PEPTIDE DEFORMYLASE AS A NOVEL TARGET OF ANTIMICROBIALS

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ABSTRACT

During the preceding decades various pathogenic microorganism have developed multidrug resistance. Discovery and development of new antimicrobial with novel targets in microbes are in great demand in order to reduce multiresistance infection and duration of chemotherapy. Peptide deformylase is an essential enzyme involved in bacterial protein biosynthesis and maturation. Its absence in eukaryotic organisms makes it an attractive target for antimicrobial chemotherapy. A number of peptide deformylase inhibitors have been discovered. Peptide deformylase inhibitors such as BB-81384, BB-83698 and VIC-104959 (LBM-415; NVP-peptide deformylase-713) have entered into clinical trials in humans as oral and parenteral agents for treatment of infections caused by Gram-positive and Gram-negative bacteria.

Keywords: Peptide deformylase inhibitors, Antimicrobial agent, BB-81384, BB-83698, VIC-104959.

INTRODUCTION

Resistance to antibacterial agents is a significant problem ever since decades. In recent years, there has been a relentless increase in the occurrence of antibiotic resistance to many common bacterial pathogens such as Methicillin resistant Staphylococcus aureus, Penicillin resistant pneumococcus and Vancomycin resistant Enterococcus faecalis\(^{[1]}\). There are many contemporary antimicrobial resistance concerns that the critical care clinician face in managing the pharmacotherapy of infection\(^{[2]}\). Beta lactamase resistance in Enterobacteriaceae, multidrug resistance in Pseudomonas aeruginosa and Acinetobacter species, fluoroquinolone resistance in Escherichia coli and fungal resistance are among the most widespread issues that intensive care unit clinician face in managing infection\(^{[3]}\). Consequently there is an urgent need for new discovery strategies and pharmaceutical industry has taken advantage of the wealth of novel targets available as a result of
Development of antibiotics now stresses on identification, validation and exploitation of new targets to discover novel antibiotics. Many thriving antibiotics inhibit protein synthesis, however antimicrobial agent that inhibits protein modification are infrequently reported. The extensive occurrence, conservation and essential nature of deformylase in bacteria, coupled with the absence of its activity in mammalian cells, make it a gorgeous target for antibacterial drug discovery. On this ground, peptide deformylase inhibition is an attractive approach for antibacterial chemotherapy. A number of peptide deformylase inhibitors such as BB-81384, BB-83698 and VIC-104959 (LBM-415; NVP-peptide deformylase-713) have shown in vivo and in vitro activity against some Gram-positive and Gram-negative bacteria.

PEPTIDE DEFORMYLASE AS NOVEL TARGET OF ANTIMICROBIAL:

Inhibition of protein synthesis is the most frequent mode of action of antibiotic. Peptide deformylase is an essential enzyme involved in bacterial protein biosynthesis and maturation. An X-ray crystal and solution structure of peptide deformylase has identified peptide deformylase as a new class of metalloenzyme related in structure to the metalloproteinase superfamily. The structure of Ec-peptide deformylase (zinc containing E. coli peptide deformylase) has been initially solved by NMR methods and by X-ray crystallography. When iron act as the active site metal ion, the enzyme is extremely unstable; however iron can be replaced by nickel or cobalt to yield a stable enzyme that retains full activity. In each case, the metal is coordinated by two histidines of an active site HEXXH motif, a conserved cysteine and a water molecule. Peptide deformylase deformylates the N-formylmethionine of newly synthesized polypeptides. This gene encoding peptide deformylase (def) is present in all sequenced pathogenic bacterial genomes and has no mammalian counterpart, making it an attractive target for antibacterial chemotherapy. M. tuberculosis peptide deformylase has been validated as a drug target and it has been suggested that this class of compounds has the potential to be developed as novel antitycobacterial agents. A lead compound 5-bromoindolyl-3-acetohydroxamic acid has been identified as a potent inhibitor of bacterial peptide deformylases.
MODE OF ACTION OF PEPTIDE DEFORMYLASE IN PROKARYOTE:

Peptide deformylase is a prokaryotic metalloenzyme essential for bacterial growth and serve as a new target for the development of antibacterial agents\(^{18}\). The enzyme is broadly conserved in more than 90 sequenced bacterial genomes and gene knock out experiments showed that it is essential for the growth of *Escherichia coli*, *Streptococcus pneumoniae* and *Staphylococcus aureus*. In bacteria, protein synthesis is initiated with a formylated methionyl tRNA\(^*_\text{f} \text{Met}\) so that the newly synthesized proteins are formylated at the amino terminus: Peptide deformylase is required to remove the formyl group from a newly synthesized polypeptide chain. This activity is essential for subsequent N-terminal processing by methionine aminopeptidase (Map)\(^{19}\).

![Figure 1](https://www.pharmasm.com/doi/fig/1)

Peptide deformylase in bacterial protein biosynthesis

PEPTIDE DEFORMYLASE INHIBITORS:

The N-terminal protein processing pathway is essential machinery in all organisms and deformylase enzyme is involved in this process in bacteria\(^{20}\). Peptide deformylase inhibitors such as BB-3497, BB-81384 and VIC-104959 (LBM415; NVP peptide deformylase-713) provide broad spectrum activity and its little interaction with the
human protein synthesis component provides the peptide deformylase inhibitors to have favorable selectivity to bacteria and therefore safety in humans.

**BB-3497:**

N-formyl hydroxylamine derivative (BB-3497, I) is a potent and selective inhibitors of peptide deformylase. It shows activity against Gram positive bacteria including Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant *Enterococcus faecalis* and some Gram negative bacteria. The X ray crystal structure of BB-3497 has facilitated the design of novel inhibitors with improved pharmacokinetic and antibacterial properties. The crystal structures of BB-3497 bound to *Escherichia coli* peptide deformylase shows that active site metal atom is pentacoordinated by the side chains of Cys 90, His 132 and His 136 and the two oxygen atoms of N-formyl hydroxylamine or hydroxamate. This data validates Peptide deformylase as a novel target for the design of a new generation of antibacterial agents. N Formyl hydroxylamine BB-3497 is an effective inhibitor (IC\textsubscript{50}=7 nM) against *Escherichia coli* peptide deformylase Ni enzyme and exhibits potent antibacterial activity both in vitro and in vivo.

![Chemical Structure of BB-3497](image)

**BB-3497:**

Peptide deformylase inhibitors have shown potent in vitro and in vivo activity against Gram-positive organisms including drug resistant isolates. P\textsubscript{2} and P\textsubscript{3} substituents (II) substantially improve antipneumococcal activity relative to BB-3497 in therapeutic optimization for respiratory infections.
Gang Shen et al. have synthesized and evaluated peptide deformylase inhibitors as a novel class of macrocyclic peptidyl hydroxamates from commercially available 5-hexenoic acid. The most potent compound exhibits tight, slow binding inhibition of *Escherichia coli* peptide deformylase and had potent antibacterial activity against Gram-positive bacterium *Bacillus subtilis* (MIC = 2–4 µg/mL). Further optimization of the ring size and P₂ side chain may lead to highly potent, selective peptide deformylase inhibitors.[27]

BB-83815, BB-84518 and BB-83698 provided by British Biotech Pharmaceuticals Ltd (Oxford, UK) have minimum inhibitory concentrations in the range 0.06–2 mg/L. Testing on the three most promising compounds with an additional 17 isolates of *M. tuberculosis* has identified BB-3497 as the most active peptide deformylase inhibitor with a median minimum inhibitory concentration of 0.25 mg/L. Isoniazid, the control drug for this experiment, had a median minimum inhibitory concentration of 0.031 mg/L. The study demonstrated that BB-3497 has potent in vitro activity against *M. tuberculosis*.[28]

Prompted by the structure of BB-3497 a new series of N-formylhydroxylamines has been screened. In view of the hydrolyzable amide bonds present in BB-3497, a series of compounds have been synthesized for antibacterial activities in the expectation of discovering novel lead compounds bearing a substituted aniline moiety in place of amide fragment of BB-3497 to work as an amide isostere. Several anilines have been chosen for incorporation into new compounds in view of their ability to act as both, hydrogen bond

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IC Value – 4.01
acceptor and donor while maintaining the proper orientation of the side chain (II) with excellent bioactivity. All the compounds exhibited potent in vitro inhibitory activity against Staphylococcus aureus and relatively weak antibacterial activity against Klebsiella pneumonia which confirmed the mode of action of the target compounds and provided direction for the design of molecules in the future[29].

Peptidomimetic modification of BB3497: BB-81384:

N-formylhydroxylamine type inhibitor previously described (BB-3497)[30] exhibits poor activity against Staphylococcus pneumoniae. Cross et al has synthesized, characterized orally active novel peptide deformylase inhibitors and demonstrated the anti-pneumococcal oral efficacy of a novel peptide deformylase inhibitor (BB-81384, III) in multiple animal models of infections. BB-81384 selectively inhibited peptide deformylase with an IC$_{50}$ \(-10$ nM and with MICs $< 0.5$ mg/L against most Staphylococcus pneumonia pathogens. Pharmacokinetic analysis revealed good oral bioavailability. Single oral administration efficacy in a mouse peritonitis model is evident with an ED$_{50}$ of 30 mg/kg. BB-81384 reduces the bacterial load by 5 and 3 log units in organ burden models of lung and thigh infection respectively. Activity has been evaluated against a broad range of organisms such as Methicillin susceptible Staphylococcus aureus, Penicillin resistant Staphylococcus pneumoniae, Penicillin susceptible Staphylococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis, Bacillus cereus and Streptococcus pyogene[31].
Jean Marie et al has recently demonstrated that the activity of different peptide deformylase is strongly dependent on the accumulation of the active molecules by using permeabilizing agents, efflux inhibitors or efflux mutated strains and observed maximum effect when combining actinonin with a dual inhibitor of methionine aminopeptidase and peptide deformylase, this molecule is also able to interact with the target while actinonin is bound to the peptide deformylase active site\cite{32}.

VIC-104959 (LBM 415; NVP Peptide deformylase -713):

LBM415 is the first of the peptide deformylase Inhibitor to advance to clinical trials for the oral and parenteral treatment of respiratory tract and skin structure infections caused by susceptible Gram-positive and Gram-negative organisms\cite{33}. LBM415 (IV) has shown in vitro activity against a wide spectrum of pathogens including Streptococcus pneumoniae, staphylococci, enterococci, Mycoplasma pneumoniae, Chlamyphila pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Neisseria meningitides\cite{34-36}. Thomas et al have developed LBM415 as class of peptide deformylase Inhibitor for clinical trials as a parenteral and oral agent for treatment of community acquired respiratory tract disease and serious infections caused by antimicrobial-resistant Gram-positive cocci. Cross-resistance with other classes of antimicrobials has not been identified\cite{37}.
Antimicrobial activity against staphylococci (153 strains; MIC at which 90% of isolates were inhibited [MIC\(_{90}\), 2 µg/ml], Streptococcus pneumoniae (170 strains; MIC\(_{90}\), 1 µg/ml), other streptococci (150 strains; MIC\(_{90}\), 1 µg/ml), enterococci (104 strains; MIC\(_{90}\), 4 µg/ml), Moraxella catarrhalis (103 strains; MIC\(_{90}\), 0.5 µg/ml) and Legionella pneumophila (50 strains; MIC\(_{90}\), 0.12 µg/ml) were inhibited at \(\leq 8\) µg of LBM415/ml, as were 97% of Haemophilus influenzae isolates (300 strains; MIC\(_{90}\), 4 to 8 µg/ml).

NVP peptide deformylase 713 appears to be a promising clinical candidate among the peptide deformylase inhibitors for the treatment of infections caused by Gram-positive organisms that possess resistances to oxazolidinones or streptoGramin combinations\(^{[38]}\). Ronald et al have evaluated the potency of a novel NVP peptide deformylase Inhibitor 713 against Gram-positive organisms having resistances to Linezolid or Quinupristin/Dalfopristin. Quinupristin/Dalfopristin resistant E. faecium (MIC range 1–2 mg/L) and staphylococci (MIC range 0.12–2 mg/L) were also inhibited by NVP peptide deformylase-713. These results indicate that NVP peptide deformylase-713, among the new candidate peptide deformylase inhibitors have excellent activity (all MICs \(\leq 4\) mg/L) against emerging Gram-positive clinical isolates that have become resistant to oxazolidinones (Linezolid and AZD-2563)\(^{[39]}\). Mycoplasma pneumoniae is a major cause of upper and lower respiratory tract infections in children and adults and is responsible for as many as 40% of cases of community-acquired pneumonia\(^{[40-43]}\). Monica et al demonstrated LBM415 has excellent in vitro activity against 100 Mycoplasma pneumoniae\(^{[44]}\). LBM415 retains activity against many antimicrobial-resistant Gram-positive organisms including Methicillin resistant Staphylococcus aureus, Penicillin nonsusceptible Streptococcus pneumoniae and Vancomycin and linezolid resistant enterococci\(^{[45]}\).

CONCLUSION

Various peptide deformylase inhibitors have shown potent in vitro and in vivo activity against Gram-positive organisms including resistant microorganism. Some of the peptide deformylase inhibitors have entered into clinical trials as a parenteral and oral agent for treatment of community acquired respiratory tract disease and serious infections caused by antimicrobial resistant Gram-positive cocci. Several peptide deformylase inhibitors
have shown in vitro activity against Chlamydia species, gonococci, spirochetes, Haemophilus ducreyi, Streptococcus pneumoniae, enterococci, Staphylococcus aureus, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Escherichia coli. Because of the compound's novel mechanism of action there will no cross resistance with currently available antimicrobials. Peptide deformylase inhibitors are at an early stage of development but have generated much interest due to the need for novel innovative antibacterial.

REFERENCES


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