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Original article

Synthesis, antibacterial activity, and biological evaluation of formyl hydroxyamino derivatives as novel potent peptide deformylase inhibitors against drug-resistant bacteria

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ABSTRACT

Peptide deformylase (PDF) has been identified as a promising target for novel antibacterial agents. In this study, a series of novel formyl hydroxyamino derivatives were designed and synthesized as PDF inhibitors and their antibacterial activities were evaluated. Among the potent PDF inhibitors (**1o**, **1q**, **1o′**, **1q′**, and **1x**), in vivo studies showed that compound **1q** possesses mild toxicity, a good pharmacokinetic profile and protective effects. The good in vivo efficacy and low toxicity suggest that this class of compounds has potential for development and use in future antibacterial drugs.

1. Introduction

The increased prevalence of multi drug-resistant (MDR) bacteria from clinical isolates has made the search for new antibacterial agents with novel modes of action even more important. One of the new targets currently receiving widespread attention from both academic and industrial research groups is peptide deformylase (PDF) [1–4]. PDF is an iron-containing metalloenzyme involved in the post-translational modification of nascent polypeptides in bacterial cells [4–8]. The catalytic mechanism of PDF enzymes containing zinc, iron, cobalt, and nickel dications was studied by Nino Russo in 2006 [9]. Protein synthesis in bacterial cells is initiated by ribosomal binding to a formyl methionine-charged transfer RNA, but most mature proteins do not retain the N-formyl group or the terminal methionine residue. Therefore, the removal of the N-formyl moiety catalyzed by PDF is a crucial step in bacterial protein biosynthesis and growth [10]. On the other hand, mammalian cytosolic protein synthesis does not produce N-formylated polypeptides and does not need the PDF enzyme [11]. The difference between bacterial and mammalian protein synthesis makes PDF an attractive and unique target for treating resistant bacteria [12,13].

Previous studies have shown that the potency of PDF inhibitors is closely linked to the additive effects of several chemical groups (Fig. 1): (a) metal-binding group: studies indicate that the best metal-binding group for most peptide deformylase inhibitors are hydroxamate or N-formyl hydroxylamine [14]; (b) P1′ group: substituents in the P1′ position that mimic the methionine residue in the natural substrate closely, such as n-butyl and cyclopentylmethyl, resulting in potent PDF inhibitors that display promising antibacterial activity [15]; (c) P2′ group: many active substituents have been described at the P2′ position and some inhibitors with proline at this position result in the desired combination of antibacterial activity and low toxicity [16–18], also the preliminary evaluation of our synthesized PDF inhibitors with proline derivatives at the P2′ position showed good to excellent antibacterial activity [19,20]; (d) P3′ group: the P3′ position is more amenable to different substitutions, and appropriate modifications

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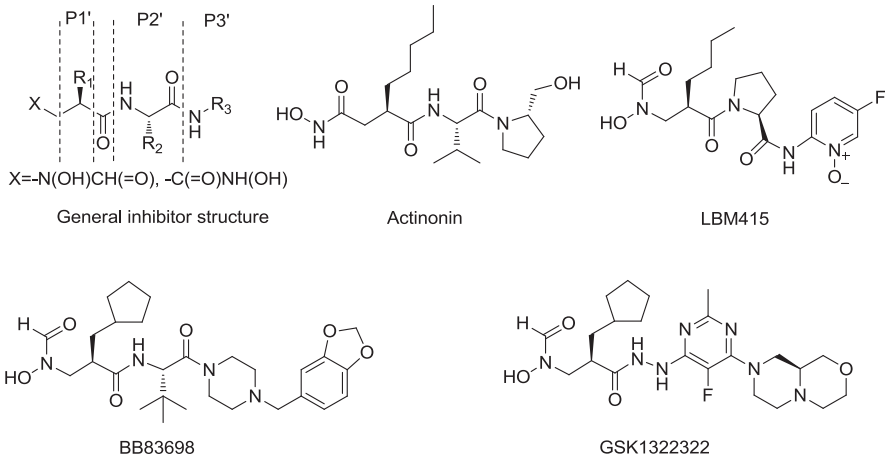


Fig. 1. Chemical structures of PDF inhibitors.

at the P3′ position could significantly improve the antibacterial activity of PDF inhibitors with only minor effects to the binding affinity for the enzyme. The first PDF inhibitor, actinonin (Fig. 1), was isolated from an actinomycete by Gordon in 1962 [21]. Actinonin exhibited moderate antibacterial activity against several Gram-positive and Gram-negative bacteria [22], but it did not show good in vivo antibacterial activity due to poor pharmacokinetic properties, which could be attributed to either poor absorption or quick clearance [23]. In order to overcome these drawbacks, novel PDF inhibitors were developed by many pharmaceutical companies and academic institutions [24–27]. LBM415 (Fig. 1, discovered by Vicuron Pharmaceuticals in collaboration with Novartis) [28,29] and BB83698 (Fig. 1, discovered by British Biotech in collaboration with Genesoft) [8,30] were the first two PDF inhibitors to undergo human clinical trials, and they exhibited much better in vitro and in vivo efficacies when compared to the original lead compound actinonin, but drawbacks of LBM415 were still founded in the further study [31]. GSK1322322 (Fig. 1, developed by GlaxoSmithKline, Brentford, UK) completed a phase II trial for acute bacterial skin and skin structure infections in April 2012 [32]. This compound not only possesses potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), but also exhibits activity against the respiratory pathogens *Haemophilus influenzae* and *Streptococcus pneumoniae* [33]. However, no PDF inhibitors are currently marketed.

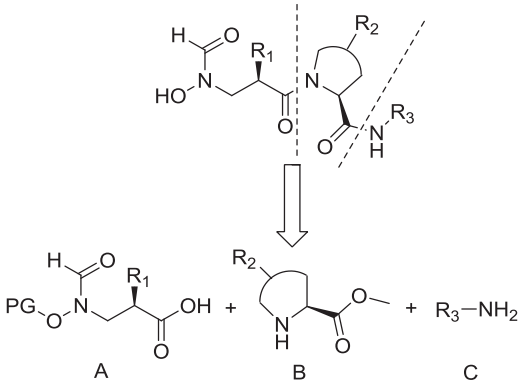
Among the PDF inhibitors that have undergone human clinical trials, LBM415 gained widespread attention for its good activity in vitro against a range of pathogens [34]. However, some concerns remain about its in vivo stability (e.g., proteolysis of the peptide bonds), solubility, and bioavailability, as these properties are closely linked to medical efficacy. In continuation of our previous study [19,20], we describe the design, synthesis, and biological evaluation of a series of PDF inhibitors based on the modification of LBM415. Five proline derivatives widely used in many biologically active products that usually play positive roles in promoting pharmacological activities and other medicinal properties, specifically (2*S*)-2,5-dihydro-1H-pyrrole-2-carboxylic acid, (2*S*)-4-methylene-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-methylpyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-fluoropyrrolidine-2-carboxylic acid, and (2*S*,3a*R*,7a*S*)-octahydro-1H-indole-2-carboxylic acid, were carefully selected to replace the proline at the P2′ position of LBM415 [35–37]. Novel PDF inhibitors were prepared by introducing various amines, such as aliphatic amines, aromatic amines, and heterocyclic aromatic amines, into the P3′ position. The in vitro antibacterial activities of all these compounds were evaluated for a primary selection, and the in vivo antibacterial activities, acute toxicity, solubility and stability, plasma protein binding rate, pharmacokinetic properties, and bioavailability of the selected representative compounds were evaluated to identify new compounds with an improved antibacterial profile and pharmacological properties.

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines the retrosynthetic analysis of the PDF inhibitors. The illustrated bond disconnection resulted in three fragments: **A**, **B**, and **C**. The target compound was assembled using the intermolecular amide coupling reaction of **A** and **B**, followed by hydrolysis of the ester group of the coupling product and subsequent coupling of the obtained carboxyl acids with amine **C**. The last step was deprotection to obtain the desired target compounds.

Following the route elucidated by Joel Slade [38], the synthesis of fragment **A** is illustrated in Scheme 2. Diethyl malonate was chosen as the starting material to synthesize fragment **A** bearing n-butyl (**A_1**) or cyclopentylmethyl (**A_2**) groups. Taking the synthesis of fragment **A_1** as an example, the reaction of diethyl malonate with 1-bromobutane in the presence of sodium ethoxide resulted in dimethyl 2-butylmalonate **6** (61% yield). The dimethyl 2-butylmalonate **6** was hydrolyzed with 25% aqueous sodium hydroxide to obtain 2-butylmalonic acid **7** (85% yield). Compound **7** was then converted to 2-methylenehexanoic acid **8** via treatment with formaldehyde in the presence of diethylamine. The reaction of



Scheme 1. Retrosynthetic analysis of PDF inhibitors.