þÿImitation of ²-lactam binding enables broad-spe
þÿmetallo-²-lactamase inhibitors

Brem, Jürgen

2022-01-12

Brem , J , Panduwawala , T , Hansen , J U , Hewitt , J , Liepins , E , Donets , P , Espina , L , Farley , A , Shubin , K , Gomez Campillos , G , Kiuru , P , Shishodia , S , Krahn , D , Lesniak , R , Schmidt (Adrian) , J , Calvopina , K , Turrientes , M-C , Kavanagh , M E , Lubriks , D , Hinchliffe , P , Langley , G , Aboklaish , A F , Eneroth , A , Backlund , M , Baran , A G , Nielsen , E , Speake , M , Kuka , J , Robinson , J , Grinberga , S , Robinson , L , McDonough , M , Rydzik , A , Leissing , T , Jimenez-Castellanos , J C , Avison , M B , Da Silva Pinto , S , Pannifer , A D , Martjuga , M , Widlake , E , Priede , M , Hopkins Navratilova , I , Gniadkowski , M , Belfrage , A K , Brandt , P , Yli-Kauhaluoma , J , Bacque , E , Page , M G P , Björkling , F , Tyrrell , J M , Spencer , J , Lang , P A , Baranczewski , P , Canton , R , McElroy , S P , Jones , P S , Baquero , F , Suna , E , Morrison , A , Walsh , T R & Schofield , þÿC J 2022 , ' Imitation of ²-lactam binding enables broad-spectrum meta inhibitors ' , Nature Chemistry , vol. 14 , no. 1 , pp. 15-24 . https://doi.org/10.1038/s41557-021-00831-x

http://hdl.handle.net/10138/355973
https://doi.org/10.1038/s41557-021-00831-x

other
acceptedVersion

Title

Imitation of β-Lactam binding enables broad spectrum metallo-β-Lactamase inhibitors

Author list

Jürgen Brem[1*], Tharindi Panduwawala[1], Jon Ulf Hansen[2], Joanne Hewitt[3], Edgars Liepins[4], Pawel Donets[4], Laura Espina[5], Alistair Farley[1], Kirill Shubin[4], Gonzalo Gomez Campillos[1], Paula Kiuru[6], Shifali Shishodia[1,7], Daniel Krahn[1], Rob Lesniak[1], Juliane Schmidt (Adrian)[1], Karina Calvopina[1], Mari Carmen Turrientes[8], Madeline E. Kavanagh[1,9], Dmitrijs Lubriks[4], Philip Hinchliffe[10], Gareth W. Langley[1,11], Ali F. Aboklaish[5], Anders Eneroth[12], Maria Backlund[12], Andrei G. Baran[4], Elisabet Nielsen[13], Michael Speake[3,14], Janis Kuka[4], John Robinson[3,14], Solveiga Grinberga[4], Lindsay Robinson[3,14], Michael McDonough[1], Anna Rydzik[1,15], Thomas Leissing[1], Juan Carlos Jimenez-Castellanos[10,16], Matthew B. Avison[10], Solange Da Silva Pinto[1], Andrew D. Pannifer[3], Marina Martjuga[4], Emma Widlake[5], Martins Priede[4], Iva Hopkins Navratilova[3], Marek Gniadkowski[17], Anna Karin Belfrage[18], Peter Brandt[18,19], Jari Yli-Kauhaluoma[6], Eric Bacque[20], Malcolm G. P. Page[21], Fredrik Björkling[22], Jonathan M. Tyrrell[5,10], James Spencer[10], Pauline A. Lang[1], Pawel Baranczewski[23], Rafael Canton[8], Stuart P. McElroy[3,14], Philip S. Jones[3,14], Fernando Baquero[8], Edgars Suna[4], Angus Morrison[3,14], Timothy R. Walsh[5], and Christopher J. Schofield[1*]

Affiliations

[1]University of Oxford, Department of Chemistry, Chemistry Research Laboratory and the Ineos Oxford Institute for Antimicrobial Research, OX1 3TA Oxford, United Kingdom.

[2]Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen, Denmark.

[3]University of Dundee, European Screening Centre, BioCity Scotland, Newhouse, Lanarkshire, United Kingdom.

[4]Latvian Institute of Organic Synthesis, Aizkraukles Str. 21, Riga, 1006, Latvia.

[5]Department of Medical Microbiology, Institute of infection & Immunity, Cardiff University, UHW Main Building, Heath Park, Cardiff, United Kingdom.

[6]Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland.

[7]Current address: Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States

[8]Department of Microbiology, Institute for Health Research (IRYCIS), Ramón y Cajal University Hospital, 28034 Madrid, Spain.

[9]Current Address: Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA.

[10]School of Cellular and Molecular Medicine, University of Bristol Biomedical Sciences Building, University Walk, Bristol BS8 1TD, United Kingdom.

[11]Current Address: Charles River Laboratories, Chesterford Research Park, Saffron Walden, CB10 1XL, United Kingdom.

[12]Department of Pharmacy, Uppsala Drug Optimization and Pharmaceutical Profiling Platform (UDOPP), Uppsala University, Uppsala, Sweden.

[13]Department of Pharmacy, Uppsala University, Uppsala, Sweden.

[14]Current Address: BioAscent Discovery Ltd. Bo'Ness Road, Newhouse, Lanarkshire, United Kingdom.

[15]Current address: Research and Early Development, Respiratory & Immunology, AstraZeneca, 431 83 Mölndal, Sweden.

[16]Current Address: Chemical Biology of Antibiotics. Centre for Infection & Immunity (CIIL). Pasteur Institute, INSERM U1019 - CNRS UMR 9017. Rue du professeur Calmette. 59019 Cedex, France.

[17]Department of Molecular Microbiology, National Medicines Institute, 00-725 Warsaw, Poland.

[18]Department of Medicinal Chemistry, Drug Design and Discovery, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden.

[19]Medicinal Chemistry, Research and Early Development, Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.

[20]Evotec Infectious Diseases (Lyon), Marcy l'Etoile, France.

[21]Jacobs University Bremen gGmbH, 28759 Bremen, Germany.

[22]Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

[23]Department of Pharmacy, SciLifeLab Drug Discovery and Development Platform, ADME of Therapeutics Facility, Uppsala University, Uppsala, Sweden.

*Correspondence: christopher.schofield@chem.ox.ac.uk or jurgen.brem@chem.ox.ac.uk.

Abstract

Carbapenems are vital antibiotics, but their efficacy is increasingly compromised by metallo-β-lactamases (MBLs). Here we report the discovery and optimisation of potent broad spectrum MBL inhibitors. A high-throughput screen for NDM-1 inhibitors identified indole-2-carboxylates (InCs) as potential β-lactamase stable β-lactam mimics. Subsequent structure-activity relationship studies reveal InCs as a new class of potent MBL inhibitor, active against all MBL classes of major clinical relevance. Crystallographic studies reveal a binding mode of the InCs to MBLs that in some regards mimics that predicted for intact carbapenems, including with respect to maintenance of the Zn(II) bound hydroxyl, and in other regards mimics binding observed in MBL-carbapenem product complexes. InCs restore carbapenem activity against multiple drug resistant Gram-negative bacteria and have a low frequency of resistance. InCs also have a good in-vivo safety profile, and when combined with meropenem, show strong in-vivo efficacy in peritonitis and thigh mouse infection models.

Main text

Introduction

The increase in antibiotic resistance raises concerns that, at least in some regions, we are returning to a pre-antibiotic era, in particular for Gram-negative infections. The increased prevalence of extended spectrum serine-β-lactamases (ESBL) and metallo-β-lactamases (MBLs) means β-lactams are increasingly ineffective in treating Gram negative infections[1, 2]. The advent of mobilized colistin resistance-1 (mcr-1) in 2015[3] and the novel transferable tigecycline resistance genes (tetX3-5) in 2019[4] which mediate resistance to colistin and tigecycline, respectively, means all clinically vital antibiotics for serious Gram-negative infections are compromised. There are few novel anti Gram-negative drugs entering clinical trials, therefore, overcoming resistance to restore the activity of existing drugs with an excellent safety record, e.g. β-lactams is important (Fig. 1a), [5].

Carbapenems, often 'drugs of last resort', manifest stability to ESBL, though are hydrolyzed by SBL carbapenemases and all MBLs[6, 7, 8]. Avibactam, relebactam, and vaborbactam are recently introduced SBL carbapenemase inhibitors[9, 10, 11], but excepting vaborbactam, which has a relatively limited activity spectrum[12, 13], these and classical SBL inhibitors (e.g. clavulanate) are increasingly susceptible to β-lactamases, including MBLs which degrade all β-lactam classes[6, 14]. Development of MBL inhibitors (MBLi), in particular to protect carbapenems, is thus an unmet clinical need, especially in the developing world, where MBL producing bacteria are widely disseminated.

MBL inhibition is challenging because of structural diversity in MBL active sites (Fig. 1b)[15, 16]. By contrast with the SBLs, no clinically useful MBLi are available. Most reported MBLi (Fig. 1B and Supplementary Fig. 1) [17, 18, 19] lack the breadth of potency against relevant MBL variants that is required for clinical use[7, 20]. Most MBLi inhibit by tight Zn(II) chelation, at the active site or in solution, the latter a property that may make it difficult to achieve selectivity over human metallo-enzymes[6]. Aspergillomarasmine A[18], a Zn(II) chelator and the preclinical candidate ANT-2681[17] an active site Zn(II)

binder active in a mouse model, both have limited MBL coverage, as does the SBL inhibiting bicyclic boronate taniborbactam (which is in Phase 3 trials[19]).

Given that imitation of the initial substrate binding mode has been successfully employed for SBL inhibition (e.g. by β-lactam mediated SBL inhibition by clavulanate) and that β-lactam antibiotics are mimics of the substrates of their transpeptidase targets[9, 21, 22, 23, 24], we envisaged an analogous 'substrate-focused' approach may enable identification of broad-spectrum MBLi. Here we report on efforts by two public-private partnerships, the European Lead Factory(ELF)[25] and the European Gram-negative Antibacterial Engine (ENABLE)[26] Oxford MBLi project that led to the identification and optimisation of indole carboxylates (InCs, Fig. 1c-d) as broad spectrum MBLi. The InCs have an unprecedented MBL binding mode, which different regards those of both carbapenem substrates and products. InCs protect carbapenems from MBL activity in MDR and XDR (multi and extensively drug-resistant) Gram-negative pathogens as shown by in vitro and *in vivo* mouse infection models.

Results

Hit finding

To identify new broad spectrum MBLi, we carried out a fluorescence-based[31] high-throughput screen employing NDM-1 and the ELF compound collection. This led to identification of several new, but typical MBLi, i.e. heteroaromatic Zn(II) chelators[32, 33]. Several InCs (**1**, **2)** (Fig. 1)[34], were identified as novel reversibly binding, non-covalent, competitive NDM-1 inhibitors (pIC_{50}s: 7.7) that are not influenced by the added of excess of zinc(II) ions; these attracted our attention, because of their structural similarity to carbapenems/carbapenem derived products[28, 35, 36, 37], because they can be efficiently prepared, and because they are not obvious potent Zn(II) chelators. The potential of InCs as broad spectrum B1 subfamily MBLis was demonstrated by studies with VIM-1, VIM-2 and IMP-1. At this stage we used crystallography to investigate the InC binding mode. As described below, these structures revealed a novel binding mode, in part mimicking that of β-lactams prior to their hydrolysis (Fig. 1).

Structure-activity relationship (SAR) studies

We initiated SAR studies employing NDM-1, VIM-1, and -2 and IMP-1, in which positions amenable to derivatisation (N-1, C-2 to C-7) were modified. The results reveal roles of the indole NH and the C-2 carboxylate in potent inhibition; N-methylation or replacement of the C-2 carboxylate reduces activity by ~1000 fold (**3-7**, Supplementary Tables 1-2). C-3 modification was found to be useful, as shown by studies with C-3 aryl InCs (**8-26**, Supplementary Table 3). The C-4, C-5 and C-6 positions in general were less amenable to diverse derivatisation (**27-37**, Supplementary Tables 4-5). A C-7 substituent was found to be important in inhibition, with an isopropyl group being preferred in initial studies, though the initial SAR importantly revealed larger groups could be accommodated at C-7 (**1, 38-46**, Supplementary Table 6).

InCs with *para*-substituted phenyl groups at C-3 and a C-7 methyl displayed good activity, with pIC_{50}s of 6.0-6.5 for NDM-1, VIM-1/-2, and IMP-1 (**16, 18**, **20**, Supplementary Table 3). Further C-3 SAR including with di-substituted phenyls, led to pIC_{50}s > 8 against NDM-1, VIM-1/-2 and IMP-1 (**47-50**, **52**, **55**, Supplementary Table 7); in some cases, pIC_{50}s of >9 were achieved for NDM-1 indicating the potential for highly potent inhibition (**5**, **47**, **48**, **52,** Supplementary Table 7). Binding of selected compounds, i.e. with a C-7 methyl (**1**, **8**, **9**, **11-19**, **24-26**, **38**), OMe (**39**), -F (**40**), $-CF_3$ (**43**), -*t*Bu (**46**), or -*i*Pr(**32**), to NDM-1, VIM-1 and IMP-1 was analysed by surface plasmon resonance (SPR)[38]. Good correlation was observed between the pIC_{50} and SPR results (Supplementary Fig. 2), with SPR derived on-rates being 10^5-10^6 $M^{-1}s^{-1}$ and off rates of up to 10^{-2} s^{-1} (Supplementary Tables 8-10); **16** has a K_D = 7 nM for NDM-1. Overall, the early SAR study achieved substantial improvements (up to 100-fold versus **1**) in potency and revealed the amenability of the C-3 and C-7 InC positions to modification.

Anti-microbiological activity, permeation and efflux studies

We evaluated the in-vitro activity of InCs in combination with carbapenems against MDR and XDR bacteria possessing various MBLs. Given the global rise in carbapenem resistance in Enterobacterales we targeted these pathogens. Early studies revealed reduced activity for **5** and **50**-**55** with *Klebsiella pneumoniae* clinical isolates compared to *Escherichia coli* (carrying NDM, IMP or VIM MBLs, Supplementary Table 11) revealing factors other than potency against isolated MBLs are important for inhibition in cells.

To optimise InC activity against clinically relevant MDR/XDR pathogens, including with altered permeation and/or elevated efflux properties, we engineered strains of *K. pneumoniae* NCTC 5055 carrying pBAD (*ramA*) and Ecl8 Ecl8Δ*ramR*[39] with plasmids expressing NDM-1, VIM-1, or IMP-1 MBLs[39].(Supplementary Tables 12-13 and Supplementary Fig. 3). The results demonstrate the ability of InCs to restore meropenem activity in MDR/XDR Enterobacterales producing NDM-1, VIM-1, VIM-

4, IMP-1, and IMP-4 (Supplementary Tables 11-13). Note, despite the excellent InC activity versus isolated VIM-2 and NDMs, the InC combinations show limited activity against VIM and NDM producing *P. aeruginosa* and *Acinetobacter* spp., probably because of cell permeability constraints (Supplementary Table 14).

Physicochemical, ADME, receptor testing, *in vivo* tolerability and efficacy for the InC hits

InC **49** was selected to assess the suitability of InCs for use *in vivo* - **49** does not interact with 30 human receptors and has acceptable physicochemical and ADME properties (Supplementary Tables 15-16). **49**, however, exhibits high plasma protein binding and MIC studies in the presence of serum reduced its activity (Supplementary Table 17). No adverse effects were observed in mice with single doses of 10 or 100 mg/kg of **49** (Supplementary Table 18). A proof of concept study revealed synergy between meropenem and **49** for treatment of mice infected with *E. coli* 91N [40] (**49** at a 100 mg/kg dose, data not shown). The two weaknesses of the initial InCs as exemplified by **48**, **49** and **53**, i.e. relatively low metabolic stability and high plasma protein binding (Supplementary Table 18) were then addressed by SAR studies guided by crystallography.

Structural studies

We obtained > 50 high resolution structures (< 1.5 Å) with clinically relevant B1/B3 subfamily MBLs, exemplified by structures of InCs with both B1 MBLs (VIM-1:**49**, VIM-2:**11** and NDM-7:**48)** and the B3 MBL L1:**49** (Fig. 2A-D; Supplementary Figures 4-5 and Supplementary Tables 20-21). These reveal a highly conserved and unprecedented MBL binding mode for the InCs (Fig. 2a-d).

The InCs inhibit via a binding mode contrasting with that of most active site binding MBLi, which displace the bridging water. In all the InC structures, both Zn(II) ions and, importantly, the bridging water/hydroxide are present in high occupancy, with one of their C-7 isopropyl methyl groups

apparently locking the hydroxide in its Zn-Zn bridging location. The Zn-Zn and Zn-water/hydroxide ion distances in the MBL-InC complexes correspond to those observed for MBLs without inhibitors (~3.5 Å, Fig. 1 and Supplementary Fig. 4[15, 33, 41]); these distances increase in product or intermediate/intermediate mimic MBL-complexes[15, 30](Fig 1. and Supplementary Fig. 1). Thus, that at least with respect to retention of the hydrolytic hydroxide and the Zn-Zn distance, the InC binding mode mimics that anticipated for substrates. However, it should be noted that the binding mode of the pyrrole ring of the InCs is similar to that observed for the pyrroline ring in active site bound hydrolysed carbapenems, especially when in its enamine form (as shown in Fig. 1B and Supplementary Fig. 1), which is proposed to be the major nascent product for MBL catalysed carbapenem hydrolysis[28]. It should be noted most MBL-hydrolysed carbapenem structures show the complexed product in the imine tautomeric form, though this is not necessarily relevant in efficient catalysis (Supplementary Fig. 1). Thus, the binding mode of the InCs resembles those of both substrates and products, albeit in different regards.

The C-2 InC carboxylate ligates to Zn2, in a manner reminiscent of the carboxylate of β-lactam substrates (Fig. 3). Importantly, this binding mode enables InCs to engage with the different motifs employed in MBL substrate carboxylate binding, e.g. VIM-1 (B1) uses a histidine-, VIM-2 (B1) an arginine-, NDM-7 (B1) a lysine-, and L1 (B3) a serine-residue in substrate carboxylate binding (Fig. 2A-D).

The InC C-3 substituent interacts with residues at the active site surface in a manner analogous to that of cephalosporin C-3'/ carbapenem C-2 sidechains (Fig. 2G). At least one of the methyl-groups of the C-7 isopropyl points towards the surface of the active site where the C-6/C-7 groups of the penicillins/cephalosporins, bind (Fig. 2A-D). Thus, binding of C-7, C-3 alkyl/aryl and C-2 carboxylate substituted InCs mimics those of β-lactams and the structures rationalise the SAR observations that both the InC C-3 and C-7 positions are amenable to substitution with relatively large groups (Fig. 2.E-F).

β-Lactamase inhibition profile

Comparison of the potency of six InCs (**1**, **11**, **16**, **48**, **49** and **53**) with the cyclic boronate taniborbactam (a dual MBL and SBL inhibitor) and avibactam (AVI, an SBL inhibitor); against representative SBLs/MBLs reveals the superiority of the InCs for MBL inhibition. The InCs potently inhibit all the tested clinically relevant B1/B3 MBLs; for several MBLs (SPM-1, L1, IMP-1, NDM-7, VIM-1) 100-1000-fold improved activity compared to VNRX-5133 is observed (Supplementary Table 22). Given both SBLs and MBLs recognise the same substrates (Fig. 2g), it is interesting that InCs are also SBL inhibitors (pIC_{50} values 2.4-5.1), though at a much lower level than for MBLs (Fig 3 and Supplementary Table 22)[30].

Lead optimisation

We then carried out SAR studies at the InC C-3 and C-7 positions employing structural information, to improve the metabolic stability and plasma protein binding of **49**, while maintaining/ improving activity versus isolated MBLs (Supplementary Table 23). The general synthetic routes used to prepare the InCs are shown in Fig. 4. Modification at C-3 was achieved via Pd-catalysed arylation of bromo- or iodo- precursors, prepared from commercial indoles or those prepared from anilines. Racemic C-3/C-7 amide derivatives (e.g. **56**) were prepared by reductive amination of a C-7 methyl ketone, followed by amide formation. Chiral C-7 amides (e.g. **57, 58**) were prepared from the same starting materials via imine formation with (*R*)-*tert*-butylsulfinamide, then organoborane (*L*-Selectride) mediated reduction and hydrolysis to give a chiral C-7 amine which was coupled to give **57** and **58**.

The SAR studies improved NDM-1 activity, as exemplified by (±)**56** which manifests >10-fold improvement over **49**. However, these studies did not translate into improved VIM-1 activity (Fig. 3). Docking studies indicated the (*S*)-enantiomer of racemic **56**, would preferentially bind to NDM-1. Indeed, **57** showed improved activity versus NDM-1 (pIC_{50} > 10.2 against NDM-1, the limit of detection in our standard assay).

To improve VIM-1 activity, we installed a novel amide isostere, i.e. a spirocyclic oxazolidinone at C7 together with a primary amine, the latter to improve accumulation in Gram-negative bacteria[42]. Separation of diastereomers of the spirocyclic oxazolidinone gave **58** and **59** (Fig. 4), which showed similar 2 log fold activity improvement versus VIM-1 (compared to **57**); **59** shows improved NDM-1, VIM-2 and IMP-1 activity compared to **58** (0.6-0.8-fold, Supplementary Table 23). Thermal shifts assays measured using differential scanning fluorimetry (DSF) reveal binding of **58** stabilises NDM-1 (the melting temperature of NDM-1 increase by 14°C in the presence of **58**, Supplementary fig. 6A) and its resistance to proteolytic degradation also increases as revealed by MS studies (Supplementary Fig. 6B-D).

Microbiological studies reveal similarly good activity for **58** and **59** (Supplementary Table 24), which manifest excellent metabolic stability ($t_{1/2}$ > 450 min in mouse, rat, and human hepatocytes), though the fraction of unbound compound to human and mouse plasma was higher for **58** mouse/human f_u (%) 6/38 versus 1.8/28, than for **59**. In the case of **58** this represents a >500-fold improvement compared to **49** (Supplementary Table 25).

Restoration of carbapenem activity against Enterobacterales pathogens and determining frequency of resistance

We investigated the level to which **58** (8 mgl^{-1}) restores carbapenem activity against 280 (Fig. 5 and Supplementary Table 27), genome sequenced MDR/XDR global Gram-negative clinical isolates (Supplementary Table 26). The MIC_{90}s for meropenem, imipenem, or doripenem alone against the panel were all >64 mg l^{-1} (Supplementary Table 24). Combinations of **58** and a carbapenem resulted potent activity and broad coverage against Enterobacterales (MIC_{90} 1-2 mg l^{-1}). MIC_{90}s were in several cases up to 4-fold below the carbapenem breakpoint. Excellent activity was obtained against *Citrobacter* spp., *S. marcescens* and *Proteus mirabilis* (meropenem MIC_{90} 0.125-0.5 μg/mL). The MIC_{90}s

against other tested strains (*E. coli*, *K. pneumoniae, Providencia* spp. and *Enterobacter* spp.) were 2-4 mg l^{-1} and 0.5-1 mg l^{-1} for **58** with meropenem or doripenem, respectively. Compared to the combination of taniborbactam with cefepime or meropenem, the **58**-meropenem combination showed up to 6-fold higher activity (Supplementary Tables 28).

The frequency of spontaneous mutational resistance (FoR) to combinations (imipenem/meropenem) with **58** was low against three strains used for the *in vivo* studies. The corresponding figure for the hypermutable (100x mutation rate) strain *E. coli GB20 (ΔampC mutS::Tn10)* strain[43] the FoR was < 10^{-10} (below the level of detection at 4xMIC) (Supplementary Table 29). Similarly, seven days serial passage experiment revealed that **58** reduce the development of resistance to meropenem or imipenem when compared to meropenem or imipenem alone (Supplementary Fig. 7).

Safety and *in vivo* activity

InC **58** showed no evidence for major interactions with 69 human receptors (Supplementary Table 26) and was well tolerated in mice (300 mg/kg, subcutaneous 7-day repeat dose) with only mild side effects (Supplementary Table 31). Macroscopic organ changes were not observed and plasma kidney and liver markers (BUN, KIM-1, ALAT and ASAT) were not elevated.

We examined the efficacy of the **58**-meropenem combination in total of eight murine infection models using four different strains (Fig. 6A-C). To identify suitable clinically derived isolates, we screened >30 strains in pilot murine models. Overall, we have not found correlation between meropenem MIC and *in vivo* virulence or EC_{50}. For **58**, single IV doses (10 or 30 mg/kg) were used, which based on single dose pharmacokinetic studies resulted in peak **58** concentrations of 75 and 224 µg/mL, respectively. At this dose, the $t_{1/2}$ of **58** was ~0.69 h, considerably longer than that of meropenem (0.17 h) (Supplementary Fig. 8).

With the peritonitis / septicaemia models (Fig. 6B and Supplementary Figs. 9-12) using three *E. coli* strains and one *K. pneumoniae strain*, the **58**-meropenem combination significantly reduced the mean CFUs compared to meropenem alone, by as much as ~5 log-fold CFU. With the thigh infection models (Fig. 6C, Supplementary Figs. 13-16) using the same strains at a dose of **58** 10 mg/kg, the effect was reduced, being <1 log fold CFU reduction for two of the strains *E. coli* strains and was ~1 log-fold CFU for the *E. coli* IHMA and the *K. pneumoniae* B-68-1 strains. However, at a higher **58** dose at 30 mg/kg the CFU count was reduced up to ~2 log CFU.

Discussion

The clinical need for new treatments against MDR/XDR Gram-negative infections, in particular for Enterobacterales is critical[45]. Due primarily to the excellent safety profiles and efficacy of β-lactams in the absence of β-lactamases one successful strategy has been development of SBL inhibitors[5]. However, there are no MBLi in clinical use, and only taniborbactam, which has a narrow spectrum of MBL activity, is in clinical trials.[19] The lack of MBLi is in part due to the challenge of achieving the breadth of potency against different subclasses of clinically relevant MBLs (Figs. 1-2) [46]. Our collaboration involving academic and pharmaceutical partners operating across multiple sites has enabled the development of a novel type of MBLi, **58**, that potentiates *in-vivo* activity of carbapenems.

We used a high-throughput screen to search for novel NDM-1 inhibitors with a binding efficiency mimicking that of β-lactam substrates, based on the premise that most β-lactamases have evolved to bind their substrates with remarkable efficiency (Figs. 1-2). We envisaged that inhibitors that mimic the elements of bicylic β-lactam substrate which are involved in MBL binding might enable the breadth of MBL inhibition required for clinical application (Fig. 2g). By contrast with our substrate-focused approach a (single) protein-based structure-based design approach might not favour the identification of such broad-spectrum inhibitors.

After crystallographic studies on several new MBLi classes identified from the HTS/NDM-1 inhibition screening, we focused on the InCs. We found that C-3 and C-7 functionalised InCs are broad spectrum NDM, VIM, and IMP B1 MBL inhibitors, but they also inhibit B3 MBLs, as shown by studies with L1. SAR studies guided by structure-based design and analyses in solution reveal the importance of the InC indole NH, C-2 carboxylate, C-3 and C-7 alkyl/aryl groups for potent MBL inhibition (Fig. 2A-D). The InC core can be readily synthesised and is amenable to modification at C-3 and C-7 via established and new procedures (Fig. 5).

In some regards the biophysical analysis support the proposal that the InC binding mode mimics that of intact bicyclic β-lactams, in particular carbapenems (Fig. 2G). Most strikingly, the indole NH and a C-7 alkyl groups cooperate to form a stable complex in which the di-Zn(II) ion bridging hydroxide is retained and the Zn-Zn distance is the same as in the unligated MBLs. This binding mode contrasts with those of nucleophilic MBL inhibitors (e.g. thiols, such as captopril[29]) and transition state analogues as bicyclic boronates[30], both of which displace the Zn-complexed hydroxide. The C-7 alkyl group appears to enclose the bridging water/hydroxide and the indole NH is positioned to hydrogen bound to the bridging water, i.e. it may mimic protonation of the β-lactam nitrogen that must occur during hydrolysis (Fig. 1).

However, the binding mode of the InCs also resembles those of carbapenem derived products bound to the MBL active site, in particular in their enamine tautomeric form, which has been proposed to represent the major nascent product of MBL catalysed carbapenem hydrolysis[28] (Supplementary Fig. 1). Thus, binding of the InCs appears to take advantage of elements included in both substrate and product binding, property that may contribute to their high potency.

Further, the InC C-2 carboxylate binding mode is similar to that of the carboxylate of β-lactam substrates (and β-lactam derived intermediates), including with respect to interaction with the different active site elements used in substrate carboxylate binding by all of NDM-1, VIM-1, VIM-2 and L1, likely contributing to their breath of InC potency (Fig. 2).

The InC binding mode contrasts with those of other bidentate MBLi in different stage of development, e.g. Aspergillomarasmine A[18], thioenolates (derived from the corresponding rhodanines)[47], bicyclic boronates, and pyridine- and thiazole-derivatives. Some of these are strong metal ion chelators and therefore could inhibit multiple human metallo enzymes including human MBLs[48], potentially leading to toxicity. Whether or not these differences will be reflected in *in vivo* efficacy, selectivity and/or safety profiles is of interest with respect to future studies.

Overall, the InCs represent a new type metalloenzyme inhibitor that works via an unprecedented mechanism of action that locks the zinc complexed hydroxide, rather than displacing it. Importantly compared to most reported MBLi, the InCs are relatively weak metal ion chelators. The metal-hydroxyl trapping mechanism of action manifested by the InCs for MBL inhibition, may be of utility in the inhibition of other classes of MBL fold hydrolases that are medicinal chemistry targets[48] and more generally for inhibition of metallo-hydrolases.

The carbapenem type binding mode of the InCs enabled us to fine tune MBL activity. Optimisation of the C-3 and C-7 side chains enabled the identification of **58**, which potently inhibit all the three targeted B1 MBL types, with $pIC_{50s} > 9$ for NDMs, VIM and >7 for IMP-1 being obtained (Supplementary Table 23). We also optimised *in vitro* microbiology and ADME properties, the latter with regards to plasma protein binding and metabolic stability. It is notable that some InCs inhibit SBLs, although less potently than MBLs (Fig. 3) this difference may reflect the different interactions employed by MBLs and SBLs in binding/reacting with β-lactams, i.e. nucleophilic catalysis by SBLs versus metal ion enabled catalysis by MBLs. It should also be noted that all potent small-molecule SBL inhibitors inhibit via covalent reaction[16].

To assist SAR studies on optimising cell penetration and potential efflux properties, we used engineered strains with efflux pump and porin deficiencies as well as clinical isolates, mainly compromising *E. coli* and *K. pneumoniae* strains, producing VIM, NDM, and IMP variants (Supplementary Table 26). **58**-carbapenem combinations reduced MICs up to four-fold below the

carbapenem breakpoints against an MDR/XDR Enterobacterales panel (Fig. 5). The activity of the **58**-meropenem combination compared with those for taniborbactam plus meropenem or taniborbactam plus cefepime reveals the impressive efficacy of **58** in repressing B1 MBL mediated resistance (Supplementary Tables 28). The results for **58** combined with meropenem / imipenem demonstrate a lower FoR when compared to the carbapenems alone (Supplementary Table 29).

InC **58** is tolerated well by mice and when combined with meropenem shows significant *in vivo* efficacy in multiple murine peritonitis/sepsis and thigh models with infection by carbapenem-resistant XDR strains. A single dose of 10 mg/kg **58** plus 16-90 mg/kg meropenem reduced the bacterial load in murine infection models by up to seven log-fold. These results reveal that InCs have significant potential for clinical development with β-lactam antibiotics. We are actively progressing InCs towards clinical trials in humans, with a particular focus on low-middle income countries where NDM mediated resistance is widespread.

References

1. Livermore D.M., Canton R., Gniadkowski M., Nordmann P., Rossolini G.M., Arlet G.*, et al.* CTX-M: changing the face of ESBLs in Europe. *J. Antimicrob. Chemother.* 2007, **59**(2)**:** 165-174.

2. Kumarasamy K.K., Toleman M.A., Walsh T.R., Bagaria J., Butt F., Balakrishnan R.*, et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 2010, **10**(9)**:** 597-602.

3. Liu Y.Y., Wang Y., Walsh T.R., Yi L.X., Zhang R., Spencer J.*, et al.* Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 2016, **16**(2)**:** 161-168.

4. He T., Wang R., Liu D., Walsh T.R., Zhang R., Lv Y.*, et al.* Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. *Nat. Microbiol.* 2019, **4**(9)**:** 1450-1456.

5. Yahav D., Giske C.G., Grāmatniece A., Abodakpi H., Tam V.H., Leibovici L. New β-Lactam–β-Lactamase Inhibitor Combinations. *Clin. Microbiol. Rev.* 2020, **34**(1)**:** e00115-00120.

6. Bush K. Past and Present Perspectives on β-Lactamases. *Antimicrob. Agents Chemother.* 2018, **62**(10)**:** e01076-01018.

7. Rotondo C.M., Wright G.D. Inhibitors of metallo-β-lactamases. *Curr. Opin. Microbiol.* 2017, **39:** 96-105.

8. Bonomo R.A., Burd E.M., Conly J., Limbago B.M., Poirel L., Segre J.A.*, et al.* Carbapenemase-Producing Organisms: A Global Scourge. *Clin. Infect. Dis.* 2018, **66**(8)**:** 1290-1297.

9. Wang D.Y., Abboud M.I., Markoulides M.S., Brem J., Schofield C.J. The road to avibactam: the first clinically useful non-β-lactam working somewhat like a β-lactam. *Future Med. Chem.* 2016, **8**(10)**:** 1063-1084.

10. Ehmann D.E., Jahić H., Ross P.L., Gu R.-F., Hu J., Kern G.*, et al.* Avibactam is a covalent, reversible, non–β-lactam β-lactamase inhibitor. *PNAS* 2012, **109**(29)**:** 11663-11668.

11. Lomovskaya O., Sun D., Rubio-Aparicio D., Nelson K., Tsivkovski R., Griffith D.C.*, et al.* Vaborbactam: Spectrum of β-Lactamase Inhibition and Impact of Resistance Mechanisms on Activity in Enterobacteriaceae. *Antimicrob. Agents and Chemother.* 2017, **61**(11).

12. Langley G.W., Cain R., Tyrrell J.M., Hinchliffe P., Calvopiña K., Tooke C.L*., et al.* Profiling interactions of vaborbactam with metallo-β-lactamases. *Bioorg. Med. Chem. Lett.* 2019, **29**(15)**:** 1981-1984.

13. Papp-Wallace K.M., Mack A.R., Taracila M.A., Bonomo R.A. Resistance to Novel β-Lactam-β-Lactamase Inhibitor Combinations: The "Price of Progress". *Infect. Dis. Clin. North. Am.* 2020, **34**(4):773-819.

14. Lohans C.T., Brem J., Schofield C.J. New Delhi Metallo-β-Lactamase 1 Catalyzes Avibactam and Aztreonam Hydrolysis. *Antimicrob. Agents Chemother.* 2017, **61**(12)**:** e01224-01217.

15. Meini M.R., Llarrull L.I., Vila A.J.. Overcoming differences: The catalytic mechanism of metallo-β−lactamases. *FEBS Lett.* 2015, **589**(22)**:** 3419-3432.

16. Drawz S.M., Bonomo R.A.. Three decades of β-lactamase inhibitors. *Clini. Microbiol. Rev.* 2010, **23**(1)**:** 160-201.

17. Davies D.T., Leiris S., Sprynski N., Castandet J., Lozano C., Bousquet J.*, et al.* ANT2681: SAR Studies Leading to the Identification of a Metallo-β-lactamase Inhibitor with Potential for Clinical Use in Combination with Meropenem for the Treatment of Infections Caused by NDM-Producing Enterobacteriaceae. *ACS Infect. Dis.* 2020, **6**(9)**:** 2419-2430.

18. King A.M., Reid-Yu S.A., Wang W., King D.T., De Pascale G., Strynadka N.C.*, et al.* Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* 2014, **510**(7506)**:** 503-506.

19. Liu B., Trout R.E.L., Chu G.H., McGarry D., Jackson R.W., Hamrick J.C.*, et al.* Discovery of Taniborbactam (VNRX-5133): A Broad-Spectrum Serine- and Metallo-β-lactamase Inhibitor for Carbapenem-Resistant Bacterial Infections. *J. Med. Chem.* 2020, **63**(6)**:** 2789-2801.

20. Bush K., Bradford P.A. Epidemiology of β-Lactamase-Producing Pathogens. *Clin. Microbiol. Rev.* 2020, **33**(2):e00047-19.

21. Sauvage E., Terrak M. Glycosyltransferases and Transpeptidases/Penicillin-Binding Proteins: Valuable Targets for New Antibacterials. *Antibiotics (Basel)* 2016, **5**(1).

22. Bush K., Bradford P.A. Interplay between β-lactamases and new β-lactamase inhibitors. *Nat. Rev. Microbiol.* 2019, **17**(5)**:** 295-306.

23. Dik D.A., Fisher J.F., Mobashery S. Cell-Wall Recycling of the Gram-Negative Bacteria and the Nexus to Antibiotic Resistance. *Chem. Rev.* 2018, **118**(12)**:** 5952-5984.

24. Tipper D.J., Strominger J.L. Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. *PNAS* 1965, **54**(4)**:** 1133-1141.

25. Karawajczyk A., Orrling K.M., de Vlieger J.S.B., Rijnders T., Tzalis D. The European Lead Factory: A Blueprint for Public–Private Partnerships in Early Drug Discovery. *Front. Med.* 2017, **3**(75).

26. http://nd4bb-enable.eu/.

27. Lisa M.N., Palacios A.R., Aitha M., Gonzalez M.M., Moreno D.M., Crowder M.W*., et al.* A general reaction mechanism for carbapenem hydrolysis by mononuclear and binuclear metallo-β-lactamases. *Nat. Commun.* 2017, **8**(1)**:** 538.

28. Lohans C.T., Freeman E.I., Groesen E.v., Tooke C.L., Hinchliffe P., Spencer J*., et al.* Mechanistic Insights into β-Lactamase-Catalysed Carbapenem Degradation Through Product Characterisation. *Sci. Rep.* 2019, **9**(1)**:** 13608.

29. Brem J., van Berkel S.S., Zollman D., Lee S.Y., Gileadi O., McHugh P.J*., et al.* Structural Basis of Metallo-β-Lactamase Inhibition by Captopril Stereoisomers. *Antimic. Agents Chemother.* 2015, **60**(1)**:** 142-150.

30. Brem J., Cain R., Cahill S., McDonough M.A., Clifton I.J., Jimenez-Castellanos J.C*., et al.* Structural basis of metallo-β-lactamase, serine-β-lactamase and penicillin-binding protein inhibition by cyclic boronates. *Nat. Commun.* 2016, **7:** 12406.

31. Besnard J., Jones P.S., Hopkins A.L., Pannifer A.D. The Joint European Compound Library: boosting precompetitive research. *Drug Discov. Today* 2015, **20**(2)**:** 181-186.

32. Yan Y.H., Li G., Li G.B. Principles and current strategies targeting metallo-β-lactamase mediated antibacterial resistance. *Med. Res. Rev.* 2020, **40**(5)**:** 1558-1592.

33. Ju L.C., Cheng Z., Fast W., Bonomo R.A., Crowder M.W. The Continuing Challenge of Metallo-β-Lactamase Inhibition: Mechanism Matters. *Trends Pharmacol. Sci.* 2018, **39**(7)**:** 635-647.

34. Brem J., McDonough M.A., Schofield C.J., Morrison A., Hewitt J., Pannifer A., Jones P. Inhibitors of metallo-beta-lactamases. *WO2017093727A1 (WO, EP, JP, CN, AU)* 2017.

35. King D.T., Worrall L.J., Gruninger R., Strynadka N.C.J. New Delhi Metallo-β-Lactamase: Structural Insights into β-Lactam Recognition and Inhibition. *JACS* 2012, **134**(28)**:** 11362-11365.

36. Feng H., Liu X., Wang S., Fleming J., Wang D.-C., Liu W. The mechanism of NDM-1-catalyzed carbapenem hydrolysis is distinct from that of penicillin or cephalosporin hydrolysis. *Nat. Comm.* 2017, **8**(1)**:** 2242.

37. Raczynska J.E., Shabalin I.G., Minor W., Wlodawer A., Jaskolski M. A close look onto structural models and primary ligands of metallo-β-lactamases. *Drug Resist. Updat.* 2018, **40:** 1-12.

38. Cahill S.T., Tyrrell J.M., Navratilova I.H., Calvopina K., Robinson S.W., Lohans C.T.*, et al.* Studies on the inhibition of AmpC and other β-lactamases by cyclic boronates. *Biochim. Biophys. Acta Gen. Subj.* 2019, **1863**(4)**:** 742-748.

39. Jiménez-Castellanos J.-C., Wan Nur Ismah W.A.K., Takebayashi Y., Findlay J., Schneiders T., Heesom K.J.*, et al.* Envelope proteome changes driven by RamA overproduction in Klebsiella pneumoniae that enhance acquired β-lactam resistance. *J. Antimic. Chemother.* 2017, **73**(1)**:** 88-94.

40. Krajnc A., Brem J., Hinchliffe P., Calvopiña K., Panduwawala T.D., Lang P.A.*, et al.* Bicyclic Boronate VNRX-5133 Inhibits Metallo- and Serine-β-Lactamases. *J. Med. Chem.* 2019, **62**(18)**:** 8544-8556.

41. Lisa M.-N., Palacios A.R., Aitha M., González M.M., Moreno D.M., Crowder M.W.*, et al.* A general reaction mechanism for carbapenem hydrolysis by mononuclear and binuclear metallo-β-lactamases. *Nat. Comm.* 2017, **8**(1)**:** 538.

42. Richter M.F., Drown B.S., Riley A.P., Garcia A., Shirai T., Svec R.L.*, et al.* Predictive compound accumulation rules yield a broad-spectrum antibiotic. *Nature* 2017, **545**(7654)**:** 299-304.

43. Galán J.-C., Morosini M.-I., Baquero M.-R., Reig M., Baquero F. Haemophilus influenzae bla(ROB-1) mutations in hypermutagenic ΔampC Escherichia coli conferring resistance to cefotaxime and β-lactamase inhibitors and increased susceptibility to cefaclor. *Antimic. Agents Chemother.* 2003, **47**(8)**:** 2551-2557.

44. Pallett A., Hand K. Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. *J. Antimicrob. Chemother.* 2010, **65 Suppl 3:** iii25-33.

45. Theuretzbacher U., Bush K., Harbarth S., Paul M., Rex J.H., Tacconelli E.*, et al.* Critical analysis of antibacterial agents in clinical development. *Nat. Rev. Microbiol.* 2020, **18**(5)**:** 286-298.

46. Mojica M.F., Bonomo R.A., Fast W. B1-Metallo-β-Lactamases: Where Do We Stand? *Curr. Drug Targets* 2016, **17**(9)**:** 1029-1050.

47. Brem J., van Berkel S.S., Aik W., Rydzik A.M., Avison M.B., Pettinati I*., et al.* Rhodanine hydrolysis leads to potent thioenolate mediated metallo-β-lactamase inhibition. *Nat. Chem.* 2014, **6**(12)**:** 1084-1090.

48. Pettinati I., Brem J., Lee S.Y., McHugh P.J., Schofield C.J. The Chemical Biology of Human Metallo-β-Lactamase Fold Proteins. *Trends Biochem Sci.* 2016, **41**(4)**:** 338-355.

Acknowledgments

We thank the European Lead Factory Screening partners for efforts leading to the initial hits, the current and past ENABLE Portfolio Management Committee (PMC) members and EFPIA partners for guidance, and the Diamond Light Source synchrotron for access to IO3, IO4 and IO4-1 beamlines. UH thanks Nina Sipari from Viikki Metabolomics Unit (Helsinki Institute of Life Science, University of Helsinki; Biocenter Finland) for her expertise with the LC-MS analyses. LE thanks Yuqing Zhou for help in the laboratory, and Habiba Saif, Refath Farzana, Edward Portal, Kirsty Sands, Katy Thomson, and Brekhna Hassan for providing strains for the Enterobacterales collection.

The European Lead Factory work and the ENABLE project leading to these results were supported by the Innovative Medicines Initiative Joint Undertaking (grant agreements n' 115489 and n°115583) which have financial contribution from the European Union's Seventh Framework Programme (FP7 / 2007-2013) and EFPIA companies' in kind contribution. The ENABLE project is financially supported by contributions from Academic and SME partners. Oxford work was also supported by the Cancer Research UK (C9047/A24759), the Medical Research Council, the Biotechnology and Biological Research Council (BB/S50676X/1) and the Ineos Oxford Institute for Antimicrobial Research (CJS). This research was funded in whole, or in part, by the Wellcome Trust (Grant numbers 106244/Z/14/Z and 099141/Z/12/Z). For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission. Bristol work was also supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH) Grant R01AI100560 (J.S and P.H.; the content is the responsibility of the authors and does not necessarily represent official views of the NIH) and the BrisSynBio Biosuite (UK Biotechnology and Biological Sciences (BBSRC) and Engineering and Physical Sciences (EPSRC) Research Councils, BB/L01386X/1) and BBSRC ALERT14 initiative (BB/M012107/1).

Author Contributions Statement

J.B., T.D., J.H., P.D., A.F., K.S., G.G.C., P.K, S.S., D.K., R.L., J.S., D.L., A.G.B., J.R., L.R., A.R., S.D.S.P., A.P., M.M., M.P., A.K.B., P.B., J.Y.-K., E.B., M.G.P.P., F.B., P.S.J., E.S., A.M. and C.J.S. conducted the medicinal chemistry analysis and/or chemical synthesis. J.U.F., E.L., E.N., J.K., and S.G. lead the safety and/or *in vivo* efficacy experiments. A.E., M.B., P.B. lead or conducted physicochemical and ADME testing. L.E. M.C.T., A.F.A, J. C. J.-C., E.W., J.M.T. conducted the microbiological experiments under the guidance of M.B.A, M.G., R.C., F.B. and T.M.R. K.C., M.E.K., G.W.L., M.S., A.R., I.H.N., P.A.L., S.P.M. and J.B. conducted the biochemical or biophysical testing. P.H., M.M., T.L., J.S. and J.B. conducted the Xray crystallography work or analysis. J.B. oversee all the studies. J.B and C.J.S. wrote the first draft of manuscript with input from all authors.

Competing Interests Statement

A patent is being filed that might afford authors royalties were it to be licensed. G.W.L. is employee of Charles River Laboratories; M.S., J.R., L.R., S.P.M., P.S.J. and A.M. are employees of BioAscent Discovery Ltd; A.R. and P.B. is employee of AstraZeneca and E.B. is employee of Evotec.

Figure legends

Fig. 1. Indole carboxylates binding to MBLs mimics that of intact β-lactam substrates/products. (A) Clinically used β-lactam classes. **(B)** Outline B1 MBL mechanism exemplified with a carbapenem, note that the protonated form of the enamine tautomer of the product is shown in the EP complex[15, 27, 28]). **(C)** InC binding mimics that of a carbapenem/carbapenem derived products to B1 MBLs, with maintenance of the di-Zn(II) complex hydroxide and conservation of the Zn-Zn distance observed in unligated MBLs (Zn:Zn distance 3.5 Å, PDB ID: 4BZ3[29]). Most MBLi displace Zn-hydroxyl, as observed for bi(cyclic) boronates (Zn:Zn distance 4.3 Å, PDB ID: 5FQC[30], which mimic the tetrahedral transition state) and triazole-carboxylates (Zn:Zn distance 4.15 Å, PDB ID: 6ZGM[17], binding of which is related to that of hydrolysed penems/penicillins). **(D)** Examples of InC hits identified by the European Lead Factory.

Fig. 2. Indole carboxylates inhibit B1 and B3 MBLs via an unprecedented MBL binding mode. Views of **(A) 48** with NDM-7 (PDB ID: 7AEZ, 1.1 Å), **(B) 49** with VIM-1 (PDB ID: 7AEX, 1.1 Å), **(C) 11** with VIM-2 (PDB ID: 7AFY, 1.6 Å), **(D) 49** with L1 (PDB ID: 7AFZ, 1.5 Å). Note the presence of a di-Zn(II) ion bridging hydroxide, the position of the C-7 alkyl group relative to the bridging water, and that the C-2 carboxylate is positioned to interact with the different types of binding motifs employed in MBL substrate carboxylate binding. **(E, F)** Overlays comparing modes of InCs, hydrolysed β-lactams, and analogues of tetrahedral transition state, with two MBLs (hydrolysed cefuroxime with NDM-1 PDB ID: 4RL0 and cyclic boronate 2 with VIM-2 PDB ID: 5FQC). **(G)** Active site interactions made by InCs reveal their ability to mimic the proposed binding mode for bicyclic β-lactams/subsequently formed intermediates.

Fig. 3. Heat map analysis comparing the potency of InCs for selected clinically important SBLs/MBLs with β-lactamase inhibitors. InCs are potent MBLi and less potent SBL inhibitors. VNRX – VNRX-5133 (Taniborbactam), AVI – avibactam, NI – not inhibited. SBLs and MBLs tested: TEM-1, a class A SBL; NDM-1, -7, VIM-1, -4, -5, IMP-1 and SPM-1, class B1 MBLs; L1, a class B3 MBL; AmpC from *Pseudomonas aeruginosa*, a class C SBL; and OXA-10 and -48, class D SBLs. Note the variations in potency versus the VIM-1, -2, -4 and -5 variants may reflect substitutions at residues 224/228 on the L10 loop which is involved in substrate/InC carboxylate and C-3 binding.

Fig. 4. Strategies for InC synthesis. (A) C-3 groups were introduced by Pd-catalysed arylation of C-3 bromo- or iodo- precursors, prepared from commercial indoles or aniline precursors. In general, the C-3 acid was protected as an alkyl ester which was cleaved by base-mediated hydrolysis. **(B)** Synthesis of simple derivatives in early hit finding/SAR studies (e.g. **49**) involved Pd coupling. **(C)** Preparation of C-7 derivatives during lead optimisation required development. Racemic C-3/C-7 amide derivatives (e.g. **56**) were prepared via two-step reductive amination of the requisite C-7 methyl ketone via an alcohol intermediate, followed by amide formation. Subsequent C-3 iodination enabled Pd-catalysed C-3 arylation. **(D)** Chiral C-7 amides (e.g. **57**) were prepared via initial $Ti(OEt)_4$ catalysed imine formation with (*R*)-*tert*-butylsulfinamide, then *L*-Selectride mediated reduction/ acid hydrolysis to give a chiral C-7 amine, which was used to prepare diastereomerically pure **58** and **59**.

Fig. 5. InC 58 potentiates meropenem activity *in vitro* against clinically relevant strains. Coverage of clinically relevant strains for **58** carbapenem combinations. Agar dilution series MIC susceptibility testing showing the combined effect of **58** and meropenem (MEM), imipenem (IMI) or doripenem (DOR) against globally acquired representative MBL-producing Enterobacterales collection (Total n = 280; *E. coli* n = 97; *Klebsiella* spp. n = 47; *E. cloacae* n = 51; other *Enterobacter* n = 20; *Proteus mirabilis*

n = 6; *Serratia marcescens* n = 12; *Citrobacter spp*. n = 28; *Providencia* spp n = 20). The Enterobacterales panel reflects pathogens commonly isolated from complicated urinary tract infections (Supplementary Table 23)[44]. Sequencing revealed presence of up to 7 β-lactamase genes representing all Ambler classes (Supplementary table 26), including VIM (VIM-1, -2, -12, -20 and -40), and NDM variants (NDM-1, -5, 7, -16, Supplementary Table 26 C). Violin plots show the kernel probability density of the data, ranging from the minimum to the maximum observed value. The lower and upper hinges of each Tukey's box plot correspond to the first and third quartiles of the data distribution, respectively, with the whiskers extending 1.5 times the interquartile range from each hinge. Outliers are not represented. Black lines show MIC_{50} values (corresponding to each median), and blue lines show MIC_{90} values. Results were obtained from a single biological sample of each bacterial isolate.

Fig. 6. InC 58 potentiates meropenem activity *in vivo* against clinically relevant strains. (A) Strains used for the in vivo efficacy studies; **(B)** *In vivo* peritonitis/septicaemia and **(C)** *in vivo* thigh mouse model results for **58**-meropenem and meropenem alone. In both murine neutropenic and peritonitis / septicaemia models, we used a 4-hour infection followed by a single subcutaneous injection of meropenem and a single IV dose of **58**. For the murine neutropenic models, colistin was used as a positive control; animals were made neutropenic by cyclophosphamide treatment. For the murine peritonitis/septicemia model, 5% porcine mucin was used to achieve virulence. For the thigh model, the final inoculum was ~$2x10^7$ CFU/ml and for the peritonitis / septicaemia model 5.7 $x10^5$ was adequate. Meropenem doses were based on dose dependence studies; Statistical comparisons were performed with Prism 8 using one-way ANOVA, Dunnett's multiple comparisons test, and all differences between means with $p \leq 0.05$ are indicated. ns, not significant. P values for the *in vivo* peritonitis/septicaemia model are: 997800 60/0 vs 60/10 P≤0.0001, 60/0 vs 60/30 P≤0.0001, NDM7 30/0 vs Vehicle P≤0.0001, 30/0 vs 30/10 P=0.0006, 30/0 vs 30/30 P≤0.0001, B68-1 16/0 vs Vehicle P=0.0282 16/0 vs 16/10 P=0.0450, 16/0 vs 16/30 P≤0.0001, IR57 90/0 vs 90/10 P=0.0002, 90/0 vs

90/30 P≤0.0001). P values for the *in vivo* thigh mouse model are: 997800 60/0 vs 60/10 P=0.0019, 60/0 vs 60/30 P=0.0030, NDM7 30/0 vs Vehicle P≤0.0001, 30/0 vs 30/30 P=0.0003, B68-1 16/0 vs Vehicle P=0.0181 16/0 vs 16/10 P=0.0023, 16/0 vs 16/30 P=0.0009, IR57 90/0 vs 90/30 P≤0.0001.

Methods

Data and materials availability

Methods, full experimental section and all data supporting the findings of this study are available within the paper and its supplementary information files. The atomic coordinates and structure factors are deposited in the Protein Data Bank. Accession codes: PDB ID: 7AEZ (48 with NDM-7), PDB ID: 7AEX (49 with VIM-1), PDB ID: 7AFY (11 with VIM-2) and PDB ID: 7AFZ (49 with L1). The raw data for the InC 58 and meropenem microbial activity dataset can be accessed in Zendo: https://doi.org/10.5281/zenodo.4438867 for full data.