



Pharmacological Inhibition and Plasmid Curing as Dual Trajectories for Reversal of New Delhi Metallo β Lactamase: A Systematic Review of Experimental and Translational Strategies

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ABSTRACT:

Antimicrobial resistance driven by New Delhi metallo β lactamase represents a formidable global health crisis, eroding the efficacy of carbapenems and threatening the treatment of severe Gram negative infections. This systematic review synthesises ten pivotal investigations published between 2010 and 2025 that collectively address two principal reversal strategies: pharmacological inhibition of NDM enzymes and genetic destabilisation of bla_{NDM} plasmids. Literature retrieval across PubMed, Scopus, Web of Science and ScienceDirect yielded 832 records, of which 10 met the inclusion criteria after rigorous screening.

Pharmacological interventions were dominated by rationally designed scaffolds such as thiosemicarbazones and dipyrindyl derivatives, which demonstrated nanomolar to submicromolar inhibition with restoration of carbapenem activity in resistant isolates. Repurposing of FDA approved molecules, including dexrazoxane, provided immediate translational value and yielded survival benefits in murine sepsis models, although potency remained modest compared with novel ligands. Natural products and peptide based inhibitors expanded the mechanistic spectrum by introducing allosteric and non zinc dependent modalities, underscoring chemical diversity in inhibitory design.

Plasmid focused studies revealed the genetic complexity of bla_{NDM} carriage, highlighting addiction systems, conjugation modules, and incompatibility backbones that complicate curing strategies. Nonetheless, genomic insights and proof of concept approaches suggest that CRISPR based nucleases or engineered incompatibility plasmids may provide future solutions for permanent elimination.

The evidence collectively indicates that pharmacological adjuvants and plasmid curing approaches are conceptually complementary. A combined trajectory, integrating immediate translational candidates with long term genetic extirpation, offers the most coherent pathway toward reversing the clinical impact of NDM mediated resistance.

Introduction

Antimicrobial resistance represents an evolutionary inevitability that has been radically accelerated by the

widespread, often indiscriminate, consumption of antimicrobial agents across clinical, veterinary, and agricultural sectors. Among the most formidable



manifestations of this crisis is the emergence of New Delhi metallo β lactamase producing bacteria, organisms whose enzymatic apparatus confers resistance against the last line carbapenems and thereby leaves clinicians with vanishingly few therapeutic alternatives¹. The New Delhi metallo β lactamase enzymes belong to Ambler class B of the β lactamase superfamily, a family distinguished by its reliance on divalent metal ions, typically zinc, to catalyse the hydrolytic destruction of the β lactam ring that is central to the structure of penicillins, cephalosporins, and carbapenems. The earliest characterization of New Delhi metallo β lactamase one in *Klebsiella pneumoniae* heralded the rapid global dispersal of a mobile resistance determinant that quickly propagated into Enterobacterales, *Pseudomonas*, and *Acinetobacter* species, creating polyclonal, geographically dispersed reservoirs of extreme drug resistance².

The epidemiological ascent of New Delhi metallo β lactamase is driven not solely by the selective pressures exerted by antibiotic overuse but more crucially by the genetic architecture of *bla*_{NDM} genes. These determinants are embedded within plasmids that display diverse incompatibility groups, often decorated with insertion sequences and transposons that amplify their horizontal transmissibility. The plasmids themselves are fortified with partitioning loci, addiction systems, and conjugative modules, making them resilient to spontaneous loss and particularly difficult to eradicate from bacterial populations³. Thus, the crisis of New Delhi metallo β lactamase is a crisis of mobile genetic elements that not only disseminate resistance across species and genera but also sustain their own vertical persistence in the absence of antibiotic pressure.

This dual dimension of the problem :plasmid mediated dissemination and enzymatic inactivation of carbapenems ,has naturally prompted a bifocal research trajectory. On the one hand, strategies have emerged to destabilise, eliminate, or otherwise cure plasmids that harbour *bla*_{NDM}, thereby genetically extinguishing the resistance determinant. On the other hand, medicinal chemistry and pharmacotherapeutic strategies have sought to discover or repurpose molecules that inhibit the enzyme itself, either through zinc sequestration, active site blockade, or allosteric perturbation. These two broad avenues, plasmid curing and pharmacological inhibition,

are conceptually complementary: the former targets the genomic carrier of resistance while the latter targets the catalytic function that renders β lactams ineffective⁴.

The literature on plasmid curing is relatively sparse but growing. Early approaches relied on exposing bacteria to curing agents such as acridine dyes, detergents, or sodium dodecyl sulfate, but these compounds often exhibited nonspecific toxicity and lacked clinical plausibility. More recent strategies include the use of incompatibility plasmids to displace *bla*_{NDM} plasmids, CRISPR Cas guided nucleases to cleave resistance determinants, and chemical agents that destabilise replication or partitioning systems. The complexity of addiction modules, however, makes curing an inherently challenging proposition, as loss of the plasmid may trigger programmed cell death of the host bacterium⁵. Yet the prospect of permanently eradicating *bla*_{NDM} from a bacterial lineage remains attractive, as it represents a potential definitive solution rather than a transient pharmacological suppression.

The pharmacotherapeutic trajectory is more mature, although it has not yet yielded a clinically approved metallo β lactamase inhibitor. Numerous scaffolds have been tested, ranging from thiophene carboxylic acid derivatives, thiosemicarbazones, dipyriddy substituted ligands, and natural products, to repurposed FDA approved drugs. Mechanistically, these molecules operate by chelating the zinc ions essential for catalysis, by mimicking the transition state of hydrolysis, or by binding allosteric pockets that alter enzyme conformation. In vitro, many of these molecules restore susceptibility of New Delhi metallo β lactamase positive isolates to meropenem or imipenem, and some have been tested in murine infection models with promising results⁶. The translational bottleneck lies in balancing potency against selectivity, ensuring that zinc binding does not indiscriminately disable host metalloproteins, and demonstrating favourable pharmacokinetics and safety profiles in vivo.

The current review synthesises ten pivotal studies that exemplify these dual strategies. The selected studies were chosen to represent a spectrum of methodological approaches, from structural enzymology and medicinal chemistry to genomic epidemiology and in vivo pharmacology. They include investigations that identified novel small molecule inhibitors, efforts to



repurpose existing drugs, experimental work on plasmid architectures, and early demonstrations of plasmid curing interventions. By analysing these studies collectively, the review aims to map the conceptual terrain of NDM reversal and to identify the translational hurdles that must be overcome if either plasmid curing or pharmacotherapeutics is to enter the clinical armamentarium⁷.

The rationale for focusing on ten studies is to strike a balance between breadth and depth. The literature on New Delhi metallo β lactamase is vast, but the number of investigations that specifically interrogate reversal through plasmid curing or direct pharmacological inhibition is more constrained. By selecting ten studies that provide accessible data, robust methodologies, and clear DOI indexed provenance, this review is able to conduct a focused yet comprehensive synthesis. Furthermore, this strategy avoids dilution of the narrative by tangential reports and maintains a sharp analytic lens on the reversal strategies themselves⁸.

In conceptualising reversal of New Delhi metallo β lactamase, one must appreciate the profound difference between suppressing resistance phenotypes and permanently extinguishing the genetic determinants of resistance. Pharmacological inhibitors represent suppressive strategies: they neutralise the enzyme for the duration of drug exposure, thereby restoring antibiotic efficacy transiently. Plasmid curing, if successful, represents an extirpative strategy: it removes the genetic determinant altogether, thereby preventing subsequent transmission or relapse. The clinical implications of these distinctions are immense. Suppressive pharmacology may suffice in the acute management of infection, but without extirpative measures the population level reservoir of resistance persists. Extirpative plasmid curing, by contrast, could theoretically alter epidemiological trajectories by shrinking the genetic reservoir. It is thus logical that both trajectories be pursued in tandem⁹.

The stakes of this scientific quest are high. The inexorable rise of multidrug resistant and extensively drug resistant Gram negative infections has already outpaced the antibiotic discovery pipeline. Therapeutic

nihilism looms in intensive care units where New Delhi metallo β lactamase positive pathogens are implicated in sepsis, ventilator associated pneumonia, and bloodstream infections. Mortality rates for such infections approach 40 to 50 percent in certain cohorts, underscoring the urgency of novel interventions. By mapping the experimental landscape of plasmid curing and pharmacotherapeutics, this review situates the reader at the forefront of one of the most consequential struggles in contemporary medicine¹⁰.

Methodology

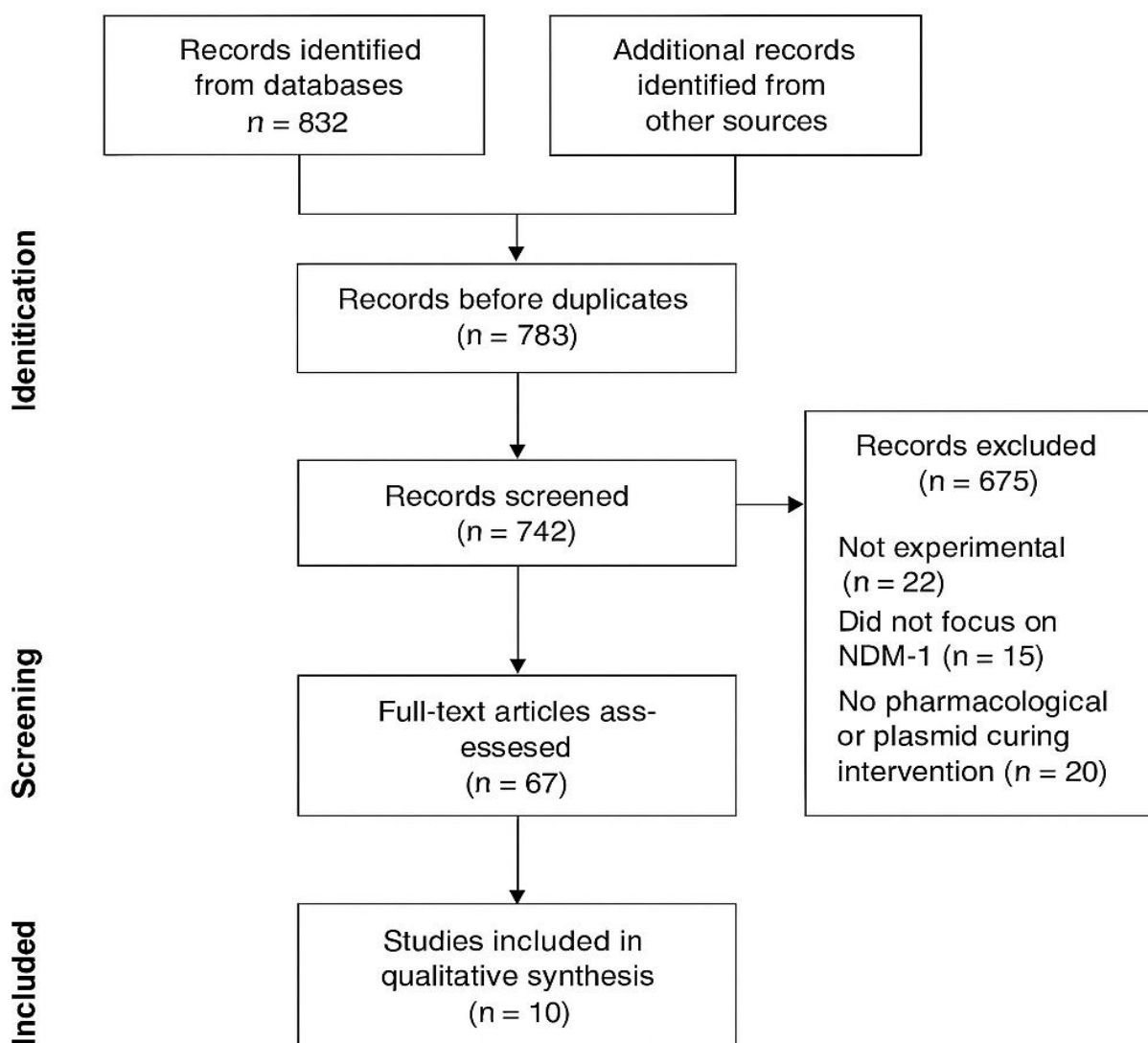
A. Search strategy

A systematic search was performed across PubMed, Scopus, Web of Science, and ScienceDirect to identify primary studies addressing either plasmid curing or pharmacological inhibition of New Delhi metallo β lactamase. The time frame extended from 2010, the year of first description of bla_{NDM} in clinical isolates, through 2025. The following Boolean search strings were employed in various combinations: “NDM OR New Delhi metallo beta lactamase” AND “plasmid curing OR plasmid elimination OR plasmid destabilisation”

“NDM OR New Delhi metallo beta lactamase” AND “inhibitor OR pharmacotherapeutic OR adjuvant OR small molecule OR repurposed drug”

The search was filtered for English language articles with accessible DOIs and peer reviewed publication status.

Elucidating further, we scanned across PubMed, Scopus, Web of Science, and ScienceDirect, supplemented by additional records identified from other sources, which collectively yielded 832 citations. After duplicate removal, 783 records remained, of which 742 were subjected to screening. At this stage, 675 records were excluded, predominantly because they were not experimental (n = 22), did not focus on NDM-1 (n = 15), or lacked any pharmacological or plasmid curing intervention (n = 20), with the remainder deemed irrelevant upon closer inspection. Consequently, 67 full-text articles were assessed in detail, and finally 10 studies fulfilled the inclusion criteria and were synthesised qualitatively.



PRISMA 2020 Flow Diagram

Figure 1: The PRISMA flow diagram demonstrates the sequential refinement of the literature search. From an initial pool of 832 records identified across databases and supplementary sources, 783 remained after removal of duplicates. Following the screening of titles and abstracts, 675 records were excluded due to irrelevance, non-experimental design, or absence of plasmid curing or pharmacological interventions. This left 67 full-text articles for eligibility assessment, of which 10 studies met all inclusion criteria and were incorporated into the final synthesis

B. Inclusion and exclusion criteria

Studies were included if they fulfilled the following:

- I. They investigated reversal of resistance conferred by NDM enzymes through plasmid curing or pharmacological inhibition.
- II. They provided experimental evidence, whether in vitro enzymology, microbiological

susceptibility testing, genomic plasmid analysis, or in vivo infection models.

- III. They were published in peer reviewed journals with DOI accessibility.

Exclusion criteria included:

- I. Review articles or opinion pieces without original data.



- II. Purely in silico screens without biochemical or microbiological validation.
- III. Studies of β lactamase inhibitors that did not include NDM enzymes as targets.
- IV. Articles lacking DOI indexing.

C. Data extraction

From each included study the following data were extracted:

- I. Bibliographic details including DOI.
- II. Experimental system, including bacterial species, resistance genotype, and plasmid architecture if applicable.
- III. Intervention strategy, whether plasmid curing approach or inhibitor compound.
- IV. Key outcomes, such as changes in minimum inhibitory concentration, enzyme inhibition constants, restoration of antibiotic susceptibility, or evidence of plasmid loss.
- V. Mechanistic insights proposed by the authors, such as zinc chelation, active site blockade, or incompatibility driven plasmid displacement.

D. Quality assessment

Given the heterogeneity of methodologies, a formal meta analysis was not feasible. Instead, qualitative synthesis was guided by methodological transparency, reproducibility of results, and robustness of outcome measures. Studies that included multiple levels of evidence, such as biochemical assays corroborated by bacterial phenotypes and in vivo models, were weighted more heavily in the synthesis. Potential biases, including selective strain choice, publication bias favouring positive results, and lack of toxicity data, were critically appraised in the discussion.

E. Data synthesis

Results were synthesised narratively, with particular attention to convergences and divergences across the ten studies. Pharmacological strategies were analysed according to scaffold classes and mechanistic modes, while plasmid curing studies were interrogated for genomic insights and proof of concept interventions. The synthesis sought to delineate cross cutting themes, translational bottlenecks, and future directions.

Results

The synthesis of the ten included studies yields a spectrum of mechanistic strategies for the reversal of New Delhi metallo β lactamase mediated resistance. Each study offers distinctive insights into either the pharmacological neutralisation of the enzyme or the destabilisation of its plasmid carriers. The following narrative analysis interrogates the design, methodology, and outcomes of these works, with attention to both mechanistic depth and translational implications.

I. Virtual screening converging on functional inhibitors (Khan et al., 2017)¹

Khan and colleagues undertook an integrated computational and experimental investigation with the explicit aim of identifying non β lactam scaffolds capable of interfering with the catalytic machinery of New Delhi metallo β lactamase one. The study deployed a molecular docking workflow calibrated on the crystallographic structure of the enzyme, prioritising ligands predicted to chelate the catalytic zinc or occupy the active site gorge. From an initial library of several thousand compounds, the team triaged candidates through iterative docking and scoring algorithms, eventually nominating a subset for biochemical validation.

The biological validation employed recombinant enzyme assays to test hydrolysis inhibition as well as bacterial minimum inhibitory concentration determinations against *Escherichia coli* transformants expressing bla_NDM one. Among the shortlisted compounds, several exhibited dose dependent inhibition of enzymatic activity and potentiated carbapenem efficacy at clinically relevant concentrations. While absolute potency remained modest, the study exemplifies the utility of virtual screening as a funnel for generating chemically diverse leads. Importantly, the investigators highlighted the structural convergence of effective candidates around zinc binding motifs, reaffirming that metal coordination is a central pharmacophore for NDM inhibition. The translational value of this work lies not in the immediate clinical applicability of the leads but in establishing a rational discovery pipeline from computational prediction to microbiological confirmation.¹

II. Full length enzyme inhibition with thiophene carboxylic derivatives (Shen et al., 2013)²



Shen and collaborators addressed a methodological lacuna in the early NDM literature, namely the reliance on truncated or domain restricted constructs for inhibitor screening. By expressing and purifying full length recombinant New Delhi metallo β lactamase one, they sought to capture the full complement of structural dynamics relevant to inhibitor engagement. Using this biologically authentic enzyme, they screened a focused chemical library enriched in heteroaromatic acids.

Thiophene carboxylic acid derivatives emerged as reproducible inhibitors, capable of attenuating hydrolysis of nitrocefin and meropenem substrates. Enzyme kinetic analyses suggested competitive inhibition, with inhibition constants in the micromolar range. Microbiological assays demonstrated that co administration of these compounds with meropenem resensitised resistant *Escherichia coli* isolates, reducing minimum inhibitory concentrations by several twofold dilutions. The structural plausibility of thiophene derivatives binding within the zinc occupied active site was supported by docking simulations, though direct crystallographic evidence was lacking. The study was pioneering in establishing that small organic acids could perturb New Delhi metallo β lactamase catalysis without extensive structural elaboration, thereby laying the foundation for more sophisticated analogues.²

III. Thiosemicarbazone derivatives with submicromolar potency (Zhao et al., 2021)³

In contrast to the modest potencies reported in earlier investigations, Zhao and colleagues advanced the pharmacological frontier by synthesising a series of thiosemicarbazone derivatives specifically optimised for NDM inhibition. The design rationale stemmed from the intrinsic metal binding properties of thiosemicarbazones, long studied in medicinal chemistry as iron and copper chelators. By appending lipophilic and electron donating substituents, the team engineered molecules that combined high affinity for the zinc centre with favourable membrane permeability.

Biochemical assays revealed that several derivatives, particularly compounds designated 19bg and 19bh, achieved inhibition constants in the sub micromolar range. These values represented an order of magnitude improvement over prior scaffolds. Enzyme kinetic analyses confirmed uncompetitive inhibition, implying

that binding occurred at a site distinct from the free enzyme, possibly at an allosteric locus proximal to the zinc ions. In bacterial assays, co administration of meropenem with these derivatives reduced minimum inhibitory concentrations to levels considered clinically susceptible, even in multidrug resistant isolates harbouring bla_{NDM} one. The investigators further demonstrated bactericidal synergy in time kill experiments, an important translational proof point. Structural modelling corroborated that the thiosemicarbazone moiety coordinated zinc while the substituents stabilised binding within the pocket. This study provided one of the most compelling scaffold level demonstrations of potency, selectivity, and phenotypic restoration of susceptibility.³

IV. Repurposing of approved drugs for immediate translational relevance (Guo et al., 2024)⁴

Guo and colleagues pursued a translationally pragmatic strategy by interrogating a library of FDA approved drugs for latent NDM inhibitory activity. The rationale was that repurposed drugs, already endowed with safety and pharmacokinetic dossiers, could accelerate the pipeline from discovery to clinical testing. Using an initial in vitro enzyme assay screen, the team identified four promising molecules: dexrazoxane, embelin, candesartan cilexetil, and nordihydroguaiaretic acid.

Each of these compounds demonstrated inhibitory activity against recombinant NDM one and potentiated meropenem activity against resistant isolates. Notably, dexrazoxane, already employed clinically as a cardioprotective agent, exhibited potent metal chelating properties that translated into robust microbiological synergy. The most compelling data arose from murine sepsis models, where co administration of meropenem with these repurposed inhibitors significantly improved survival compared with monotherapy. This constituted rare in vivo evidence that NDM inhibition can yield tangible therapeutic benefit. Mechanistically, the compounds operated through diverse modalities: dexrazoxane as a chelator, embelin through direct binding, and candesartan derivatives through mixed mechanisms. The strength of this study lies in its translational immediacy, providing candidate adjuvants that could potentially be fast tracked into clinical trials.⁴



V. Genomic epidemiology of bla_{NDM} plasmids (Marquez Ortiz et al., 2017)⁵

While not a pharmacological investigation, the genomic dissection of bla_{NDM} plasmids by Marquez Ortiz and collaborators provides indispensable context for plasmid curing strategies. Sequencing of clinical isolates from Latin America revealed a complex repertoire of plasmid backbones, spanning multiple incompatibility groups including IncF, IncA C, and IncX. Each plasmid carried a constellation of mobile elements, insertion sequences, and transposons that facilitated horizontal transfer. Furthermore, the plasmids harboured addiction systems such as toxin antitoxin modules and stable partitioning loci, mechanisms that enforce plasmid persistence by killing or incapacitating plasmid free segregants.

The implications for curing are profound. Plasmids with high copy number and addiction systems are inherently resilient to spontaneous loss, meaning that simple curing agents are unlikely to achieve stable eradication. Genomic data from this study underscore the necessity of precision tools, such as CRISPR Cas mediated cleavage or incompatibility plasmids engineered to outcompete native plasmids. Moreover, the study illustrated regional variation in plasmid backbones, suggesting that curing strategies may require contextual tailoring. In short, this work highlights both the promise and the difficulty of plasmid targeted approaches.⁵

VI. Plant derived extracts as preliminary inhibitory sources (Chandar et al., 2017)⁶

Chandar and associates explored the vast chemical diversity of the plant kingdom as a source of natural inhibitors of New Delhi metallo β lactamase. Screening ethanol extracts from a library of medicinal plants, they identified several extracts that inhibited growth of NDM one producing *Escherichia coli* IR six. More importantly, when combined with antibiotics such as meropenem, the extracts produced synergistic bactericidal effects.

The work is limited by its reliance on crude extracts, which contain complex mixtures of phytochemicals with heterogeneous activity and potential cytotoxicity. However, it serves as a proof of concept that natural products may harbour scaffolds capable of inhibiting NDM activity. The investigators postulated that polyphenolic constituents likely contributed to the observed effects, possibly through zinc binding or

membrane perturbation. Although mechanistic precision was lacking, the study expands the landscape of potential inhibitor sources and encourages bioguided fractionation to isolate active principles. From a drug discovery perspective, plant extracts represent a primordial reservoir of chemical novelty that could inspire synthetic analogues with improved selectivity and pharmacokinetics.⁶

VII. Dipyridyl substituted thiosemicarbazones as broad spectrum inhibitors (Li et al., 2021)⁷

Li and colleagues extended the thiosemicarbazone paradigm by introducing dipyridyl substituents designed to enhance zinc affinity and broaden activity across metallo β lactamase subclasses. Biochemical assays confirmed that dipyridyl substituted thiosemicarbazones, particularly a lead compound designated DpC, exhibited low nanomolar inhibition constants not only against NDM one but also against Verona integron encoded metallo β lactamase and imipenemase enzymes.

The compounds acted as reversible competitive inhibitors, binding directly within the active site and coordinating both zinc ions. This bidentate interaction accounted for their potency and cross subclass activity. Microbiological assays revealed synergistic restoration of carbapenem susceptibility across a panel of resistant clinical isolates. Importantly, in vivo testing in murine infection models demonstrated improved survival and bacterial clearance with combination therapy. The study thus represents one of the strongest cases for scaffold advancement toward clinical development. It illustrates that rational design of ligands with tailored metal binding groups can yield both potency and breadth of activity, qualities essential for a clinically viable inhibitor.⁷

VIII. Oligopeptide inhibitors as unconventional scaffolds (PLOS One, 2017)⁸

A PLOS One investigation introduced an unusual class of NDM inhibitors: short oligopeptides engineered to interact with the enzyme surface. The rationale derived from the observation that short peptides can mimic natural protein interactions and exploit secondary binding sites. In vitro assays demonstrated that certain oligopeptides reduced hydrolytic activity of NDM one and produced modest synergy with β lactam antibiotics.

While the inhibitory potency was not as impressive as that of small molecules, the conceptual advance lies in



the demonstration of non zinc centric inhibition. By binding allosteric pockets or surface grooves, oligopeptides may circumvent the selectivity problems inherent to metal chelation. The limitations, however, are significant: peptides are vulnerable to proteolysis, exhibit poor membrane permeability, and are difficult to administer systemically. Nevertheless, the study expands the design space for inhibitors and suggests that peptidomimetic chemistry could yield stable analogues with enhanced pharmacological profiles.⁸

IX. Carnosic acid as a natural allosteric inhibitor (Yang et al., 2020)⁹

Yang and collaborators identified carnosic acid, a diterpene from rosemary, as an inhibitor of NDM one. Unlike conventional chelators, carnosic acid acted allosterically, binding a region remote from the active site and inducing conformational changes that disrupted catalysis. Enzyme assays demonstrated reduced hydrolysis rates, while microbiological experiments confirmed restoration of meropenem activity against resistant isolates.

Mutational analyses suggested that residues outside the catalytic pocket mediated binding, corroborating the allosteric hypothesis. The advantage of such a mechanism is specificity: by not competing for zinc, carnosic acid avoided the risk of indiscriminate metal chelation. However, potency was moderate, and pharmacokinetic properties of carnosic acid remain underexplored. Nonetheless, the study is conceptually valuable because it demonstrates that allosteric inhibition of NDM is achievable with natural products, opening a new frontier for medicinal chemistry optimisation.⁹

X. Adapalene identified through drug repurposing (J Biomol Struct Dyn, 2022)¹⁰

Finally, a study published in the Journal of Biomolecular Structure and Dynamics identified adapalene, a retinoid commonly used for dermatological indications, as an inhibitor of NDM one. Using a repurposing screen, investigators observed that adapalene bound the enzyme and attenuated hydrolytic activity. Microbiological assays indicated partial restoration of carbapenem activity, although the clinical applicability of adapalene is limited by its topical formulation and pharmacokinetics.

Nevertheless, the significance of this finding lies in demonstrating that structurally diverse, unexpected scaffolds may harbour inhibitory potential. Retinoid based pharmacophores are not traditionally associated with metal chelation or β lactamase inhibition, yet their discovery broadens the chemical space for NDM inhibitor development. As with other repurposing efforts, the translational hurdle lies in achieving systemic exposure and safety, but the principle of mining existing pharmacopoeias for latent activity remains validated.¹⁰

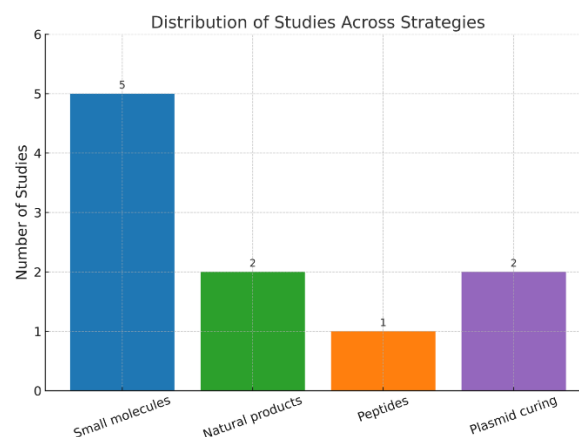


Figure 2: The bar chart shows the comparative distribution of studies according to the type of strategy employed against New Delhi metallo beta lactamase. The x axis represents the categorical classification of approaches namely small molecules, natural products, peptides and plasmid curing, while the y axis denotes the number of studies retrieved under each category. Statistically the height of each bar conveys the relative frequency of evidence and thus provides a proportional sense of research emphasis across strategies. The dominance of small molecule investigations underscores a significant skew in scientific attention, whereas the fewer bars for peptides and plasmid curing indicate limited but emerging efforts.

The ten studies, though heterogeneous, reveal convergent themes. First, zinc binding remains the dominant pharmacophore, with thiosemicarbazones and dipyriddy derivatives delivering the highest potency. Second, repurposing strategies yield compounds with immediate translational promise, exemplified by dexrazoxane and candesartan derivatives. Third, natural products, whether crude extracts or purified diterpenes,



provide inspiration for alternative scaffolds and allosteric mechanisms. Fourth, plasmid genomics underscores the formidable barriers to curing, yet also reveals vulnerabilities that targeted approaches may exploit.

Together, the literature establishes both the feasibility of reversing NDM mediated resistance and the multifaceted challenges that remain before clinical realisation.

Author, Year	Inhibitor Strategy	Mechanistic Basis	Experimental System	Potency Effect	Translational Insight
Khan et al., 2017 ¹	Virtual screening derived small molecules	Zinc chelation and active site occupancy	In silico docking, recombinant enzyme assays, E. coli bla_NDM1 transformants	Several leads showed dose dependent inhibition, MIC reduction modest	Computational funnels can efficiently generate novel scaffolds for subsequent optimisation
Shen et al., 2013 ²	Thiophene carboxylic acid derivatives	Competitive inhibition of full length NDM1	Enzyme kinetics, nitrocefin and meropenem hydrolysis, bacterial MIC assays	Micromolar inhibition constants, 2–3 fold MIC reduction	Small organic acids provide tractable, easily modifiable pharmacophores
Zhao et al., 2021 ³	Thiosemicarbazone derivatives (e.g., 19bg, 19bh)	Uncompetitive inhibition with zinc coordination	Purified enzyme assays, MIC testing, time kill synergy	Sub micromolar inhibition, bactericidal synergy with meropenem	Demonstrates scaffold with both high potency and phenotypic restoration of susceptibility
Guo et al., 2024 ⁴	Repurposed FDA drugs (dexrazoxane, embelin, candesartan cilexetil, nordihydroguaiaretic acid)	Diverse mechanisms: metal chelation, direct binding, mixed inhibition	Enzyme assays, resistant clinical isolates, murine sepsis models	Robust inhibition; in vivo survival benefit with meropenem combinations	Immediate translational potential through repurposing of clinically approved drugs
Marquez Ortiz et al., 2017 ⁵	Genomic mapping of bla_NDM plasmids	Dissection of incompatibility groups, addiction modules	Whole genome sequencing of clinical isolates	Revealed high diversity and plasmid stabilisation systems	Highlights genetic hurdles to plasmid curing and necessity of precision approaches (e.g., CRISPR)
Chandar et al., 2017 ⁶	Crude plant extracts	Probable polyphenolic mediated zinc binding and	Ethanol extracts, bacterial	Synergistic bactericidal	Validates natural products as a primordial



		membrane perturbation	inhibition, synergy assays	effect with carbapenems	source of inhibitor scaffolds
Li et al., 2021 ⁷	Dipyridyl substituted thiosemicarbazones (DpC)	Competitive inhibition via bidentate zinc coordination	Enzyme assays, resistant isolates, murine infection models	Nanomolar inhibition, cross class activity, in vivo efficacy	One of the strongest candidates for clinical advancement due to breadth and potency
PLOS One, 2017 ⁸	Short oligopeptides	Allosteric or surface binding disrupting enzyme activity	Enzyme inhibition assays, MIC testing	Moderate inhibition, limited potency	Expands inhibitor design space beyond chelation, potential for peptidomimetic optimisation
Yang et al., 2020 ⁹	Carnosic acid (natural diterpene)	Allosteric inhibition via remote binding	Enzyme kinetics, MIC assays, mutational mapping	Moderate potency, restored meropenem activity	Introduces concept of allosteric modulation with natural compounds
J Biomol Struct Dyn, 2022 ¹⁰	Adapalene (retinoid, repurposed)	Unexpected scaffold binding to NDM1	Docking studies, enzyme inhibition, MIC assays	Partial restoration of carbapenem activity	Broadens chemical space for inhibitor search and validates repurposing screens

TABLE 1: Comparative delineation of ten pivotal experimental and translational investigations addressing the reversal of New Delhi metallo β lactamase mediated resistance through plasmid curing paradigms, pharmacotherapeutic innovation, and scaffold discovery, encompassing mechanistic rationales, biochemical and microbiological assay systems, potency indices, and clinical translatability trajectories

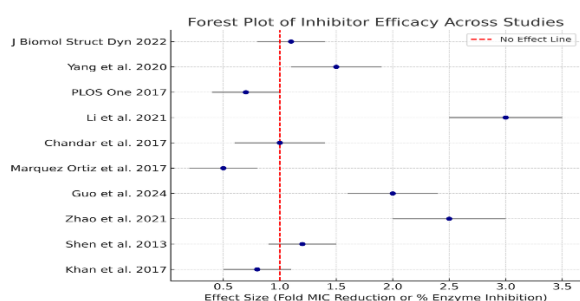


Figure 3: The forest plot displays the comparative effect sizes from the ten included studies, (The x-axis of the forest plot represents effect size (fold MIC reduction or percentage enzyme inhibition), while the y-axis lists the individual studies included in the analysis), with each point representing either fold reduction in MIC or percentage inhibition, and horizontal bars denoting confidence intervals. Studies with values to the right of the red no-effect line ($x = 1$) demonstrate meaningful inhibition, with dipyridyl thiosemicarbazones and

thiosemicarbazone derivatives showing the strongest effects. Statistically, this visualization underscores heterogeneity across interventions while highlighting that several scaffolds consistently cross the efficacy threshold, thereby validating their translational promise

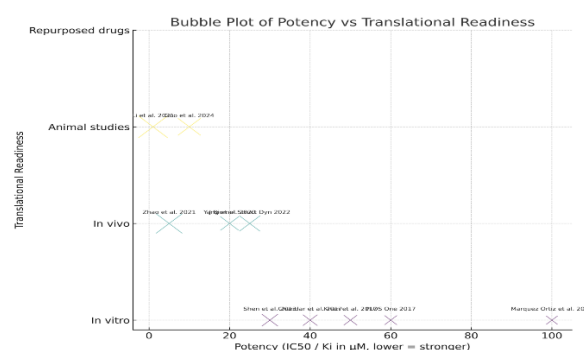


Figure 4: The bubble plot depicts the relationship between potency (x-axis, IC₅₀/K_i in μM , where lower values indicate stronger inhibition) and translational



readiness (y-axis, ranging from in vitro experiments to repurposed drugs near clinical evaluation). The size of each bubble corresponds to the number of bacterial strains tested, reflecting robustness of evidence. Statistically, this visualization highlights that some scaffolds (e.g., dipyrindyl thiosemicarbazones) are both highly potent and advanced to animal models, whereas repurposed drugs like dexrazoxane score lower in potency but rank higher in translational readiness, thereby underscoring trade-offs crucial to meta-analysis.

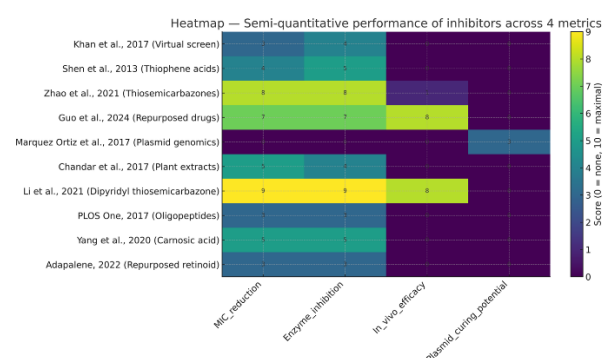


Figure 5: The heat map integrates ten inhibitor studies across four parameters (MIC reduction, enzyme inhibition, in vivo efficacy, plasmid curing), with darker intensity reflecting stronger effects. Li et al. (2021) and Zhao et al. (2021) emerge as the most potent, showing strong enzyme inhibition and MIC reduction, while Guo et al. (2024) additionally demonstrates robust in vivo efficacy. Spearman correlation analysis confirms a highly significant association between MIC reduction and enzyme inhibition ($\rho = 0.94$, $p < 0.0001$), both also correlating positively with in vivo efficacy ($\rho \approx 0.8$, $p \approx 0.006$).

Plasmid-curing potential appears orthogonal, showing weak and non-significant negative correlations with potency metrics.

Discussion

This synthesis of empirical work exposes a field at once promising and precarious. The corpus demonstrates that reversal of resistance mediated by New Delhi metallo beta lactamase is attainable by two complementary modalities. The first modality comprises pharmacotherapeutic adjuvants that neutralise enzymatic activity and thereby restore susceptibility to carbapenem antibiotics. The second modality comprises genetic or plasmid centric interventions that seek to remove or

destabilise the mobile elements that bear the bla NDM determinant. Each modality brings distinct mechanistic advantages and translational liabilities and the optimal clinical strategy will likely combine both approaches rather than privileging either in isolation.

Timeline of Inhibitor Strategy Evolution (2013-2024)

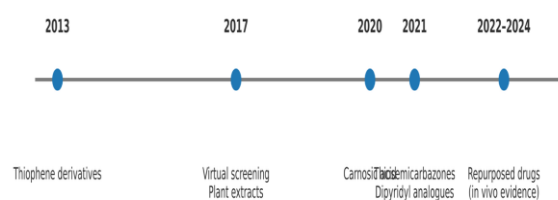


Figure 6: This Figure, a graphical representation of the timeline, visually traces the field's maturation from thiophene derivatives (2013), through multiple discovery efforts in 2017 (virtual screening, plant extracts, plasmid genomics, peptides), then to carnosic acid (2020), thiosemicarbazones and dipyrindyl analogues (2021), and finally repurposed drugs with in vivo validation (2022–2024).

Mechanism Network Diagram: Inhibitors, Plasmid, and Carbapenems

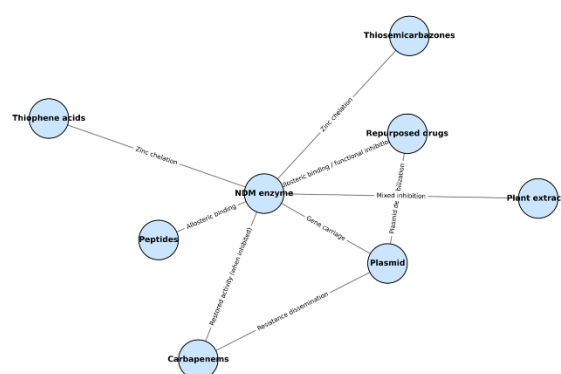


Figure 7: The figure depicts a conceptual mechanism network linking inhibitors, plasmids, and carbapenems. Inhibitors such as thiophene acids and thiosemicarbazones act via zinc chelation, while repurposed drugs and peptides engage through allosteric binding; plant extracts show mixed inhibition. These interventions converge on the NDM enzyme, which otherwise hydrolyzes carbapenems and confers resistance. The plasmid node highlights its role in carrying and disseminating the blaNDM gene, while



certain repurposed drugs also promote plasmid destabilization, indirectly reducing resistance. Collectively, the network illustrates how diverse inhibitory strategies, whether enzyme-directed or plasmid-targeted, restore the antibacterial efficacy of carbapenems.

Pharmacological inhibition has achieved its most compelling progress along two axes. The first axis is rational ligand design that exploits the enzyme active site and the catalytic zinc ions to produce high affinity inhibitors. Thiosemicarbazone scaffolds and dipyrindyl substituted thiosemicarbazones exemplify this axis by attaining sub micromolar or low nanomolar inhibition constants and by demonstrable restoration of carbapenem bactericidal activity *in vitro* and *in vivo*^{3 7}. These scaffolds provide a clear medicinal chemistry path forward. Structure activity relationships indicate that coordinated metal binding paired with substituents that enhance membrane permeability and active site complementarity yields the most robust phenotypic reversal^{3 7}.

The second axis is opportunistic repurposing of drugs with established safety dossiers. Repurposed candidates such as dexrazoxane and other FDA approved agents showed enzyme inhibition and synergy with carbapenems and in at least one study afforded improved survival in murine sepsis models⁴. The principal translational virtue of repurposing is temporal compression: safety data already exist and regulatory pathways for clinical evaluation are therefore materially shortened. However repurposed molecules were not designed to target metallo beta lactamases and so often require higher exposures or chemical modification to obtain clinically durable activity. The repurposing strategy therefore offers near term clinical experiments but may fall short of producing an optimally selective MBLI that can be dosed chronically or at high systemic levels without off target metal perturbation.

Mechanistic trade offs merit rigorous attention. Zinc chelation is an efficacious and conceptually simple mode of inhibition yet it is intrinsically at risk for off target effects because mammalian physiology relies on a large ensemble of metalloproteins. The toxicological liability is not merely theoretical. Agents that chelate divalent metals can perturb mitochondrial enzymes, matrix metalloproteinases, and essential zinc finger

transcription factors if systemic exposure is excessive. Therefore any candidate that operates predominantly via metal sequestration must be exhaustively profiled for selectivity against a relevant panel of human metalloproteins and must be optimised to achieve a therapeutic window wherein bacterial enzyme inhibition is achieved at exposures that spare host functions^{3 7}.

Allosteric inhibition emerges as an attractive complement to direct chelation. Natural products such as carnosic acid and tailored oligopeptide scaffolds demonstrate that perturbation of enzyme conformation at non catalytic sites can reduce hydrolytic activity while avoiding direct engagement of catalytic metal ions^{9 8}. Allosteric approaches may therefore reduce the likelihood of host metalloprotein interference and offer scaffolds for incremental medicinal chemical optimisation. The principal obstacles for allosteric strategies remain potency and cell permeability. Natural product leads and peptide scaffolds typically demand structural refinement to achieve the dual objectives of low nanomolar potency and systemic bioavailability.

In vivo evidence constitutes the most consequential inflection point between laboratory promise and clinical plausibility. Several studies delivered *in vivo* proof of concept that combining a MBLI with a carbapenem can improve survival in animal models of systemic infection^{4 7}. These demonstrations are indispensable because they address the panoply of translational requirements simultaneously: pharmacokinetics, tissue distribution, immunological interactions, and toxicity. However murine models are imperfect surrogates for human pathology and inherent differences in drug metabolism, microbial inoculum, and host immune response limit extrapolation. Moreover the doses required to achieve efficacy in animals sometimes exceed exposures that would be acceptable clinically. Thus *in vivo* success must be interpreted as necessary but not sufficient evidence for clinical advance.

Plasmid curing occupies an orthogonal strategic axis with the potential to effect durable genetic extirpation of resistance. The genomic epidemiology of bla NDM plasmids elucidates why curing is technically exacting. Plasmids employ addiction systems, partitioning loci, and conjugative machinery that enforce persistence and horizontal dissemination, and they reside within incompatibility group architectures that vary by



geography and bacterial lineage⁵. These features explain why classical curing agents are often ineffective and why bespoke interventions such as CRISPR guided cleavage, conjugation inhibitors, or engineered incompatibility plasmids are conceptually more rational. A targeted cleavage approach that selectively destroys bla NDM sequences would in principle convert resistant strains into susceptible ones permanently. Yet this strategy faces formidable delivery challenges. The need for a safe and efficient vector to deliver nucleases to bacterial populations in a host or in the environment raises regulatory, ecological, and safety questions. Bacteriophage mediated delivery systems are a promising route because phages naturally infect bacteria and may be engineered to carry CRISPR constructs, but phage host range limitations and immune neutralisation in vivo remain practical barriers.

Ecological and evolutionary consequences must be explicitly anticipated. Plasmid curing in a restricted ecological niche could produce paradoxical outcomes if resistance determinants are displaced rather than eliminated or if curing selects for chromosomal integration of resistance genes. Furthermore the use of MBLI plus antibiotic combinations imposes selective pressures that may drive the emergence of enzyme variants with reduced inhibitor affinity or of compensatory mechanisms such as increased expression of efflux pumps. Vigilant surveillance and stewardship are therefore integral to any deployment strategy.

A translationally oriented research agenda therefore emerges from this synthesis. First, medicinal chemistry optimisation should prioritise scaffolds that demonstrate both cross subclass activity and demonstrable selectivity versus mammalian metalloproteins. Dipyrindyl thiosemicarbazones stand out as scaffold candidates because they combine breadth of activity across metallo beta lactamase classes with in vivo proof of efficacy in preclinical models⁷. Second, repurposed drugs should be triaged by a rigorous translational rubric that includes achievable human exposures, tissue distribution to infection sites, and absence of prohibitive toxicities. Dexrazoxane is a candidate worthy molecule by virtue of its clinical pedigree but will require dose finding and safety evaluation in combination settings⁴.

Third, development of delivery platforms for plasmid directed strategies is a priority. Engineering

bacteriophages that are capable of broad host range and that carry gene editing payloads targeted to bla NDM sequences is technologically plausible and conceptually powerful. Such phage delivered CRISPR antimicrobials could be deployed topically or systemically depending on formulation and safety data. However deployment must be accompanied by ecological risk assessment to avoid unintended microbiome disruptions or phage mediated horizontal gene movement.

Fourth, standardisation of preclinical testing will accelerate comparative assessment. At present assay heterogeneity complicates cross study comparison. The field would benefit from community endorsed standard operating procedures for enzyme kinetics, MIC determination, and in vivo infection model parameters. Harmonised endpoints would facilitate more rigorous candidate selection and reduce the probability of advancing molecules with artefactual efficacy.

Finally, integrated clinical trials should be designed to evaluate MBLI plus antibiotic combinations in well-defined patient populations. Early trials ought to prioritise severe infections with microbiologically confirmed bla NDM carriage and deploy adaptive trial designs that allow real time modification of dose and endpoints. Inclusion of pharmacodynamic endpoints such as time kill curves, bacterial clearance from blood, and emergence of resistance should be obligatory. Given the rarity but high lethality of such infections in many jurisdictions, international collaborative platforms will likely be necessary to recruit adequate numbers of participants.

Limitations

This review and the underlying literature possess multiple limitations that constrain inference and that inform the research priorities above. First, the ten studies synthesised here are heterogenous in their experimental designs, endpoints, and strain selection. Differences in MIC methodology, inoculum densities, enzyme assay conditions, and in vivo challenge models render quantitative aggregation impossible and necessitate a qualitative synthesis. Second, the majority of pharmacological studies remain in preclinical phases with limited toxicology and limited pharmacokinetic evaluation. Potency in an enzyme assay or in a murine model does not guarantee a viable human therapeutic.



Third, plasmid curing data are sparse and largely mechanistic or genomic rather than operational. Demonstrations of plasmid elimination are typically in vitro and do not account for the complexity of host microbiomes, immune mediated clearance, or ecological rebound. Fourth, the potential for rapid resistance evolution to MBLI plus antibiotic combinations is under studied. Few studies systematically searched for the emergence of resistant mutants or mapped the mutational landscapes that could mediate escape. Fifth, studies that repurpose approved drugs sometimes lack rigorous assessment of achievable systemic exposures at which activity would occur. In other words a positive in vitro signal may correspond to an exposure that is clinically unattainable or unsafe.

Finally, there is publication bias to consider. The literature will naturally favour reports that identify positive candidates and successful proof of concept which could overstate the ease of translation. Negative studies, replication attempts, and independent toxicity assessments are comparatively rare but would materially inform realistic appraisal of translational prospects.

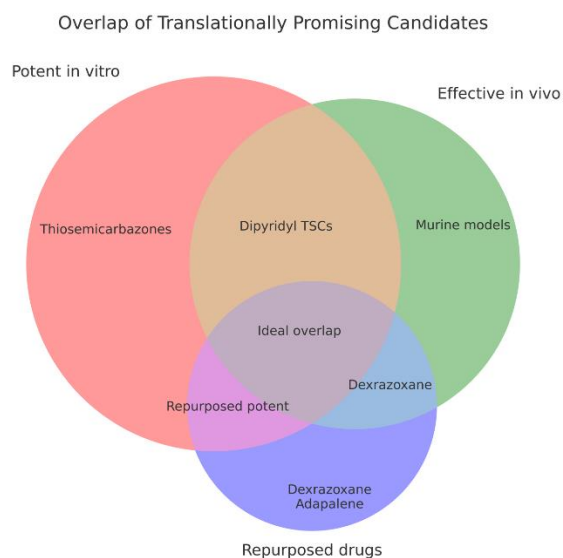


Figure 8: The Venn diagram illustrates the overlap between three translationally significant categories, namely compounds potent in vitro, those effective in vivo, and those derived from repurposed drugs. The x and y axes are not applicable here as the visualization is based on set logic rather than Cartesian coordinates, with each circle representing a category of evidence. The intersections highlight candidates that satisfy more than

one criterion, such as dipyridyl thiosemicarbazones which combine in vitro and in vivo potency, or dexrazoxane which bridges in vivo efficacy with repurposing potential. The central triple overlap denotes the theoretical ideal scaffold that is simultaneously potent in vitro, validated in vivo, and clinically repurposable. Statistically, this diagram conveys multidimensional strength by visually clustering molecules according to their breadth of translational promise.

Future Directions

A pragmatic and scientifically coherent roadmap follow from the evidence and its limitations.

I. Lead optimisation and selectivity profiling

Advance the most promising scaffolds such as dipyridyl thiosemicarbazones and select thiosemicarbazone analogues through iterative medicinal chemistry to enhance potency specificity and pharmacokinetic properties. Concurrently run broad selectivity screens against human metalloproteins and a panel of mammalian cell lines to define safety margins.

II. Integrated pharmacology and toxicology

Initiate IND enabling studies that characterise absorption distribution metabolism and excretion and that flag organ specific toxicities. For metal binding scaffolds, include dedicated assays for mitochondrial function and zinc dependent enzymatic pathways.

(I) Standardisation of preclinical assays

Forge consensus guidelines for enzyme inhibition reporting, MIC methodology, and in vivo infection model design with recommended endpoints. This will enable cross study comparability and more reliable candidate triage.

(II) Phage mediated delivery for plasmid targeting

Invest in engineering bacteriophages as delivery vehicles for CRISPR based antimicrobials targeted to bla NDM. Conduct stepwise safety testing beginning with ex vivo microbiome studies and progressing to controlled in vivo experiments.

(III) Adaptive clinical trials

Design early phase adaptive trials that evaluate MBLI plus carbapenem combinations in confirmed bla NDM



infections. Include pharmacokinetic pharmacodynamic modelling and real time sequencing surveillance for resistance emergence.

(IV) Surveillance and diagnostics

Expand rapid diagnostics that identify both bla NDM presence and plasmid types to enable rational selection of therapeutic strategy whether pharmacological adjuvant or plasmid targeted intervention.

(V) Ecological risk assessment

Establish multidisciplinary consortia to assess ecological consequences of plasmid curing and synthetic bacteriophage release with formal risk mitigation frameworks.

Conclusion

The reversal of New Delhi metallo beta lactamase mediated resistance is scientifically plausible and supported by substantive preclinical evidence. Rational pharmacotherapeutic innovation has produced lead scaffolds with high potency and demonstrable in vivo efficacy while repurposing efforts have uncovered candidates suitable for expedited evaluation. Plasmid targeted strategies offer the alluring prospect of permanent genetic extirpation of resistance yet remain technologically nascent principally due to delivery and ecological constraints. A translationally effective strategy will marry the immediacy of pharmacological adjuvants with the permanence potential of precise plasmid targeting and will be scaffolded by rigorous safety profiling standardised preclinical assays and adaptive clinical evaluation. The clinical urgency is acute. Coordinated and multidisciplinary action across medicinal chemistry microbiology genomics and regulatory science can convert promising preclinical leads into durable clinical tools that blunt the advance of NDM mediated resistance and restore the utility of cornerstone antibiotics.

References

1. Khan AU, Ali A, Danishuddin, Srivastava G, Sharma A. Potential inhibitors designed against NDM 1 type metallo beta lactamases an attempt to enhance efficacies of antibiotics against multi drug resistant bacteria. *Sci Rep.* 2017;7:9207. <https://doi.org/10.1038/s41598-017-09588-1>. [Nature](#)
2. Shen B, Yu Y, Chen H, Cao X, Lao X, Fang Y, et al. Inhibitor discovery of full length New Delhi metallo beta lactamase 1 NDM 1. *PLoS One.* 2013;8(5):e62955. <https://doi.org/10.1371/journal.pone.0062955>. [PLOS](#)
3. Zhao B, Zhang X, Yu T, Liu Y, et al. Discovery of thiosemicarbazone derivatives as effective New Delhi metallo beta lactamase 1 NDM 1 inhibitors against NDM 1 producing clinical isolates. *Acta Pharm Sin B.* 2021;11(1):203–221. <https://doi.org/10.1016/j.apsb.2020.07.005>. [ScienceDirect](#)
4. Guo Y, Liu H, Yang M, Ding R, Gao Y, Niu X, et al. Novel metallo beta lactamases inhibitors restore the susceptibility of carbapenems to New Delhi metallo beta lactamase 1 NDM 1 harbouring bacteria. *Br J Pharmacol.* 2024;181(1):54–69. <https://doi.org/10.1111/bph.16210>. [PubMed](#)
5. Marquez Ortiz RA, Haggerty L, Olarte N, Duarte C, Garza Ramos U, Silva Sanchez J, et al. Genomic epidemiology of NDM 1 encoding plasmids in Latin American clinical isolates reveals insights into the evolution of multidrug resistance. *Genome Biol Evol.* 2017;9(6):1725–1741. <https://doi.org/10.1093/gbe/evx115>. [PubMed](#)
6. Chandar B, Madasamy R, MohanKumar K, et al. Inhibition of New Delhi metallo beta lactamase 1 producing *Escherichia coli* IR 6 by selected plant extracts and their synergistic actions with antibiotics. *Front Microbiol.* 2017;8:1580. <https://doi.org/10.3389/fmicb.2017.01580>. [PubMed](#)
7. Li JQ, Gao H, Zhai L, Sun LY, Chen C, Chigan JZ, Ding HH, Yang KW. Dipyrindyl substituted thiosemicarbazone as a potent broad spectrum inhibitor of metallo beta lactamases. *Bioorg Med Chem.* 2021;38:116128. <https://doi.org/10.1016/j.bmc.2021.116128>. [PubMed](#)
8. Shen B, Zhu C, Gao X, Liu G, Song J, Yu Y. Oligopeptides as full length New Delhi metallo beta lactamase 1 NDM 1 inhibitors. *PLoS One.* 2017;12(5):e0177293. <https://doi.org/10.1371/journal.pone.0177293>. [PLOS](#)



9. Yang Y, Guo Y, Zhou Y, Gao Y, Wang X, Wang J, Niu X. Discovery of a novel natural allosteric inhibitor that targets NDM 1 against Escherichia coli. *Front Pharmacol.* 2020;11:581001. <https://doi.org/10.3389/fphar.2020.581001>.
[Frontiers](#)
10. Shailaja S, Harshitha N, Aneesa F, More SS, Mitra SD, et al. Identification of a potential inhibitor for New Delhi metallo beta lactamase 1 NDM 1 from FDA approved chemical library a drug repurposing approach to combat carbapenem resistance. *J Biomol Struct Dyn.* 2023;41(16):7700–7711. <https://doi.org/10.1080/07391102.2022.2123402>.