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Synthesis and biological properties of thiazole-analogues of pyochelin, a siderophore of *Pseudomonas aeruginosa*

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ABSTRACT

Pyochelin is a siderophore common to all strains of *Pseudomonas aeruginosa* utilized by this Gram-negative bacterium to acquire iron(III). FptA is the outer membrane transporter responsible of ferric-pyochelin uptake in *P. aeruginosa*. We describe in this Letter the synthesis and the biological properties (^{55}Fe uptake, binding to FptA) of several thiazole analogues of pyochelin. Among them we report in this Letter the two first pyochelin analogues able to bind FptA without promoting any iron uptake in *P. aeruginosa*.

Iron is a crucial element for almost all forms of life, and bacteria are no exception. Despite iron being one of the most common elements in the earth's crust, its bioavailability is very limited because of the poor solubility of iron(III) at physiological pHs under aerobic conditions. The human body contains substantial amounts of iron, all tightly associated with transport and storage proteins; consequently, iron is not freely available to pathogenic bacteria. The concentration of free iron(III) in physiological fluids is estimated to be 10^{-9} to 10^{-18} M, whereas bacteria generally require iron concentrations in the micromolar range for optimal proliferation.[1] Thus, there has been a strong evolutionary pressure for microorganisms to develop efficient iron uptake systems to acquire this essential element either from the environment or from a host.[2] One of the more efficient and prevalent iron uptake system is based on low molecular weight chelators called siderophores.[3,4] Under conditions of iron depletion, microorganisms synthesize siderophores and secrete them into the extracellular medium where they chelate iron(III).[3,4] Gram-negative bacteria express outer membrane transporters (OMTs) that each recognize, bind and transport a specific ferric-siderophore. Siderophore–iron(III) complexes are then translocated through the membranes, by a multiprotein system.[5] This uptake process is driven by the proton motive force of the inner membrane via an inner membrane complex composed of TonB, ExbB and ExbD proteins.[6] All the proteins involved in siderophore-dependent iron acquisition systems are promising, and widely untapped, targets for the design of new antibiotics.[7,8]

Siderophore OMTs are the best studied proteins of bacterial iron uptake systems, and the three-dimensional structures of several siderophore OMTs have been reported over the last 10 years.[5,9] The study of structure–activity relationships between siderophores and their specific OMTs could contribute to the development of new antibiotic strategies (particularly the design of OMTs inhibitors, and the development of siderophore-based Trojan horse strategies, for example).[10]

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium responsible for severe lung infections in cystic fibrosis patients.[11] These patients are treated regularly with antibiotics leading to a progressive and unavoidable increase of bacterial resistance.[11,12] The emergence of antibiotic-resistant strains has been exacerbated by the almost complete lack of new classes of clinically useful antibiotics reaching the market over the last decades.[7,13] The discovery of new biological targets for innovative inhibitors would make an enormous contribution to regain the upper hand against these human pathogens. Under conditions of iron starvation, *P. aeruginosa* produces two siderophores: pyochelin (Pch) **1** and pyoverdin.[14–16] Pch **1** is recognized by FptA, a specific OMT expressed in *P. aeruginosa*.[17] Therefore, the Pch-dependent iron uptake system is a promising potential target for

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antibiotic strategies dedicated to the treatment of chronic lung infection in cystic fibrosis patients.

Pch is a tricyclic siderophore bearing three chiral centres at positions C4′, C2″ and C4″.[18] Pch chelates iron(III) with a stoichiometry of 2:1.[19] One of the Pch is tetradently bound to the metal ion and the second Pch is necessary for octahedral hexacoordination.[20] The three-dimensional structure of a ternary complex between FptA and Pch-iron(III) was solved a few years ago.[9a] The structure of the FptA transporter revealed that only the iron(III) tetradently bound to Pch is necessary for the recognition by the binding site of the FptA transporter:[21] the Pch binding pocket is located on the extracellular face of FptA and is mostly constituted of hydrophobic and aromatic residues (Phe114, Leu116, Leu117) in the apical loop of the plug domain. The ferric Pch is stabilized in the binding site by several van der Waals interactions and by one hydrogen bond between its carboxylate group and the main chain of FptA.[21,9a] This three-dimensional structure was exploited for a structure–activity relationship study, unprecedented in the field of siderophores chemistry.[21,9a] First, the significance of the configuration of the three chiral centres was investigated. To do this, several stereoisomers and analogues of Pch were synthesized and tested for their biological properties.[21,22] This work demonstrated that the configuration of the C4'' chiral centre is determinant for the stereospecific recognition of the siderophore by its transporter.[9a] However, the configuration of the C4′ chiral centre seems to have only a small effect on Pch recognition.[21] Thus, the Pch thiazole analogue HPTBT **2**,[23] loaded with iron(III) binds the FptA receptor with an affinity in the same range as ferric Pch (K_i = 2.2 ± 0.5 nM for Pch_2-Fe(III), K_i = 9.8 ± 3.5 nM for $HPTBT_2$–Fe(III)) and also promotes iron(III) uptake in *P. aeruginosa* through the Pch-dependent pathway (Fig. 1).[21]

Docking experiments showed that in the FptA binding site, the aromatic ring of Pch **1** and HPTBT **2** is surrounded by several polar residues (Y356, Q395) and peptide bonds of the main chain.[21] We mapped these interactions because they may be relevant to increase affinity of Pch analogs: modifications of these structures may allow the development of specific FptA inhibitors. In this context thiazole analogues of Pch, modified at the C4 and C5 positions of the aromatic ring by the addition of polar organic functions (fluoride, hydroxyl and amine functions) were synthesized. In addition, the synthesis of thiazole analogues of Pch with methylated phenol functions, and unable to complex iron(III), was also attempted. Syntheses and biological properties of these analogues are reported in the present Letter. HPTBT was selected as a lead compound in this approach since the biological properties of this molecule and those of Pch are very similar. Moreover, the chemical synthesis of Pch leads usually to a mixture of four stereoisomers due to the partial epimerization of the C4′ chiral centre.[18] The absence of this chiral centre in HPTBT limit the number of stereoisomers and lead only to a single ferric complex after the chelation of iron(III).

We previously reported an improved protocol for the synthesis of Pch **1** and of its thiazole analogue HPTBT **2**, using 2-hydroxybenzonitrile **10** as starting material.[23,24] The same protocol was used to build the tricyclic core of thiazole analogues **3**–**9** of Pch starting from adequately functionalized 2-hydroxybenzonitriles:

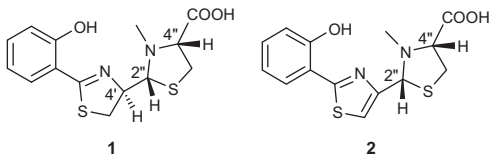


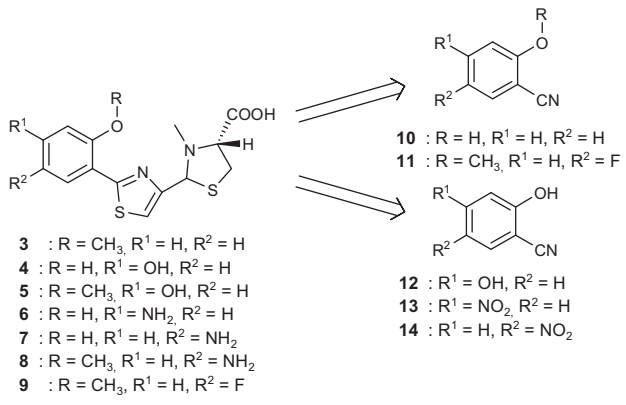
Figure 1. Structures of pyochelin **1** and HPTBT **2**.

compound **11** with a fluoride on the C5 position of the phenol ring, **12** bearing a hydroxyl group, and **13** and **14** bearing a nitro group in the C4 and C5 positions, respectively (Scheme 1).

Starting materials **10** and **11** are available commercially. Compounds **12**–**14** were synthesized according to published procedures as follows: 2,4-dihydroxy-benzonitrile **12** was synthesized in one step with a yield of 88% from the commercially available 2,4-dihydroxybenzaldehyde;[25] 2-hydroxy-4-nitro-benzonitrile **13** was prepared from benzo[1,3]dioxole with an overall yield of 60% over two steps.[26] 2-hydroxy-5-nitro-benzonitrile **14** was synthesized quantitatively from the 1,2-benzodioxazole in two steps.[27] The nitro function of compounds **13** was then reduced by catalytic hydrogenation over 10% Pd/C in ethanol leading to the expected amine **15** isolated in quantitative yield. To avoid side-reactions during the further synthetic steps, the amine function of **15** was protected under the form of a carbamate as follows: the crude amine **15** was reacted with benzyl-chloroformate in the presence of sodium hydrogeno-carbonate leading to the expected carbamate **16** (95% yield). The Boc-protected compound **17** was obtained from the nitro compound **14** in two steps, with an overall yield of 58%, by the procedure described previously by our team (Scheme 2).[28]

2-Hydroxybenzonitrile **10**, **12** and **17** were then converted to thiazoline derivatives **18**–**20** by condensation with L-cysteine in a hydromethanolic medium buffered at pH 6.4.[29] In these conditions the expected thiazolines were obtained with good yields (63–90%). However, this protocol failed to produce the expected thiazolines from compounds **16** to **11**. Therefore, harsher conditions (e.g. L-cysteine, DIPEA, *i*PrOH, 90 °C)[30] were used and allowed the production of the expected thiazolines **21** and **22** at yields of 88% and 75%, respectively. Thiazolines **18**–**22** were further converted into their corresponding Weinreb amides **23**–**27** which were obtained at poor to acceptable yields (32–76%) (Scheme 3).

The synthesis of Pch analogs **3**, **5** and **8** requires methylation of the phenol function. Various conditions were tested to introduce a methyl group onto the phenol ring of compounds **23**–**25**, but the desired methylated compounds were never isolated; this appeared to be due to the strong hydrogen bond between the phenol proton and the nitrogen atom of thiazoline ring. Analysis by NMR indicated that the phenol proton exchangeability was significantly enhanced when the thiazoline was oxidized to a thiazole. This property was used to protect the dihydroxyl compound **24** selectively. The hydroxyl function in position 4 of compound **24** reacted selectively when treated with *p*-methoxybenzyl bromide in the presence of potassium carbonate. The expected PMB phenoxide **28** was isolated with a yield of 93%. Thiazolines **23**–**28** were then further oxidized into the corresponding thiazoles **29**–**34** by



Scheme 1. Retrosynthesis of pyochelin analogs **3**–**9** starting from the functionalized 2-hydroxybenzonitrile **10** to **14**.