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The evolution of peptide deformylase as a target: Contribution of biochemistry, genetics and genomics

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ABSTRACT

Although peptide deformylase (PDF, EC 3.5.1.27) was first described in 1968, the instability of enzyme preparations prevented it from being seriously considered as a target until this problem was finally solved in 1998. PDFs essentiality was first demonstrated in *Escherichia coli* in 1994. Genomic analyses have shown this enzyme to be present in all eubacteria. PDF homologs have also been found in eukaryotes including *Homo sapiens*. The function and relevance of the human chromosomal homolog to the safety of PDF inhibitors as therapeutic agents is not clear at this stage. Although there is considerable sequence variation between the different bacterial PDFs, there are three strongly conserved motifs that together constitute a critical metal binding site. The observation that PDF is a metalloenzyme has led to the design of inhibitors containing metal chelating pharmacophores. The most potent of these synthetic inhibitors are active against a range of clinically relevant respiratory tract pathogens in vitro and in vivo, including those resistant to current antibiotics. Mutants resistant to PDF inhibitors have been obtained in the laboratory; these resulted from mutations in the genes for transformylase (EC 2.1.2.9) or PDF. The mechanism involved and its frequency were pathogen-dependent. The two most advanced PDF inhibitor leads, which are both reverse hydroxamates, have progressed to phase 1 clinical trials and were well tolerated.

1. Discovery of PDF

In 1966 Capecchi [1] proposed that *N*-formyl methionine was a universal initiator in bacterial protein synthesis. However, in the vast majority of cases the formyl group is cleaved off and in many cases the methionine is removed as well. Adams [2] was the first to demonstrate the presence of PDF (EC 3.5.1.27) activity in a bacterial extract; he noted the instability of the enzyme and its inhibition by mercaptoethanol. Although we now know that methionine plays a key role in the initiation of protein synthesis in all cells, the formylation and subsequent deformylation of methionine is peculiar to prokaryotes. It plays no role in the cytosolic protein synthesis of eukaryotes. The presence and role of these enzymes in cellular organelles, such as mitochondria, will be discussed later. All this biochemical information already suggested by the late 1960s the suitability of PDF as a potential target for antibacterial agents. However, proof of essentiality was lacking, and the instability of PDF was a serious obstacle.

2. PDF defined as an antibacterial target

Further progress on PDF had to wait more than 20 years and depended on advances in bacterial genetics and molecular biology. In 1993 the gene coding for PDF in *Escherichia coli* was

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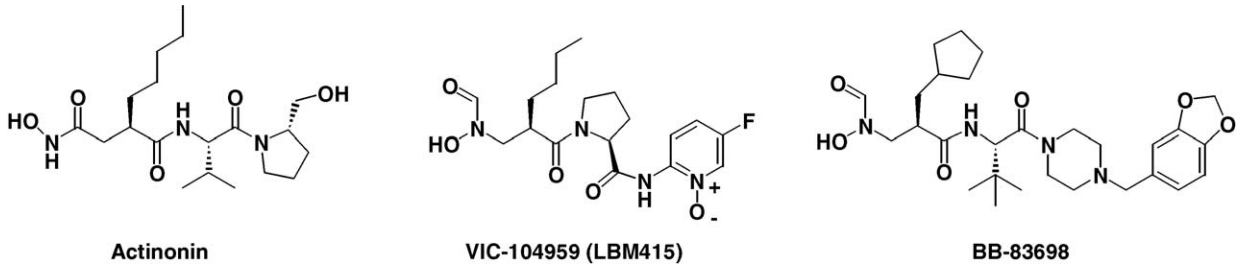

Fig. 1 – Structure of PDF inhibitors. Actinonin is a natural product isolated in 1962 with an unknown mechanism of action that was subsequently shown to be a PDF inhibitor in 2000. LBM415 and BB-83698 are synthetic PDF inhibitors that have both progressed to phase 1 clinical trials in human volunteers.

shown to belong to a single operon with the related formyl transferase (FMT; EC 2.1.2.9) gene [3]. When PDF was over-expressed and purified it was found to contain a tightly bound zinc atom (in agreement with the predicted presence of a zinc binding motif in the protein). However, the enzyme obtained had a very low specific activity. In 1994 further characterization of the PDF- and FMT-encoding genes showed that PDF was essential whereas knocking out FMT only resulted in impaired growth [4]. PDF could for the first time legitimately described as a potential target for antibacterials. Interestingly, although it is essential in an otherwise normal background, the PDF gene could be knocked out, provided that the FMT gene was knocked out as well. Such double mutants, like the FMT single mutants, were seriously growth impaired. In 1995 it was shown that PDF activity was inhibited by phenanthroline [5], a further indication of the importance of metal ions to enzyme activity. These results were reinforced by Pei's laboratory [6], which reported that several divalent metal ion chelators were potent inhibitors of PDF.

3. Solving the problem of PDF instability

The enduring problem of PDF instability was finally solved in 1998 when it was shown that the natural metal is actually ferrous ion and not zinc [7]. The ferrous ion-containing enzyme is highly active but unstable. Excluding oxygen from purification solutions stabilized enzyme activity by preventing the oxidation of the active ferrous ion to the inactive ferric form. Over-expression and purification using standard conditions results in formation of a poorly active enzyme containing zinc, which is assumed to be an artifact. It is not clear if the zinc enzyme itself possesses poor activity or whether the small amount of activity observed is due to traces of other active divalent cations. Fortunately, it was discovered that addition of other divalent cations, such as cobalt or nickel, during purification made it possible to isolate highly active, stable preparations of PDF [8,9]. These practical observations paved the way for a number of groups to initiate the synthesis of PDF inhibitors.

4. Application of mechanism-based design to PDF inhibition

The fact that PDF is a metalloenzyme suggested use of an approach to inhibitor design pioneered by researchers at Squibb. Their work on the human angiotensin converting enzyme (ACE; EC 3.4.15.1) led to the synthesis of the selective inhibitor Captopril®, which is used for the treatment of hypertension [10]. ACE is a metalloenzyme; Captopril® selectively chelates the metal ion present at the active site, inhibiting enzyme activity. The chelating function in Captopril® is a sulfhydryl group. This mechanism-based drug design approach was subsequently applied to PDF. A moiety resembling the enzyme substrate is coupled with an appropriate chelator. Researchers at Vicuron Pharmaceuticals recognized that a molecule having such characteristics had already been described: actinonin [11]. This natural product (see Fig. 1) had been discovered in 1962 [12] by empirical screening and, although it had antibacterial activity, its mechanism of action was unknown. In 1999 in vitro tests with purified PDF confirmed that actinonin was indeed a potent inhibitor of the enzyme [11]. The chelating group present in actinonin is a hydroxamate. A range of different chelators has been tried in the quest to synthesize PDF inhibitors including hydroxamates, reverse hydroxamates, and carboxylates, as well as sulfhydryls. At this stage the most potent and selective leads reported contain reverse hydroxamates. Many other important enzymes in human cells are also dependent on metal ions for their function; thus there is a risk that PDF inhibitors of this type would not provide sufficient selectivity and therefore be toxic. The mammalian matrix metalloproteases are a well-known group of enzymes that are being pursued as potential anticancer targets [13]. The availability of such enzymes, in addition to ACE, provided a suitable panel to check the selectivity of PDF inhibitors at the in vitro level. In addition, the pursuit of matrix metalloprotease inhibitors using the same mechanism-based drug design approach provided relevant insight for the design of PDF inhibitors. The first crystal structure for PDF from *E. coli* was published in 1997 [14], and further reports with and without inhibitors quickly followed [8,15,16]. The availability of this structural information has undoubtedly provided additional input on which to base inhibitor design. For a more thorough discussion of PDF inhibitors see Jain et al. [17].

5. Activity against intact bacteria

Identification of a potent PDF inhibitor is no guarantee that it will be active against intact bacterial cells. The ability of an inhibitor to access the target (by penetrating the permeability

Table 1 – Metalloenzyme selectivity and antibacterial activity of the PDF inhibitor LBM-415

	Selectivity [47]
PDF-Ni^{2+} *E. coli* (IC_{50}, μM)	0.007
MMP-7 (IC_{50}, μM)	>250
Organisms (*n*): [19–22]	MIC_{90} (μg/ml)
S. aureus (258)	2
S. pneumoniae (170)	1
Streptococcus spp. (150)	1
Enterococcus spp. (104)	4
H. influenzae (300)	4–8
M. catarrhalis (103)	0.5
H. pylori (19)	0.5
Mycoplasma (5)	0.002–0.015
Legioniella pneumophila (50)	0.12
Anaerobes: Gram-positive and negative (31)	1
Enterobacteriaceae (112)	>32
Nonfermentative bacilli (107)	>32

Taken from Refs. [19–22].

barrier), and to maintain an effective concentration (in the face of efflux pumps and inactivation) is also critical. Fortunately many potent PDF inhibitors have also proved to be active against bacteria. Typically these compounds have contained hydroxamates or reverse hydroxamates as chelators. The antibacterial spectrum of PDF inhibitors published to date has been primarily gram positive, including staphylococci and streptococci, and there is no cross-resistance with existing antibiotic classes [18]. Table 1 gives the in vitro properties of the two most advanced PDF leads [19–22]. There is some encouraging activity against fastidious gram negatives such as *Morexella catarrhalis* and *Haemophilus influenzae*. In addition there is excellent activity against the respiratory pathogens *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* [18,23,24]. The combination of these activities suggests a potential utility in the treatment of respiratory tract infections (RTIs) [25,26]. Like most other protein synthesis inhibitors, the antibacterial effect of blocking PDF is primarily bacteriostatic [11], although in some species such as *S. pneumoniae* the effect is bactericidal [27]. Examination of the bacterial proteome during inhibition of PDF revealed the formation of a new series of protein spots on 2D gels that are considered to be the *N*-formylated versions of the normal proteins [26,28]. The precise mechanism of the growth inhibition is not clear. Are *N*-formylated proteins non-functional? Does one or more of the *N*-formylated proteins interfere with the function of its normal counterpart? We do not yet know. Surprisingly, it has been found that even normal *B. subtilis* cells contain significant amounts of the *N*-formylated versions of certain proteins [28].

6. Mechanism of whole cell activity

Proof that the mechanism of growth inhibition in intact bacteria by a PDF inhibitor is mediated through PDF inhibition comes from several sources. For example, if the PDF gene is put under the control of an inducible promoter, it can be shown that the sensitivity to inhibitor correlates with the amount of PDF being expressed. At low levels of inducer, which result in low levels of PDF, the susceptibility of the bacteria increases significantly. At high levels of inducer, which result in over-expression of the PDF gene, the bacteria become resistant to PDF inhibitors but not to other inhibitors of protein synthesis [11,26]. In the case of *S. pneumoniae* it was shown that mutations in the PDF gene resulted in resistance to inhibitors [29]. Furthermore, bacterial mutants that by-pass the formylation–deformylation cycle (discussed later) are completely resistant to PDF inhibitors. All this evidence suggests that growth inhibition by these compounds is indeed due to inhibition of PDF.

7. Resistance

The first published studies on acquired resistance to PDF inhibitors were carried out with *Staphylococcus aureus* [30]. Mutants resistant to actinonin were readily obtained in vitro at the rather high frequency of 1×10^{-6}. Such mutants were highly resistant to actinonin, but normally susceptible to the other classes of antibiotics tested. There was, however, cross-resistance with other synthetic PDF inhibitors. Further examination of the resistant mutants revealed that they were growth impaired, with a growth rate about half that of the parent strain. Similar results have been reported for resistance in *E. coli* [26]. In the case of *S. pneumoniae* the resistance frequency was lower, in the range of 1×10^{-8} [29]. The reason for this difference became apparent when the mechanism of resistance in each of these bacteria was studied. In the case of *S. aureus*, resistant mutants had an altered FMT gene, whereas that encoding for PDF was unchanged. This recalled the earlier observation in *E. coli* that PDF was essential, unless the FMT-encoding gene was knocked out as well, and that such double mutants were growth impaired [4]. The high frequency obtained with *S. aureus* was presumably due to the fact that any mutation resulting in loss of FMT activity would result in resistance, albeit growth impaired. In the case of *S. pneumoniae* resistance resulted from mutations in the PDF gene itself rather than in FMT (which is essential for its growth). Such mutations must retain PDF function in addition to preventing the binding of inhibitor. This will be a less frequent event than simply inactivating an enzyme, as is the case for FMT in *S. aureus*. The relevance of the high frequency of resistance seen in the laboratory for some pathogens to the eventual clinical utility has been a matter of active debate. One published study on the virulence of a *S. aureus* actinonin-resistant mutant indicated that its growth was attenuated by four orders of magnitude in an in vivo mouse model of infection [30,31]. In another study with a mouse model of pneumococcal pneumonia, animals were treated with sub-optimal doses of a PDF inhibitor (BB-83698) and post treatment isolates from blood were checked for their susceptibility to the drug used [32]. All 14 isolates tested had the same susceptibility as the parent strain; thus resistant mutants were not taking over the population. Additional animal studies seem warranted to further explore this issue. Survival of such resistant but growth-impaired mutants, in the absence of a PDF inhibitor, could depend on the occurrence of secondary 'fitness' mutations [33]. Even relatively small differences in growth rate could rapidly result in the replacement of one strain by another. The diminished activity (intrinsic resistance) seen against *E. coli* and

H. influenzae is likely due to efflux, since in vitro results have shown that efflux defective mutants have a significantly increased susceptibility to PDF inhibitors [34–36]. Efflux is presumably playing a significant role in the intrinsic resistance of other Gram negative bacteria as well.

8. The contribution of genomics

The definition of PDF as a potential target in *E. coli* was based on biochemical and genetic evidence and just preceded the beginning of the microbial genomics era in 1995 [37]. However, the search for PDF inhibitors only began in earnest once the problem of enzyme stability was solved in 1998. At that stage a rapidly increasing number of microbial genomes was already accessible on public databases. This allowed work on characterizing the PDF gene across a wide variety of bacteria. As with any target in the post-genomic era, it was possible to answer an important series of questions: How widespread was its occurrence? How conserved was the sequence? Was there more than one copy present? Was it always paired with the FMT gene in a single operon? The genomic data confirmed the presence of a PDF gene in all eubacteria examined, and in some instances two PDF related ORFs were found. In the case of *S. pneumoniae* it was shown that only one of these was a functional PDF [29]. On the other hand, studies with *B. subtilis* revealed that both PDF-related ORFs present produced functional PDF [38]. The universal presence of PDF in clinically relevant bacterial pathogens defined PDF as a potential broad-spectrum target. Genomic studies show that although there are three highly conserved motifs (together constituting the so-called PDF signature motif), there is quite a lot of sequence variation elsewhere [39]. A comparison of the crystal structure of PDFs from different bacterial species revealed that they shared a common overall structure in spite of these differences in sequence. Although the N-terminus of PDF appears to be essential, a significant part of the C-terminal sequence is dispensable [40]. In the crystal structure the three conserved motifs are located close to each other, at the active site crevice where the metal ion is bound. In many, but not all, eubacteria the PDF gene forms part of a single operon with the FMT gene as originally shown for *E. coli* [41]. Sequence alignments for the various PDFs and phylogenetic trees indicated the presence of two sub-families [42]. One of them contains the *E. coli* enzyme and many other gram negatives; the other is composed of mostly gram positives, including *S. aureus*. However, there is no difference in sensitivity to PDF inhibitors correlating to this grouping.

Had PDF not already been identified by classical biochemical and genetic techniques, would it have been discovered by a purely genomic approach? If whole genomes had been searched for essential genes by techniques such as transposon mutagenesis [43], the essentiality of the PDF genes observed would have depended on the species studied. In *B. subtilis*, for example, the ability to individually knock out either of the two PDF genes present would have indicated non-essentiality [38]. On the other hand PDF would have turned up as an essential gene in *E. coli* or *S. pneumoniae*, where there is only a single functional copy.

The initial excitement over PDF as a target was fueled by the prevailing thought that the enzyme was unique to bacteria. As a wider net was cast using the genomics approach it became apparent that this was not true: PDF-like genes were also found in *Homo sapiens*, eukaryotic parasites, and plants [44]. The presence of PDF homologs in the organelles of eukaryotes, such as mitochondria and chloroplasts, is in agreement with their proposed evolutionary origin from prokaryotes. It has been proposed that PDF represents a suitable target for antiparisitic drugs [45]. In the case of animal cells it had been observed that mitochondrial proteins are usually *N*-formylated (discussed in Ref. [46]). Thus there appeared to be an FMT activity, but by implication no functional PDF. Expression of the human homolog showed that it shared many properties with the bacterial enzyme and was inhibited by the same compounds [47]. However, it was much less active than its bacterial counterpart, probably due to a variation in a residue that is highly conserved in bacteria. Human PDF is coded for by a chromosomal gene but has a signal sequence that targets it to mitochondria. Subsequent work published by another group [48] proposed this same human PDF as a potential anticancer target, and reported that the PDF inhibitor actinonin inhibited the growth of 16 different cancer cell lines in vitro. Surprisingly there was little effect on three normal cell lines. The basis for such selectivity is not understood. Additional data reported that actinonin was well tolerated in vivo, and was active against two animal xenograft models (human prostate and lung cancers). The implication of all these results to the safety of PDF inhibitors as antibacterials in humans is not clear at this juncture. Do eukaryotic PDFs represent non-functional vestigial remnants of prokaryotic genes, or do they have some as yet unrecognized function in human cells [47]?

9. Activity in animal models of infection

The excellent in vitro activity of PDF inhibitors has been shown to translate into in vivo efficacy using animal models of infection. Models used include systemic septicemias and localized tissue infections. Many of the inhibitors are active by the oral and parenteral routes against septicemias caused by *S. aureus*, *S. pneumoniae* and *H. influenzae* [34–36,49]. Given that the proposed indication for the most advanced leads in this class is RTIs, perhaps the more relevant models are these same infections in rodents. Several of the advanced PDF inhibitor leads have been shown to be quite active in a murine pneumococcal pneumonia model after oral or subcutaneous administration [32,35]. Tissue penetration of these compounds into the lung was generally good, giving higher levels than those in serum. In the case of BB-83698, efficacy was retained against penicillin-, quinolone-, and macrolide-resistant strains, as would be predicted by the absence of cross-resistance in vitro [32]. Area under the curve (AUC) over minimal inhibitory concentration (MIC) for the drug has been shown to be the best predictor of efficacy for PDF inhibitors in animal models [50,51].

10. Clinical results

The ultimate validation of PDF as a target will depend on the results of clinical trials. Two PDF inhibitors have been advanced to phase 1 clinical studies: BB-83698 (Oscient

Pharmaceuticals) and LBM415 (Novartis Pharmaceuticals). Detailed results for an intravenous phase 1 study on BB-83698 have been published [52]. Systemic exposure had a mostly linear relationship to dose administered. The pharmacokinetic results were consistent and predictable, and a good allometric scaling was evident when data from several animal species and man were compared. Drug exposure based on AUC estimates, coupled with knowledge of the MICs for relevant RTI pathogens, predicted that efficacious levels of drug were present and the potential for once daily dosing. Novartis has announced that they are replacing the development candidate LBM415 with an improved analog that is projected to enter phase 1 studies by the end of 2005. No clinically significant adverse effects were noted in either study. This is important since in addition to the concern about the role of human 'PDF' mentioned earlier, the chemical nature of the inhibitors themselves (reverse hydroxamates) could result in non-specific effects impinging on safety. These two drugs that have progressed to clinical trials have undergone all the required regulatory preclinical testing, including sub acute toxicology in two animal species. For the moment there do not appear to be any safety issues precluding the development of PDF inhibitors. The future plans for the development of PDF inhibitors have not been made public, as is often the case in the highly competitive arena of pharmaceutical and biotechnology R&D. The key issues concerning efficacy and resistance will only be resolved when the results to phases 2 and 3 become available.

Note: The references cited are representative and are not intended to be comprehensive. Further information on PDF as a target can be obtained from other published review articles [17,46,53–57].

REFERENCES

[1] Capecchi MR. Initiation of *E. coli* proteins. Proc Natl Acad Sci USA 1966;55(6):1517–24.
[2] Adams JM. On the release of the formyl group from nascent protein. J Mol Biol 1968;33:571–89.
[3] Meinnel T, Blanquet S. Evidence that peptide deformylase and methionyl-tRNA(fMet) formyltransferase are encoded within the same operon in *Escherichia coli*. J Bacteriol 1993;175(23):7737–40.
[4] Mazel D, Pochet S, Marliere P. Genetic characterization of polypeptide deformylase, a distinctive enzyme of eubacterial translation. EMBO J 1994;13(4):914–23.
[5] Meinnel T, Blanquet S. Enzymatic properties of *Escherichia coli* peptide deformylase. J Bacteriol 1995;177(7):1883–7.
[6] Rajagopalan PT, Datta A, Pei D. Purification, characterization, and inhibition of peptide deformylase from *Escherichia coli*. Biochemistry 1997;36(45):13910–8.
[7] Rajagopalan PTR, Yu XC, Pei D. Peptide deformylase, a new type of mononuclear iron protein. J Am Chem Soc 1997;119:12418–9.
[8] Groche D, Becker A, Schlichting I, Kabsch W, Schultz S, Wagner AF. Isolation and crystallization of functionally competent *Escherichia coli* peptide deformylase forms containing either iron or nickel in the active site. Biochem Biophys Res Commun 1998;246(2):342–6.
[9] Becker A, Schlichting I, Kabsch W, Groche D, Schultz S, Wagner AF. Iron center, substrate recognition and mechanism of peptide deformylase. Nat Struct Biol 1998;5(12):1053–8.
[10] Cushman DW, Ondetti MA. History of the design of captopril and related inhibitors of angiotensin converting enzyme. Hypertension 1991;17(4):589–92.
[11] Chen DZ, Patel DV, Hackbarth CJ, Wang W, Dreyer G, Young DC, et al. Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. Biochemistry 2000;39(6):1256–62.
[12] Gordon JJ, Kelly BK, Miller GA. Actinonin: an antibiotic substance produced by an Actinomycete. Nature 1962;195:701–2.
[13] Docherty AJ, Crabbe T, O'Connell JP, Groom CR. Proteases as drug targets. Biochem Soc Symp 2003;70:147–61.
[14] Chan MK, Gong W, Rajagopalan PT, Hao B, Tsai CM, Pei D. Crystal structure of the *Escherichia coli* peptide deformylase. Biochemistry 1997;36(45):13904–9 [published erratum appears in Biochemistry 1998;37(37):13042].
[15] Guilloteau JP, Mathieu M, Giglione C, Blanc V, Dupuy A, Chevrier M, et al. The crystal structures of four peptide deformylases bound to the antibiotic actinonin reveal two distinct types: a platform for the structure-based design of antibacterial agents. J Mol Biol 2002;320(5):951–62.
[16] Baldwin ET, Harris MS, Yem AW, Wolfe CL, Vosters AF, Curry KA, et al. Crystal structure of type II peptide deformylase from *Staphylococcus aureus*. J Biol Chem 2002;277(34):31163–71.
[17] Jain R, Chen D, White RJ, Patel DV, Yuan Z. Bacterial peptide deformylase inhibitors: a new class of antibacterial agents. Curr Med Chem 2005;12(14):1606–21.
[18] Apfel C, Banner DW, Bur D, Dietz M, Hirata T, Hubschwerlen C, et al. Hydroxamic acid derivatives as potent peptide deformylase inhibitors and antibacterial agents. J Med Chem 2000;43(12):2324–31.
[19] Fritsche TR, Sader HS, Cleeland R, Jones RN. Comparative antimicrobial characterization of LBM415 (NVP PDF-713), a new peptide deformylase inhibitor of clinical importance. Antimicrob Agents Chemother 2005;49(4):1468–76.
[20] Credito K, Lin G, Ednie LM, Appelbaum PC. Antistaphylococcal activity of LBM415, a new peptide deformylase inhibitor, compared with those of other agents. Antimicrob Agents Chemother 2004;48(10):4033–6.
[21] Lopez S, Wu C, Blais J, Hackbarth C, Gomez M, Kubo A, et al. In vitro profiling of the new peptide deformylase inhibitor LBM415(VIC-104959). In: Proceedings of the 44th Annual ICAAC Meeting; 2004 (Abstract No. F1960).
[22] Ryder NS, Dzink-Fox J, Kubik B, Mlineritsch W, Alvarez S, Bracken K, et al. LBM415, a new peptide deformylase inhibitor with potent in vitro activity against drug-resistant bacteria. In: Proceedings of the 44th Annual ICAAC Meeting; 2004 (Abstract No. F-1959).
[23] Robin P, Hammerschlag M. In vitro activity of a novel new antibiotic, NVP-PDF386 (VRC4887), against *Chlamydia pneumoniae*. In: Proceedings of the 42nd Annual ICAAC Meeting; 2002 (Abstract No. F-1674).
[24] Waites KB, Reddy NB, Crabb DM, Duffy LB. Comparative in vitro activities of investigational peptide deformylase inhibitor NVP LBM-415 and other agents against human mycoplasmas and ureaplasmas. Antimicrob Agents Chemother 2005;49(6):2541–2.
[25] Wise R, Andrews JM, Ashby J. In vitro activities of peptide deformylase inhibitors against gram-positive pathogens. Antimicrob Agents Chemother 2002;46(4):1117–8.
[26] Apfel CM, Locher H, Evers S, Takacs B, Hubschwerlen C, Pirson W, et al. Peptide deformylase as an antibacterial drug target: target validation and resistance development. Antimicrob Agents Chemother 2001;45(4):1058–64.

[27] Ednie LM, Pankuch G, Appelbaum PC. Antipneumococcal activity of LBM415, a new peptide deformylase inhibitor, compared with those of other agents. Antimicrob Agents Chemother 2004;48(10):4027–32.
[28] Bandow JE, Becher D, Buttner K, Hochgrafe F, Freiberg C, Brotz H, et al. The role of peptide deformylase in protein biosynthesis: a proteomic study. Proteomics 2003;3(3): 299–306.
[29] Margolis P, Hackbarth C, Lopez S, Maniar M, Wang W, Yuan Z, et al. Resistance of *Streptococcus pneumoniae* to deformylase inhibitors is due to mutations in defB. Antimicrob Agents Chemother 2001;45(9):2432–5.
[30] Margolis PS, Hackbarth CJ, Young DC, Wang W, Chen D, Yuan Z, et al. Peptide deformylase in *Staphylococcus aureus*: resistance to inhibition is mediated by mutations in the formyltransferase gene. Antimicrob Agents Chemother 2000;44(7):1825–31.
[31] Chen D, Hackbarth CJ, Ni ZJ, Wang W, Wu C, Young DC, et al. In vivo evaluation of VRC3375 a potent peptide deformylase inhibitor. In: Proceedings of the 40th Annual ICAAC Meeting; 2000 (Abstract No. 2175).
[32] Azoulay-Dupuis E, Mohler J, Bedos JP. Efficacy of BB-83698, a novel peptide deformylase inhibitor, in a mouse model of pneumococcal pneumonia. Antimicrob Agents Chemother 2004;48(1):80–5.
[33] Bjorkman J, Andersson DI. The cost of antibiotic resistance from a bacterial perspective. Drug Resist Update 2000;3(4):237–45.
[34] Hackbarth CJ, Chen DZ, Lewis JG, Clark K, Mangold JB, Cramer JA, et al. *N*-Alkyl urea hydroxamic acids as a new class of peptide deformylase inhibitors with antibacterial activity. Antimicrob Agents Chemother 2002;46(9):2752–64.
[35] Gross M, Clements JM, Beckett P, Thomas W, Taylor S, Lofland D, et al. Oral anti-pneumococcal activity and pharmacokinetic profiling of a novel peptide deformylase inhibitor. Antimicrob Agents Chemother 2004.
[36] Clements JM, Beckett RP, Brown A, Catlin G, Lobell M, Palan S, et al. Antibiotic activity and characterization of BB-3497, a novel peptide deformylase inhibitor. Antimicrob Agents Chemother 2001;45(2):563–70.
[37] Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 1995;269(5223):496–512.
[38] Haas M, Beyer D, Gahlmann R, Freiberg C. YkrB is the main peptide deformylase in *Bacillus subtilis*, a eubacterium containing two functional peptide deformylases. Microbiology 2001;147(Pt 7):1783–91.
[39] Meinnel T, Lazennec C, Villoing S, Blanquet S. Structure–function relationships within the peptide deformylase family. Evidence for a conserved architecture of the active site involving three conserved motifs and a metal ion. J Mol Biol 1997;267(3):749–61.
[40] Meinnel T, Lazennec C, Dardel F, Schmitter JM, Blanquet S. The C-terminal domain of peptide deformylase is disordered and dispensable for activity. FEBS Lett 1996;385(1–2):91–5.
[41] Mazel D, Coic E, Blanchard S, Saurin W, Marliere P. A survey of polypeptide deformylase function throughout the eubacterial lineage. J Mol Biol 1997;266(5):939–49.
[42] Serero A, Giglione C, Meinnel T. Distinctive features of the two classes of eukaryotic peptide deformylases. J Mol Biol 2001;314(4):695–708.
[43] Akerley BJ, Rubin EJ, Camilli A, Lampe DJ, Robertson HM, Mekalanos JJ. Systematic identification of essential genes by in vitro mariner mutagenesis. Proc Natl Acad Sci USA 1998;95(15):8927–32.
[44] Giglione C, Serero A, Pierre M, Boisson B, Meinnel T. Identification of eukaryotic peptide deformylases reveals universality of N-terminal protein processing mechanisms. EMBO J 2000;19(21):5916–29.
[45] Meinnel T. Peptide deformylase of eukaryotic protists: a target for new antiparasitic agents? Parasitol Today 2000;16(4):165–8.
[46] Giglione C, Pierre M, Meinnel T. Peptide deformylase as a target for new generation, broad spectrum antimicrobial agents. Mol Microbiol 2000;36(6):1197–205.
[47] Nguyen KT, Hu X, Colton C, Chakrabarti R, Zhu MX, Pei D. Characterization of a human peptide deformylase: implications for antibacterial drug design. Biochemistry 2003;42(33):9952–8.
[48] Lee MD, She Y, Soskis MJ, Borella CP, Gardner JR, Hayes PA, et al. Human mitochondrial peptide deformylase, a new anticancer target of actinonin-based antibiotics. J Clin Invest 2004;114(8):1107–16.
[49] Chen D, Hackbarth C, Ni ZJ, Wu C, Wang W, Jain R, et al. Peptide deformylase inhibitors as antibacterial agents: identification of VRC3375, a proline-3-alkylsuccinyl hydroxamate derivative, by using an integrated combinatorial and medicinal chemistry approach. Antimicrob Agents Chemother 2004;48(1):250–61.
[50] Craig W, Andes D. In vivo pharmacodynamics of BB-83698, a deformylase inhibitor. In: Proceedings of the 41st Annual ICAAC Meeting; 2001 (Abstract No. F-355).
[51] Craig W, Andes D. In vivo pharmacodynamic activity of LBM415 against multiple bacterial pathogens. In: Proceedings of the 14th European Congress of Clinical Microbiology and Infectious Diseases; 2004 (Poster No. P-922).
[52] Ramanathan-Girish S, McColm J, Clements JM, Taupin P, Barrowcliffe S, Hevizi J, et al. Pharmacokinetics in animals and humans of a first-in-class peptide deformylase inhibitor. Antimicrob Agents Chemother 2004;48(12):4835–42.
[53] Yuan Z, Trias J, White RJ. Deformylase as a novel antibacterial target. Drug Discov Today 2001;6(18):954–61.
[54] Waller AS, Clements JM. Novel approaches to antimicrobial therapy: peptide deformylase. Curr Opin Drug Discov Dev 2002;5(5):785–92.
[55] Clements JM, Ayscough A, Keavey K, East SP. Peptide deformylase inhibitors, potential for a new class of broad spectrum antibacterials. Curr Med Chem 2002;1(3):239–49.
[56] Johnson KWLD, Moser HE. PDF Inhibitors: an emerging class of antibacterial drugs. Curr Drug Targets Infect Disord 2005;5(1):39–52.
[57] Chen D, Yuan Z. Therapeutic potential of peptide deformylase inhibitors. Expert Opin Invest drugs 2005;14(9):1107–16.