List of ten best papers of the candidate, highlighting important discoveries/contributions described in them briefly (not to exceed 3000 words)

Total Publications: 124

Research Articles:106 (82 as corresponding author) + 14 Review articles (13 as corresponding author) + 4 Editorials (4 as corresponding author) + 9 Book Chapters (8 as corresponding author)
Citations: 6841, h-index: 46, i-10 index: 98 (According to Google Scholar as on 15th July, 2024)

I. Sarkar, P.; De, K.; Modi, M.; Dhanda, G.; Priyadarshini, R.; Bandow, J. E.; **Haldar, J.***. Next-generation membrane-active glycopeptide antibiotics that also inhibit bacterial cell division. *Chem Sci,* **2023**, *14*, 2386-2398.

[In our pursuit for the search of next-generation membrane-active glycopeptide antibiotics, in this study we report a new derivative of vancomycin and explored the antibacterial mechanisms beyond the conventional notion of D-Ala-D-Ala binding. These modified vancomycin derivatives show several alterations in the bacterial cells, which facilitate inactivation of the pathogens. A detailed study of the structure and activity profile gives alkyl-cationic substitutions an edge over aryl analogs. The lead candidate, $VanQAmC_{10}$ shows a significant extent of depolarization and permeabilization of bacterial membrane. Interestingly, $VanQAmC_{10}$ shows retardation of growth during cell division, which is the first of its kind observation setting $VanQAmC_{10}$ apart from other glycopeptide antibiotics. The compound also hampers the distribution of MinD, a protein which has an integral role in cell division regulation machinery. Not only *in-vitro*, $VanQAmC_{10}$ showed superior activity in a mice model of thigh infection. VanQAmC10. Overall, this study investigates multiple mechanisms of action of a novel vancomycin derivative which contribute to the negligible resistance induction and superior antibacterial properties as compared to the parent drug.]

II. Barman, S.; Mukherjee, S.; Jolly, L.; Troiano, C.; Grottesi, A.; Basak, D.; Calligari, P.; Bhattacharjee, B.; Bocchinfuso, G.; Stella, L.; **Haldar, J.*,** Isoamphipathic Antibacterial Molecules Regulating Activity and Toxicity through Positional Isomerism. *Chem. Sci.* **2023**, *14*, 4845–4856.

[We report the discovery of new isoamphipathic antibacterial molecules (IAMs: 1–3), designed with positional isomerism as a key factor. These molecules demonstrated good (MIC = 1–8 μg/mL or μM) to moderate [MIC = 32–64 μg/mL (32.2–64.4 μM)] antibacterial activity against various Gram-positive and Gram-negative bacteria. Positional isomerism significantly influenced antibacterial activity and toxicity for the ortho, meta and para isomers. Co-culture studies and membrane dynamics investigations indicated that the ortho isomer, IAM-1, had more selective activity towards bacterial membranes over mammalian membranes compared to the meta and para isomers. Detailed molecular dynamics simulations characterized the mechanism of action of the lead molecule, IAM-1. Additionally, IAM-1 was effective against dormant bacteria and mature biofilms, unlike

conventional antibiotics. Importantly, IAM-1 showed moderate in vivo activity against MRSA wound infections in a murine model without detectable dermal toxicity. Overall, this report highlights the design and development of isoamphipathic antibacterial molecules, establishing the role of positional isomerism in creating selective and potent antibacterial agents.]

III. Dey, R.; Mukherjee, S.; Mukherjee, R.; **Haldar, J.***; Small Molecular Adjuvant Repurposes Antibiotics towards Gram-negative Bacterial Infections and Multispecies Bacterial Biofilm. *Chem. Sci.* **2024**, *15*, 259 – 270.

[We have developed a small molecular adjuvant by fine-tuning various structural parameters, including the balance between hydrophilic and hydrophobic groups, the spatial arrangement of hydrophobic regions, and hydrogen bonding interactions. This design causes moderate membrane perturbation in bacterial cells without any toxicity to mammalian cells. Such moderate membrane disruption enhances the internalization of antibiotics and increases intracellular drug concentrations by inhibiting efflux mechanisms. This significantly boosts the efficacy of various classes of antibiotics by 32–512 fold without inducing toxicity. The leading combination exhibits potent bactericidal activity against A. baumannii biofilms and effectively disrupts mature multispecies biofilms composed of A. baumannii and methicillin-resistant Staphylococcus aureus (MRSA), which are typically resistant to most antibiotics. Importantly, the combination therapy demonstrates good biocompatibility and excellent in vivo antibacterial efficacy (>99% reduction) in a skin infection model of A. baumannii. Notably, A. baumannii shows a reduced tendency to develop resistance against the leading combination, highlighting its potential for treating multi-drug-resistant infections.]

IV. Ghosh, S.; Mukherjee, R.; Basak, D.; **Haldar, J.***; One-Step Curable, Covalently Immobilized Coating for Clinically Relevant Surfaces That Can Kill Bacteria, Fungi, and Influenza Virus. *ACS Appl Mater Interfaces*. **2020**, *12*, 27853-27865.

[In this work, we created one-step curable covalent coatings using two organo- and water-soluble small molecules: quaternary benzophenone-based ester and quaternary benzophenone-based amide. UV rays can cause these molecules to cross-link on surfaces. The covering killed bacteria and fungus in vitro, including drug-resistant pathogens like methicillin-resistant Staphylococcus aureus (MRSA) and fluconazole-resistant Candida albicans spp. The coating also displayed antiviral action against the influenza virus, killing it 100%. Furthermore, the coated surfaces eliminated stationary-phase MRSA cells, which are resistant to standard antibiotics. After hydrolysis, the surfaces went into an antifouling condition, which greatly reduced bacterial adhesion. To the best of our knowledge, this is the first report of an antimicrobial covering that can kill bacteria, fungus, and the influenza virus. Overall, the antimicrobial coating presented here shows great potential for further application in healthcare settings.]

V. Yarlagadda, V.; Sarkar, P.; Samaddar, S.; Haldar, J.*. Incorporation of Pyrophosphate Binding Ability to Vancomycin: A Strategy to Combat Vancomycin-resistant Bacteria. *Angew. Chem. Int. Ed.* **2016**, *27*, 7836-7840.

[This study reports the development of a potent alternative therapeutic for tackling vancomycin-resistant bacteria, in the form of a dipicolyl conjugated vancomycin derivative. This derivative displays 350-fold more in-vitro activity than vancomycin against vancomycin resistant *Enterococci* (VRE). The enhancement of activity occurs due to the binding of dipicolyl moiety to Zn^{2+} ion, which increases the binding of this derivative to pyrophosphate groups present in cell wall lipids while maintaining the inherent binding affinity for pentapeptide termini of cell-wall precursors. Furthermore, no resistance development was seen against this compound after several serial passages. Investigations of the in vivo activity revealed that the compound reduced Vancomycin resistant bacterial load by ~5 log at a concentration of 12 mg/kg in a murine model of VRB renal infection. This simple strategy of enhancing binding to pyrophosphate yielded an extremely active vancomycin derivative, with more than two orders of magnitude higher activity as compared to vancomycin, against VRE.]

VI. Dhanda, G.; Mukherjee, R.; Basak, D.; **Haldar, J.***. Small-Molecular Adjuvants with Weak Membrane Perturbation Potentiate Antibiotics against Gram-Negative Superbugs. *ACS Infect. Dis.*, **2022**, *8*, 1086–1097.

[In this work, we meticulously explore the structural parameters for the formation of an optimum membrane-targeting antibiotic adjuvant using norspermidine as a model compound. By appending different hydrophobic groups to its secondary amine, it's observed that cyclic hydrophobic moieties show good potentiating ability and are devoid of toxicity or activity. Adamantane and aryl-functionalized derivatives (NAda and NDiphe) exhibit as high as 4096-fold potentiation of multiple classes of antibiotics toward critical Gram-negative superbugs, proving to be the optimum adjuvant. The mechanism of potentiation consists of weak outer membrane permeabilization, membrane depolarization, and efflux inhibition. The lead adjuvants show a significantly increased accumulation of antibiotics in NDM-1-producing bacteria E. coli R3336 and K. pneumoniae R3934. This work is the first of its kind highlighting the importance of "weakly perturbing the membrane" by incorporating cyclic hydrophobic moieties in the chemical design.]

VII. Ghosh, C.; Manjunath, G. B.; Akkapeddi, P.; Yarlagadda, V.; Hoque, J.; Uppu, D. S. S. M.; Konai, M. M.; **Haldar, J.***. Small Molecular Antibacterial Peptoid Mimics: The Simpler The Better! *J. Med. Chem*. **2014**, *57*, 1428-1436.

[This article reports the development of simple, amino acid-based antibacterial molecules, which display potent activity against a broad range of drug-resistant bacteria. From a structural perspective, the design is extremely simple, where two positive charges contributed by the amino acid lysine and hydrophobicity, brought in by an aromatic core and an alkyl chain, are formulated in three simple steps. The lead molecule from this series possesses a naphthyl group and a decyl alkyl chain. It has been identified to be the most selective one, with high antibacterial activity, and least toxicity towards mammalian cells. This molecule is an extremely effective therapeutic, as it eradicates highly pathogenic multidrug-resistant Gram-positive and Gram-negative bacteria, including the nosocomial pathogen *P. aeruginosa*, the top priority pathogen vancomycin-resistant *E. faecium*, etc. This molecule, unlike naturally occurring antimicrobial peptides, is highly resistant to enzymatic cleavage, and retains its activity in complex biological fluids such as blood plasma. It works particularly by disrupting the bacterial cell membrane, and destabilizing its potential. It also displays rapid killing kinetics, effectively eradicating ~6 log bacteria within 60 minutes.

Altogether, these simple designs present immense potential as antibacterial therapeutics.]

VIII. Ghosh, S.; Mukherjee, R.; Mahajan, V. S.; Boucau, J.; Pillai, S.; **Haldar, J.***. Permanent, Antimicrobial Coating to Rapidly Kill and Prevent Transmission of Bacteria, Fungi, Influenza, and SARS-CoV-2. *ACS Appl. Mater. Interfaces,* **2022**, *14*, 42483–42493.

[In the current scenario of COVID-19, development of antimicrobial surface coatings for PPEs, and other biomedical surfaces will definitely play an important role in prevention of spread of infectious diseases in hospitals and community, including viral infections. Towards the same direction, we report here the development of a simple, highly active surface coating, with potent ability to reduce transmission of multidrug-resistant bacteria, fungi as well as the Influenza and SARS-CoV-2 viruses. This coating is based on a quaternary benzophenone-based small molecule, which can cross-link on a wide range of surfaces upon brief UV irradiation. The coated surfaces show very rapid inactivation of pathogens thereby diminishing their transmissibility.]

IX. Uppu, D. S. S. M.; Samaddar, S.; Ghosh, C.; Paramanandam, K.; Shome, B. R.; **Haldar, J.***; Amide Side Chain Amphiphilic Polymers Disrupt Surface Established Bacterial Biofilms and Protect Mice from Chronic Acinetobacter baumannii Infection. *Biomaterials* **2016**, *74*, 131-143.

[We report the development of novel maleic anhydride-based cationic polymers with amide side chains that effectively disrupt established multi-drug-resistant A. baumannii biofilms. Notably, these polymers significantly ($p < 0.0001$) reduce the bacterial burden in mice with chronic A. baumannii burn wound infections. Additionally, the polymers demonstrate potent antibacterial efficacy against methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), and multi-drug-resistant clinical isolates of A. baumannii, while exhibiting minimal toxicity to mammalian cells. We found that the selective toxicity to bacteria is dictated by the optimal hydrophobicity, which depends on the side chain chemical structure of these polymers. These polymers interact with bacterial cell membranes by causing membrane depolarization, permeabilization, and energy depletion. Unlike bacteria, which rapidly develop resistance to erythromycin and colistin, no detectable resistance development occurs against these polymers even after several passages. These findings suggest the potential application of these polymeric biomaterials in disinfecting biomedical device surfaces after infection has become established and for the topical treatment of chronic bacterial infections.]

X. Dey, R.; Mukherjee, R.; **Haldar, J.***. Photo-crosslinked antimicrobial hydrogel exhibiting wound healing ability and curing infections in vivo. *Adv. Healthc. Mater.* **2022**, *11*, 2200536.

[In this report, we engineer an intrinsically antimicrobial hydrogel (HyDex) in a one-pot UV crosslinking technique, employing dextran methacrylate, polyethylene glycol diacrylate, and cationic lipophilic methacrylate with varied hydrophobic chain. The optimized hydrogel exhibits potent antimicrobial efficacy against multidrug-resistant Gram-positive and Gram-negative bacteria as well as against pathogenic fungus *Candida albicans*. A major problem, traumatic or wound-associated hemorrhage is

addressed rapidly using this hydrogel in a mice liver puncture model. The hydrogel kills carbapenem-resistant Acinetobacter baumannii in a murine model of burn wound infection with >99% reduction in bacterial burden, with accelerated wound healing in rat deep wound model.]