Development of polymyxin B derivatives incorporating uncommon amino acids: synthesis and antimicrobial activity

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The rise of antimicrobial resistance, especially in multidrug-resistant (MDR) Gram-negative bacteria, has led the World Health Organization (WHO) to highlight priority pathogens, including the ESKAPE group. These organisms, frequently resistant to multiple antibiotic classes and increasingly virulent, are major causes of hospital-acquired infections. With a limited antibiotic pipeline, polymyxins B (PMB) and E (PME) have been reintroduced as last-resort antibiotics against MDR Gram-negative infections. However, their use is limited by narrow-spectrum activity (but most infections are predominantly polymicrobial), nephro- and neurotoxicity, and growing resistance. This has renewed interest in designing PM analogues with improved efficacy and lower toxicity. Yet, despite extensive modifications, a superior derivative has not been identified, underscoring the need for continued development.

We report the synthesis and antibacterial activity of six new PMB derivatives (PMBa-f) against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) strains.

Four uncommon amino acids, structurally related to Phe and Dab present in the native sequence, were incorporated at selected positions of PMB using microwave-assisted solid-phase peptide synthesis. Antibacterial activity was evaluated by MIC and MBC determination (broth microdilution) against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923.

Three PMB derivatives modified at positions 1 (PMBd), 3 (PMBe), and 6 (PMBc) demonstrated promising activity against *P. aeruginosa*, with MICs ranging from 4 to 8 mg/L. All six derivatives exhibited strong activity against *S. aureus*, comparable to or better than native PMB. Notably, derivatives with Phe substitutions at position 6 (PMBa-c) showed enhanced anti-staphylococcal potency, with MICs of 32–64 mg/L versus 128 mg/L for PMB. After adjusting for compound purity, estimated MIC and MBC values were approximately 6–7-fold and 6–14-fold lower, respectively, than those of unmodified PMB.

These results support the strategic incorporation of unnatural amino acids in PMB analogue design, to enhance antibacterial effectiveness and broad-spectrum activity. Ongoing studies aim to improve compound purity and assess nephrotoxicity profiles.

Keywords: polymyxins, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, unnatural amino acids.

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