Synthesis of polymyxin analogs with enhanced activity towards Gram-negative biofilm-associated infections

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Polymyxins (PM) B and E are last-resort drugs against Gram-negative bacterial infections (often biofilm-related), but resistance and toxicity compromise PM use. A fast-growing no. of PM analogs have been reported, but with varied success in improving PM activity and ameliorating toxicity. α,α-Dialkylglycines (DAG), unnatural amino acids, hold unique characteristics (bioactivity, metabolic resistance, hydrophobicity, stability), being innovative building blocks in PM analog design.

We report 6 new PM analogs' synthesis and activity against planktonic and biofilm cultures, including PM analog combination with common antibiotics.

Two DAGs, diisobutyl glycine (Dibg) and dibenzyl glycine (Dbng), were synthesised by Ugi multicomponent reaction and assembled in situ at positions 6 and/or 7 of native PMB/E through microwave assisted solid-phase peptide synthesis. Antibacterial/antibiofilm activities were assessed against 24-h old Pseudomonas aeruginosa ATCC 27853 cultures (broth microdilution; culturable biofilm cells), including synergy evaluation with ciprofloxacin (CIP) and tobramycin (TOB) (checkerboard assays; anti-biofilm activity).

The 2 analogs with Dibg at position 7 (PMBa/PMEa) were the most promising, with PMBa having MIC/MBC of 4 mg/L and no cytotoxicity towards A549 lung epithelial cells. Preliminary HPLC analysis indicates ~66% purity, meaning that pure analog concentrations are ~2 mg/L, close to that of PMB (1 mg/L). Furthermore, 512 mg/L (~340 mg/L pure analog) of PMBa decreased biofilm cell numbers by 3.7 log(CFU/mL), which is promising considering that PMB achieved a similar reduction, ~3.9 log(CFU/mL), for a higher effective concentration (512 mg/L). In turn, PMBb/PMEb (Dbng at position 6) and PMBc (Dbng at position 6 + Dibg at position 7) analogs were the most promising in combination scenarios, achieving synergic/additive outcomes ($0.3125<FICI<0.75$) when combined with TOB/CIP against planktonic cells. Preliminary results still show potential synergy of PMEb+TOB with decreased biofilm cell numbers ~3.4 log(CFU/mL).

We designed 6 new PM analogs, with 5 possessing antimicrobial and anti-biofilm activity against P. aeruginosa, including in combination with antibiotics, revealing the suitability of DAGs in PM analog design. Further testing include analog purification and nephrotoxicity evaluation.

Keywords: Polymyxin analogs, α,α-Dialkylglycines, anti-biofilm, combination therapy.

Acknowledgements

We acknowledge the FCT-funded project POLYmix-POLYmic (DOI:10.54499/2022.06595.PTDC), the programmatic funding to CQUM (DOI:10.54499/UIDB/00686/2020) and CEB (DOI:10.54499/UIDB/04469/2020), and the contracts of the author PJ (DOI:10.54499/2020.00194.CEECIND/CP1600/CT0021) and SL (DOI:10.54499/DL57/2016/CP1377/CT0043).

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