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Effect of replacing main-chain ureas with thiourea and guanidinium surrogates on the bactericidal activity of membrane active oligourea foldamers

Stéphanie Antunes [a,b], Jean-Philippe Corre [c], Guillain Mikaty [c], Céline Douat [a,b], Pierre L. Goossens [c,*,1], Gilles Guichard [a,b,*,1]

[a] Univ. Bordeaux, CBMN, UMR 5248, Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac, France
[b] CNRS, CBMN, UMR 5248, F-33600 Pessac, France
[c] HistoPathologie et Modèles Animaux/Pathogénie des Toxi-Infections Bactériennes, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, France

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ABSTRACT

Membrane-active foldamers have recently emerged as potential mimics of antimicrobial peptides (AMPs). Amphiphilic cationic helical *N,N′*-linked oligoureas are one such class of AMP mimics with activities *in vitro* against a broad range of bacteria including *Bacillus anthracis*, a Gram-positive sporulating bacillus and causing agent of anthrax. Here we have used site-selective chemical modifications of the oligourea backbone to gain additional insight into the relationship between structure and function and modulate anthracidal activity. A series of analogues in which urea linkages at selected positions are replaced by thiourea and guanidium surrogates have been prepared on solid support and tested against different bacterial forms of *B. anthracis* (germinated spores and encapsulated bacilli). Urea → thiourea and urea → guanidinium replacements close to the negative end of the helix dipole led to analogues with increased potency and selectivity for *B. anthracis* versus mammalian cells.

1. Introduction

The cationic amphipathic structure of a number of host defense antimicrobial peptides (AMPs) which exert their activity by interacting with and permeabilizing the bacterial membrane, has inspired the design of a whole new range of synthetic and membrane active folded molecules (foldamers) as potential antibacterial agents. Noteworthy examples include aliphatic helically folded peptidomimetic backbones such as helical β-peptides,[1–3] α,β-peptide hybrids[4], α- [5,6] and β-peptoids,[7] sulfono-γ-AApeptides[8] and oligoureas.[9,10] Abiotic arylamide foldamers invented by Degrado are another class of potent antimicrobials with remarkable activities and safety profile.[11] Cationic amphipathic short chain (8-mer) *N,N′*-linked oligoureas which reproduce the essential features of α-helical host-defense peptides (e.g. secondary structure and spatial segregation of lipophilic and cationic side-chains) have shown significant and broad antibacterial activity against Gram-negative (e.g. *E. coli*) and Gram-positive (e.g. *S. aureus* including methicilin resistant strains) bacteria with selectivity for prokaryotic versus mammalian cells.[9,10,12] Urea-oligomer **1** (Fig. 1), which was found to be active both *in vitro* against bacterial forms of *B. anthracis* (Gram-positive bacteria) encountered *in vivo*, and *in vivo* in mouse models of *B. anthracis* infection exhibited high *in vitro* stability against proteases and high resistance to metabolic degradation *in vivo*.[13]

The mode of action of this cationic amphipathic urea-based foldamer is not known in details but it is believed to be related to its ability to adopt an amphipathic helical structure in aqueous and membrane environment and to interact and disrupt bacterial membranes.[14,10]

Similar to peptides, the activity profile of oligoureas such as **1** can be modulated by varying the nature and distribution of side chains along the surface of the helix in a sequence dependent manner.[9,10] Chemical modifications of the backbone can also be introduced to subtly modify the physicochemical properties of the main chain and further explore the relationship between structure and activity. We previously reported analogues of **1** containing γ^4-amino acid residue substitutions and found that despite close similarities, the oligourea and γ-peptide backbones behave very

* Corresponding authors at: Univ. Bordeaux, CBMN, UMR 5248, Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac, France (G. Guichard). Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, France (P.L. Goossens).
E-mail addresses: pierre.goossens@pasteur.fr (P.L. Goossens), g.guichard@iecb.u-bordeaux.fr (G. Guichard).
[1] Share senior coauthorship.

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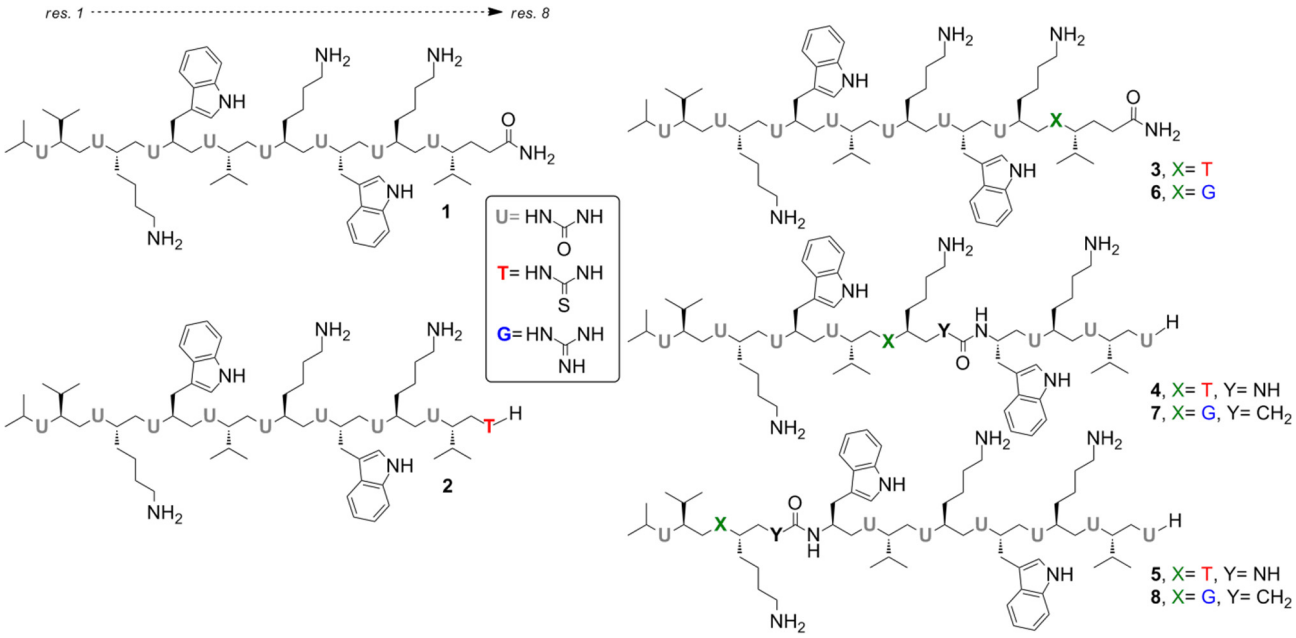

Fig. 1. Chemical structures of parent oligourea **1**[9,13] and related hybrid oligomers incorporating either thiourea (**2**–**5**) or guanidino (**6**–**8**) replacements synthesized in this work.

differently.[9] Whereas the insertion of a single γ-amino acid in the sequence resulted in potent compounds, it was found that the antimicrobial activity was decreasing with the number of γ-amino acid residues in the sequence. Even more striking was the fact that the γ-peptide analogue of **1** was virtually inactive. To gain additional insight into the antibacterial activity profile of **1**, we thought to investigate the effects of other types of isosteric urea (U) backbone replacements such as thiourea (T) and guanidine (G) at various positions in the sequence of **1** (Figure 1). The thiourea (T) moiety has distinct electronic and geometrical properties, which lead to increased hydrogen-bond-donor capacity but weaker H-bond acceptor ability compared to corresponding ureas. We have shown previously in model sequences that the oxo-to-thioxo replacement can be used to locally tune the geometry of the 2.5-helix.[15] In particular, thiourea replacements close to the positive pole of the helix tend to increase terminal flexibility and helix fraying whereas modifications at the other end of the sequence are rather stabilizing.[15] Oligoguanidinium molecules including peptides and dendrimers are well known to interact with phosphate groups on membranes and to be effective molecular transporters.[16] The insertion of a guanidinium (G) linkage[17] brings an additional cationic charge and more polarity to the oligourea backbone, two features that may substantially modify the membrane activity of compound **1**. Thiourea and guanidinium groups if properly located in the sequence of **1** could directly contribute through cooperative interactions with the anionic phosphate headgroup of membrane lipids as depicted in Fig. 2.[18] This hypothesis is further supported by the recent finding that the orientation and accessibility of the urea NHs at the positive end of the helix dipole are well suited for recognition by anionic guests.[19,20]

Here, we report the solid-phase synthesis (SPS) of seven analogues of antibacterial oligourea **1** bearing T or G units at several selected positions by employing new SPS methodologies developed in the laboratory (Fig. 1).[21] It is worth mentioning that analogues of **1** with guanidinium linkages were obtained by direct conversion of main chain thioureas in corresponding oligo(thio) ureas following activation with methyl iodide (Pulka et al. Manuscript in preparation). All prepared oligomers (**2**–**8**) have been

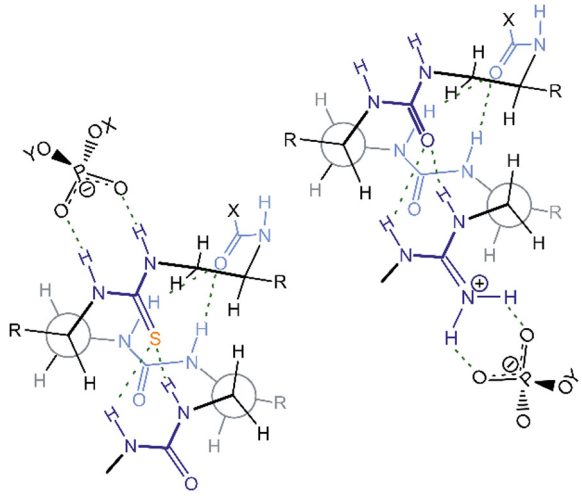

Fig. 2. Possible modes of interaction between thiourea/guanidinium linkages in the 2.5-helical structure and the anionic phosphate headgroup of membrane lipids.

tested *in vitro* for their anthracidal activity against both germinated spores and encapsulated bacilli of *B. anthracis*.

2. Results and discussion

2.1. Synthesis of hybrid (thio)urea-based foldamers **2**–**5**

We recently investigated different synthesis approaches to incorporate a thiourea unit within the backbone of a growing oligourea sequence on solid support.[21] The Boc strategy starting from a methylbenzhydrylamine (MBHA) resin combined with the use of *N*-Boc thiocarbamoyl-benzotriazole-type monomer was found to be particularly robust; yet, it lacks the operative flexibility for rapid parallel synthesis. We also developed a more modular SPS route compatible with the routine preparation of oligoureas on TFA-labile resin.[22] This route which required several chemical adjustments utilizes *N*-Fmoc-aminoalkylisothiocyanates for the clean