Structure-Guided Synthesis of Triazole-Stabilized Peptidomimetics as Inhibitors of New Delhi Metallo-β-Lactamase-1

[1,2]Msanif Msanif*, [1]M. Jewgiński, [2]F. Nuti, [3]P. Rovero, [2]A. M. Papini, [1]R. Latajka

[1]Department of Bioorganic Chemistry, Wrocław University of Science and Technology, 50-370 Wrocław, Poland
[2]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff", University of Florence, 50019 Sesto Fiorentino (FI), Italy
[3]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, 50019 Sesto Fiorentino (FI), Italy
msanif.msanif@pwr.edu.pl
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1. Introduction

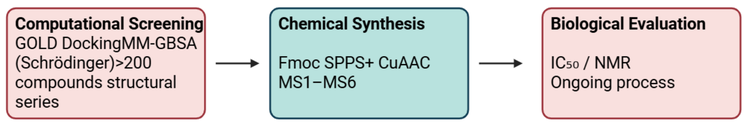
The global emergence of New Delhi metallo-β-lactamase-1 (NDM-1) has critically undermined the efficacy of β-lactam antibiotics, including last-resort carbapenems. As a zinc-dependent enzyme operating via a bimetallic Zn(II) center, NDM-1 hydrolyzes the β-lactam ring through a mechanism that renders all currently approved β-lactamase inhibitors ineffective [1,2]. Its flexible L3 loop and broad substrate-binding groove present a compelling multi-contact surface for peptidomimetic inhibitor design [3]. Triazole-constrained cyclic peptides formed via intramolecular CuAAC offer conformational rigidity, proteolytic stability, and direct zinc coordination as a structurally innovative inhibitor strategy [4,5]. No clinically approved NDM-1 inhibitor exists, making it one of the most urgent unmet targets in antimicrobial therapy and lead the urgency of structurally innovative inhibition strategies.

2. Aim of study

To design, synthesize, and biochemically evaluate triazole-constrained peptidomimetics as structurally innovative inhibitors of NDM-1 metallo-β-lactamase, guided by computational modeling and targeting the bimetallic Zn(II) active site.

3. Methods

Rational Design Strategy



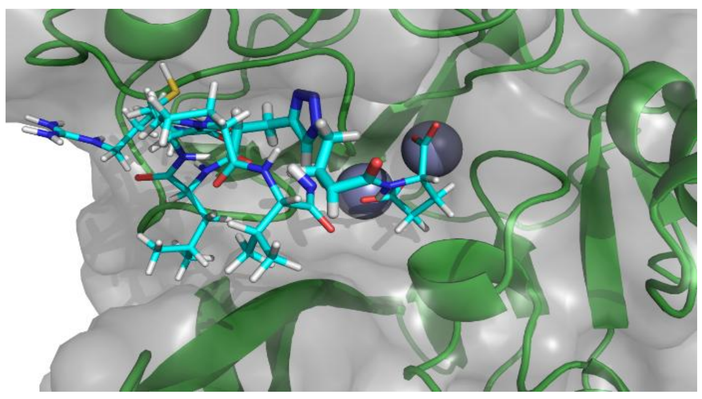


***Figure 1**. Molecular docking pose of lead compound 6xbe-pept1 within NDM-1 active site (PDB: 6XBE). The compound is accommodated in the groove formed by L1, L2, and L3 loops with docking score −9.185 and MM-GBSA ΔGbind(NS) = −116.94 kcal/mol.]*

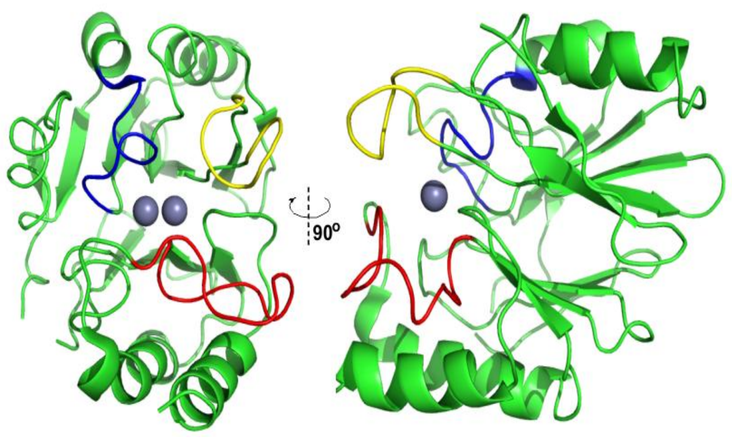


Figure 2: *Overall structure of NDM-1 (PDB: 5ZGE) shown in cartoon representation with active site loops highlighted: loop L3 (red), loop L10 (blue), and loop L7 (yellow). Zinc ions at the catalytic center are shown as grey spheres. Right panel shows a 90° rotated view.*

Table 1. **Summary of Best-Performing Compounds Across Stapled Peptidomimetic Series I–VIII**

Series	Best Compound	Staple/Linker	Docking Score	MM-GBSA ΔGbind (kcal/mol)	MM-GBSA ΔGbind(NS) (kcal/mol)
I	6xbe-peptyd_1	Direct triazole (i→i+3, C→N)	−9.932	−81.75	−102.69
II	6xbe-peptyd_2	Alkyl -$(CH_2)_5$-	−8.419	−87.46	−118.52
III	**6xbe-peptyd_3**	**Pra/Lys(N_3) direct triazole**	**−9.185**	**−76.16**	**−116.94**
IV	6xbe-peptyd_4	PEG-like -$(CH_2)_2$-O-$(CH_2)_2$-	−10.403	−67.58	−107.43
V	6xbe-2-5	Direct triazole (shortened chain)	−7.680	−53.39	−67.29
VI	6xbe-2-6	1,3-Phenyl linker	−7.310	−63.16	−75.65
VII	6xbe-peptide_2_7	N-N allyl staple	−11.104	−61.81	−86.68
VIII	6xbe-peptide_2_8	S-Ph-S thioether-phenyl	−13.78	−73.99	−114.05

ΔGbind = MM-GBSA binding free energy

4. Result

Docking, Chemical Synthesis and characterization - Compounds MS1- MS6

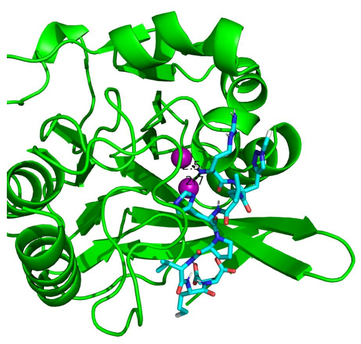


***Figure 3.** Best-scoring AutoDock Vina pose of MS5 docked into the NDM-1 active site (PDB 6XBE). The ligand (cyan sticks) engages both catalytic Zn^{2+} ions (magenta spheres) directly, with contact distances of 2.5 Å and 2.7 Å. Rendered in PyMOL.*

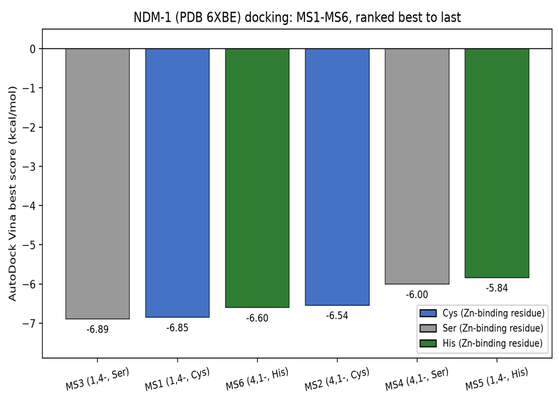


***Figure 4.** Vina predicted binding affinity by compound, grouped by position-4 zinc-binding residue (blue = Cys, grey = Ser, green = His).*

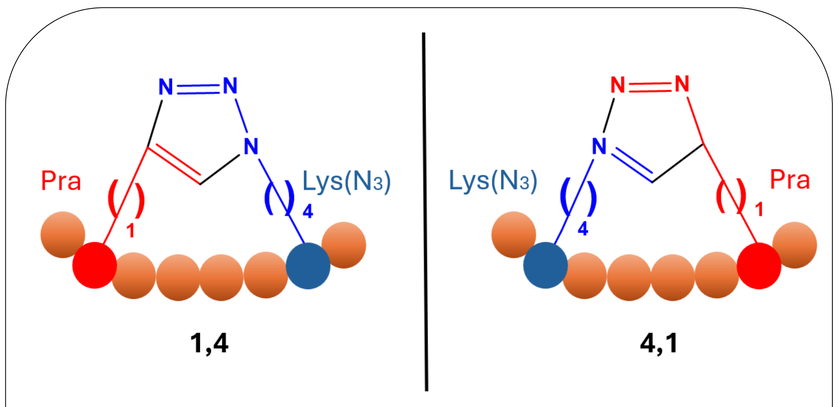


Figure 5. The triazole staple - 1,4- and 4,1- regiochemistry. Intramolecular CuAAC joins the propargylglycine (Pra) alkyne and the Nε-azido-lysine [Lys(N_3)] side chain: Pra → triazole C4 (–CH_2–, red), Lys(N_3) → triazole N1 (–$(CH_2)_4$–, blue). Both series carry the same 1,4-disubstituted 1,2,3-triazole; "1,4-" (MS1/MS3/MS5) and "4,1-" (MS2/MS4/MS6) denote the N→C order of the two handles along the backbone, set by residue placement.

Table 2 **Summary of characterized and synthesized Compounds MS1–MS6**

Compound	Triazole	Zn-Binding Residue	Purity	Yield
MS1	1,4-	Cys (-SH)	90%	8 mg
MS2	4,1-	Cys (-SH)	90%	34 mg
MS3	1,4-	Ser (-OH)	95%	35 mg
MS4	4,1-	Ser (-OH)	95%	45.8 mg
MS5	1,4-	His	95%	42 mg
MS6	4,1-	His	95%	34.9 mg

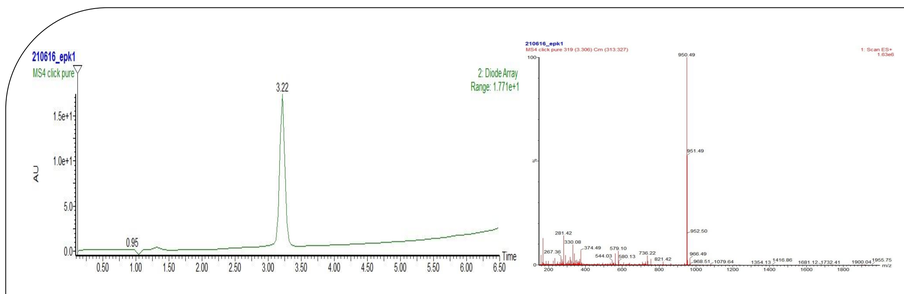


***Figure 6**. HPLC chromatogram (left) and ESI-MS (right) of peptide MS4*

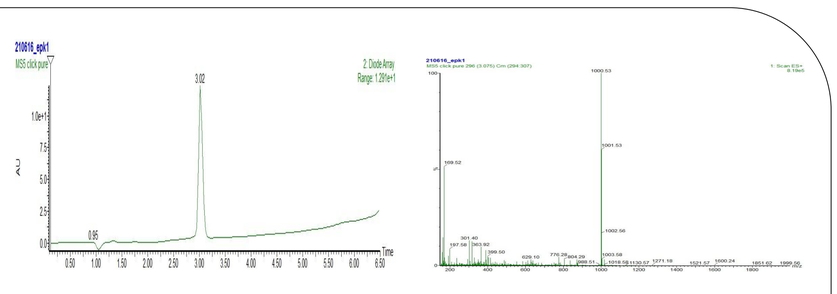


***Figure 7.** HPLC chromatogram (Left) and ESI-MS (right) of peptide MS5*

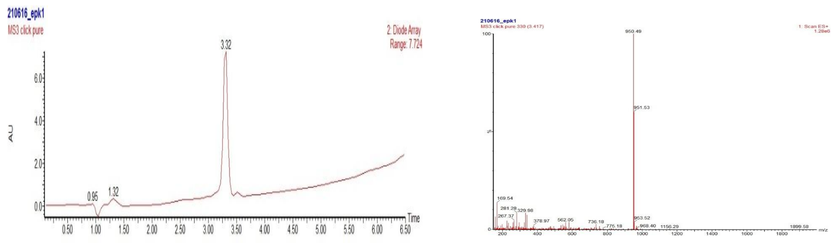


***Figure 8**. HPLC chromatogram (left) and ESI-MS (right) of peptide MS3*

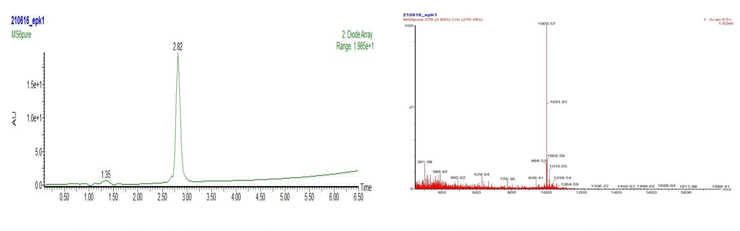


***Figure 9.** HPLC chromatogram (Left) and ESI-MS (right) of peptide MS6*

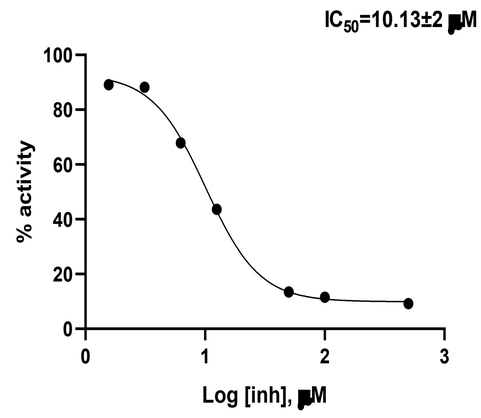


***Figure 10.** Preliminary results showing of Dose-response inhibition curve against NDM-1*

5. Conclusion

▶ A library of >200 stapled peptidomimetics across 8 structural series was computationally evaluated by GOLD docking and MM-GBSA against NDM-1 (PDB: 6XBE)
▶ Six triazole-constrained cyclic peptidomimetics (MS1-MS6) were successfully designed, synthesized, and characterized via Fmoc SPPS and on-resin CuAAC click chemistry
▶ Systematic variation of zinc-binding residue (Cys, Ser, His) and triazole orientation (1,4- vs. 4,1-) provides a structured SAR framework for NDM-1 inhibitor optimization
▶ Preliminary resazurin screening of Minimum inhibitory concentration and Inhibitory concentration of 50% identified as there are promising candidate for potentiating meropenem activity against NDM-1-producing bacteria. Further optimization and biological replicates of experiments are ongoing

6. Future direction

▶ Full IC_{50}/MIC profiling against VIM-2 and IMP-1, and replicate NDM-1 assays
▶ NMR analysis
▶ Second-generation synthesis - MM-GBSA-guided design and synthesis of optimized analogs at PeptLab, University of Florence

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