

Synthesis of conjugates between oxazolidinone antibiotics and a pyochelin analogue



Aurélien Paulen, Françoise. Hoegy, Béatrice. Roche, Isabelle J. Schalk, Gaëtan L.A. Mislin*

CNRS Equipe Transport Membranaire Bactérien, Biotechnologie et Signalisation Cellulaire, UMR7242, Pole API, 300 boulevard Sébastien Brant, CS10413, 67412 Illkirch Cedex, France
University of Strasbourg, Ecole Supérieure de Biotechnologie de Strasbourg (ESBS), F-67413 Illkirch Cedex, France

ARTICLE INFO

Article history:

Received 29 August 2017
Revised 15 September 2017
Accepted 16 September 2017
Available online 18 September 2017

This article is dedicated to the late Pr. Dieter Haas, in memory of its outstanding contributions to molecular microbiology on *Pseudomonas aeruginosa*.

Keywords:

Pyochelin
Siderophore
Oxazolidinone antibiotic
Linezolid
Trojan Horse strategy

ABSTRACT

Pseudomonas aeruginosa is a Gram-negative pathogenic bacterium responsible for severe infections, and it is naturally resistant to many clinically approved antibiotic families. Oxazolidinone antibiotics are active against many Gram-positive bacteria, but are inactive against *P. aeruginosa*. Increasing the uptake of oxazolidinones through the bacterial envelope could lead to an increased antibiotic effect. Pyochelin is a siderophore of *P. aeruginosa* which delivers external iron to the bacterial cytoplasm and is a potential vector for the development of Trojan Horse oxazolidinone conjugates. Novel pyochelin-oxazolidinone conjugates were synthesized using an unexpectedly regioselective peptide coupling between an amine functionalized pyochelin and oxazolidinones functionalized with a terminal carboxylate.

© 2017 Elsevier Ltd. All rights reserved.

Bacterial resistance is one of the major health threats of the near future and there is an urgent need for new and effective antibiotic agents. The ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteria* species) group is a “shady club” of microorganisms causing serious anxiety among health authorities.^{1–3} This group is composed of Gram-positive and Gram-negative pathogenic bacteria which can cause antibiotic-resistant infections with potentially fatal outcomes. After several decades of big pharmaceutical companies showing less interest in anti-infective drug research, innovative molecules are once again in the pipelines. However, very few of the biological targets validated and the leads investigated are relevant to the treatment of infections due to Gram-negative bacteria in general and *P. aeruginosa* in particular.^{2,3} The low permeability of the *P. aeruginosa* cell envelope provides natural resistance to many antibiotic families currently approved for use against Gram positive bacteria.

Nutrient uptake systems are gates through the bacterial envelope and therefore could be exploited by Trojan horse strategies to

allow, or improve, the delivery of antibiotics into bacteria. Iron-uptake systems are promising candidates, and could be used as gates for antibiotic conjugates with siderophores as vectors.^{4–6} Siderophores are low molecular weight secondary metabolites secreted by bacteria into the extracellular medium to scavenge iron(III).⁷ The ferric siderophore complexes formed are recognized by specific outer membrane transporters (OMT) and imported into the periplasm using energy supplied by the TonB machinery.^{8,9} The subsequent fate of the ferric siderophore complexes is very different according to the siderophore and the microorganism: some siderophores deliver iron to the periplasm whereas others cross the inner membrane and deliver the metal to the bacterial cytoplasm.¹⁰ Import across the inner membrane involves ABC transporters or proton motive force-dependent permease. The siderophores selected for Trojan horse strategies for antibiotics need to deliver the drug payload to the appropriate bacterial compartment for antibacterial activity.⁴

Siderophore-based antibiotic vectorization was once considered to be a pipedream of academic scientists. However, recent work by the groups of Elizabeth Nolan and Marvin J. Miller show that this approach could be valuable to improve the activity of β -lactam antibiotics against Gram-negative bacteria.^{11,12} Moreover, several molecules developed by pharmaceutical companies are currently

* Corresponding author at: CNRS Equipe Transport Membranaire Bactérien, Biotechnologie et Signalisation Cellulaire, UMR7242, Pole API, 300 boulevard Sébastien Brant, CS10413, 67412 Illkirch Cedex, France.

E-mail address: mislin@unistra.fr (G.L.A. Mislin).

in clinical trials. MC-1, BAL30072 and cefiderocol (formerly S-649266) are conjugates between β -lactam antibiotics from various families and catechol, or isoster, vectors.¹³ These compounds were specifically developed for the treatment of infections due to Gram-negative bacilli in general and *P. aeruginosa* in particular. Thus, it is evident that siderophore-based Trojan horse strategies are now plausible for vectorisation of antibiotics with periplasmic targets. The next challenge is to vectorise antibiotics with a cytoplasmic target.¹⁴ In this context, the group of Marvin J. Miller proved recently that the Trojan horse strategy could extend efficiently the antibiotic spectrum of daptomycin.¹⁵ The siderophore pyochelin is a promising candidate for such innovative therapeutic approach.

Pyochelin **1** is an endogenous siderophore produced, and used, by *Pseudomonas aeruginosa* and some species of the *cepacia* complex.^{16,17} The biosynthesis of pyochelin has been described exhaustively,^{18,19} but the secretion process remains unknown. This siderophore forms complexes with iron(III) with a 2:1 stoichiometry.^{20,21} Ferric pyochelin is recognized and transported through the outer membranes by a specific OMT called FptA and through the inner membrane by a proton motive force permease called FptX.^{22,23} The molecular mechanism involved in iron release from pyochelin in the bacterial cytoplasm remains unclear. Ferric pyochelin has been described to be the effector of the PchR protein responsible for the regulation of the expression of the enzymes involved in pyochelin biosynthesis, and of *fptA* and *fptX*.^{24,25} These observations strongly suggest that pyochelin enters the cytoplasm as a ferric complex, although this has not been formally proved. Nothing is known about the ferric pyochelin dissociation mechanism or the recycling, if any, of the siderophore (Fig. 1).

Oxazolidinones are antibiotics active against many Gram-positive bacteria but have only weak or moderate activity against Gram-negative bacteria.²⁶ For example, linezolid, the first oxazolidinone approved has no activity against *P. aeruginosa*.²⁷ The target of these drugs is the 50S subunit of bacterial ribosomes in the cytoplasm; the difference in activity against Gram-positive and -negative bacteria seems to be related to both impermeability of the Gram-negative outer membrane (Gram-positives have no such membrane) and efflux processes. Vectorisation of linezolid analogues with a monocatechol vector derived from aminochelin, a siderophore of *Azotobacter vinelandii*, seems to improve the antibiotic activities against *P. aeruginosa*.²⁷ These oxazolidinone

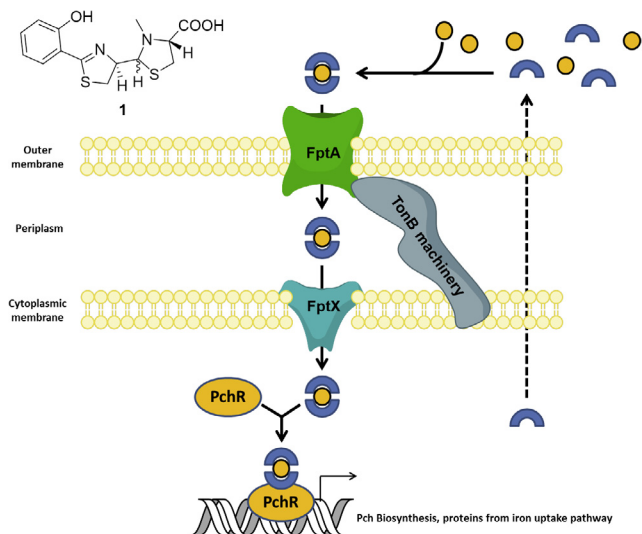


Fig. 1. Structure of pyochelin **1** and the pyochelin-dependent iron uptake pathway in *P. aeruginosa*. Iron(III) is represented as orange balls and pyochelin as blue arch.

conjugates were amongst the most active described against this microorganism, but the MICs were nevertheless much higher than those of antibiotics currently approved against *P. aeruginosa*. There is evidence that catechol siderophores, like enterobactin and azotochelin, deliver iron to the periplasm of *P. aeruginosa*.^{28,29} The iron-uptake system used by aminochelin-oxazolidinone conjugates has not been determined, but it is likely that our conjugates accumulate in the periplasm. The MICs of vectorised oxazolidinones against *P. aeruginosa* may be improved using pyochelin because it passes into the cytoplasm where the ribosomes are located. We have already described the synthesis of functionalized pyochelin **2** and of the corresponding free amine analogue **3** (Fig. 2).³⁰

The ferric form of molecule **2** binds FptA with nearly the same affinity as the natural siderophore.³¹ Analogue **3** is able to promote iron(III) accumulation in bacteria using the pyochelin-dependent iron-uptake system. Analogues **2** and **3** thus mimic natural pyochelin **1**, and efficiently vectorise NBD fluorophores into *P. aeruginosa*.³⁰ Therefore, siderophore analogue **3** has properties making it a promising vector for antibiotic Trojan horse strategies. Preliminary data based on the vectorization of fluoroquinolones were disappointing: antibiotic activities were poor, mainly due to the poor solubility of the conjugates in physiological media and to the hydrolysis of the spacer arm in culture broth.³¹ Here, we report the synthesis of conjugates between pyochelin **3** and oxazolidinone antibiotics connected by succinic and more water-soluble PEG-derived linkers. The oxazolidinone payloads were connected to functionalized pyochelin through a direct peptide bond between the amine function of vector **2** and oxazolidinones bearing a terminal carboxylate.

The synthesis of pyochelin vectors **2** and **3** has been described.^{30,31} Pyochelin vector **3** was conjugated with oxazolidinone-spacer arm building blocks bearing a terminal carboxylate. The functionalization of oxazolidinones can deeply modify the antibiotic activity. However, the morpholine moiety, present in linezolid, was shown to be replaced by many other functional groups without impairing drastically neither the binding to the target nor the antibiotic properties.^{32,33}

The synthesis of oxazolidinones **12**, **13** and **15** with terminal carboxylates started with the production of the structural elements of three spacer arms selected: these three linkers of different lengths and chemical properties were selected to avoid the solubility problems encountered with pyochelin-fluoroquinolone conjugates described previously.³¹ For the shortest linker, the synthesis started with mono-*t*-Butyl-succinate **4**.³⁰ The free carboxylate was then activated in the presence of pentafluorophenol and DCC. The expected pentafluorophenyl ester **5** was obtained at 50% yield. A longer spacer arm was prepared from commercially available *tert*-butyl-2-(2-(2-hydroxyethoxy)ethoxy)acetate **6**: it was treated with triphosgene in the presence of pyridine leading to the corresponding chloroformate **7**, which was isolated in 94% yield. A third linker fragment **9** was obtained at 95% yield by the treatment of Boc-glycine **8** with pentafluorophenol in the presence of DCC as a coupling agent. These linkers were then connected to an oxazolidinone antibiotic: the Boc protecting group of this linezolid analogue **10**²⁷ was cleaved using 20% TFA in

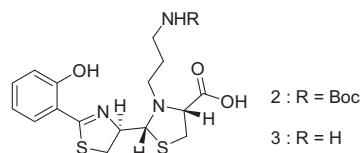


Fig. 2. Structures of pyochelin analogues **2** and **3**.

Download English Version:

<https://daneshyari.com/en/article/5155345>

Download Persian Version:

<https://daneshyari.com/article/5155345>

[Daneshyari.com](https://daneshyari.com)