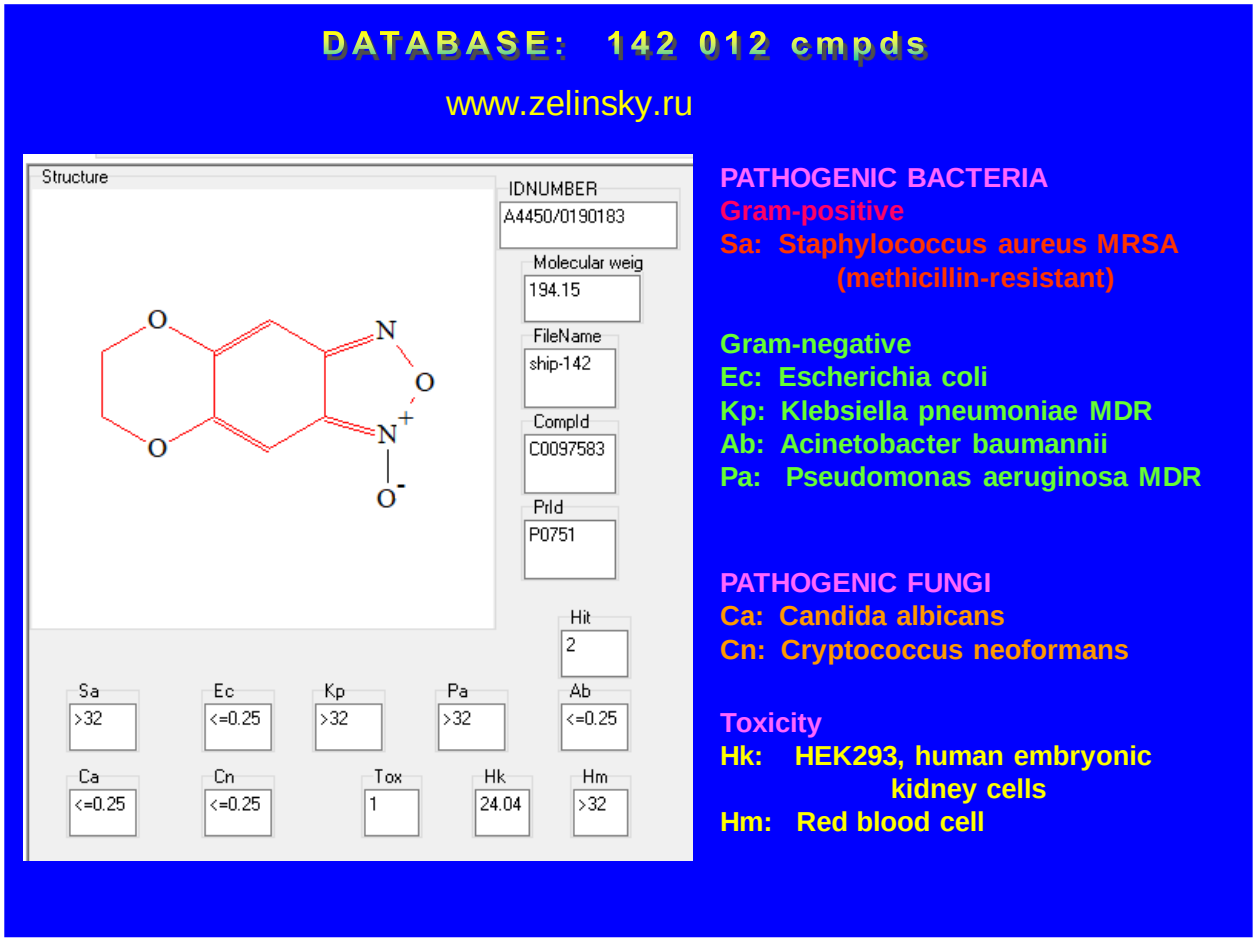

Figure S1 Screenshot of the antimicrobial activity of the structure recorded in the CheD program database.[S5]

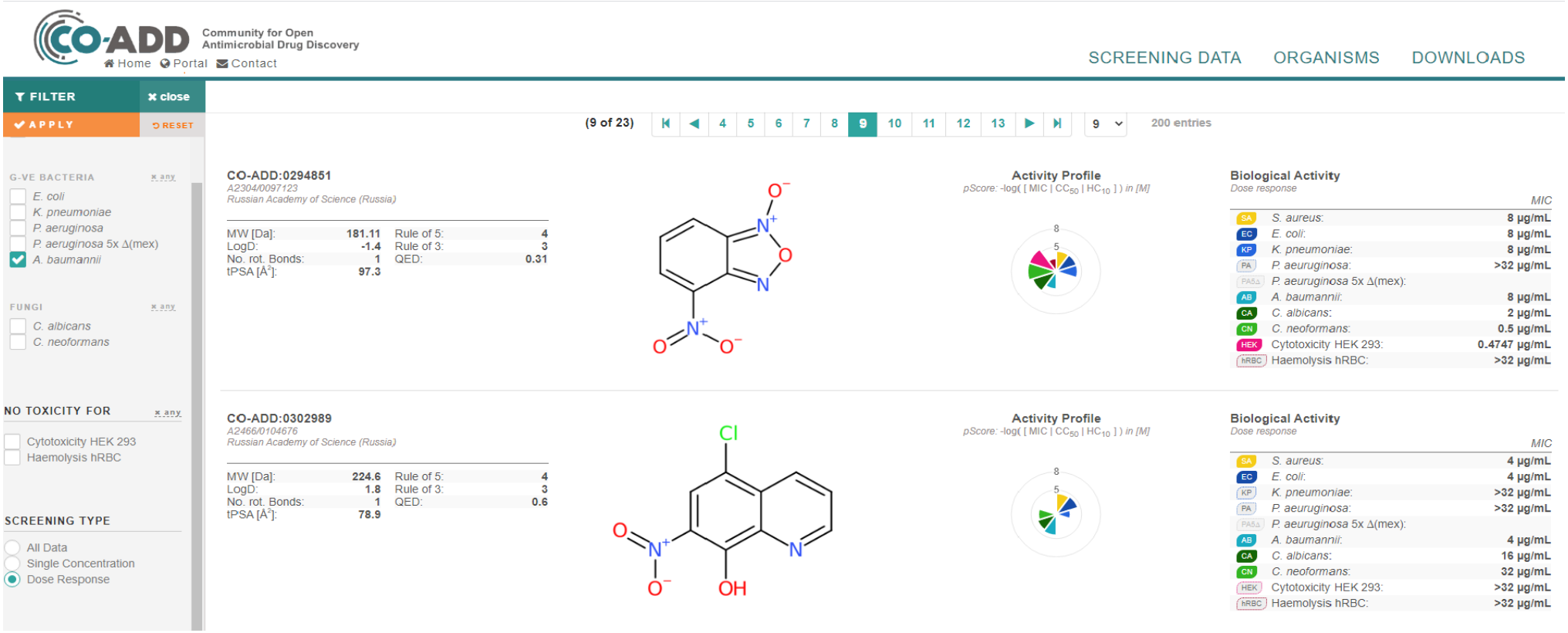

Figure S2 Screenshot of a summary overview of compound structures and associated antimicrobial data within CO-ADD open-access database.[S6]

References

S1 ChEMBL Database, ver. ChEMBL_28, 2021. https://www.ebi.ac.uk/chembl/.

S2 M. Davies, M. Nowotka, G. Papadatos, N. Dedman, A. Gaulton, F. Atkinson, L. Bellis and J. P. Overington, *Nucleic Acids Res.*, 2015, **43**, W612.

S3 Terms and conditions for provision of compounds & material to CO-ADD, Community for Open Antimicrobial Drug Discovery, December, 2018. https://www.co-add.org/content/terms.

S4 A. Frei, J. Zuegg, A. G. Eliott, M. Baker, S. Braese, C. Brown, F. Chen, C. G. Dowson, G. Dujardin, N. Jung, A. Paden King, A. M. Mansour, M. Massi, J. Moat, H. A. Mohamed, A. K. Renfrew, P. J. Rutledge, P. J. Sadler, M. H. Todd, C. E. Willans, J. J. Wilson, M. A. Cooper and M. A. T. Blaskovich, *Chem. Sci.*, 2020, **11**, 2627.

S5 CheD-program database, CHEmicalDatabase. https://www.zelinsky.ru/files/public/ChedDemoCBI.zip.

S6 Open-access Antimicrobial Screening Database, Community for Open Antimicrobial Drug Discovery, February 1, 2020. https://db.co-add.org.