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# Functional foldamers that target bacterial membranes: The effect of charge, amphiphilicity and conformation

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ABSTRACT

By varying the molecular charge, shape and amphiphilicity of a series of conformationally distinct diarylureas it is possible to control the levels of phospholipid membrane lysis using membranes composed of bacterial lipid extracts. From the data obtained, it appears as though the lysis activity observed is not due to charge, conformation or amphiphilicity in isolation, but that surface aggregation, H-bonding and other factors may also play a part. The work provides evidence that this class of foldamer possesses potential for optimisation into new antibacterial agents.



## 1. Introduction

There can no longer be any doubt that new antibacterial agents are needed, drugs which not only have potent activity against resistant strains of bacteria, but which are less susceptible to developing resistance at a later date. The crisis associated with antimicrobial resistance has generated major world-wide opportunities for science and technology to lead the way, and one area that could deliver some of the answers is the field dedicated to foldamer research.[1–5] A foldamer can be defined as 'a discrete oligomer that folds into a conformationally ordered state in solution', and contemporary research has shown that a number of foldamer constructs (in particular, antimicrobial peptides (AMPs)) can interact with, and disrupt, bacterial cell membranes thus making these agents valid candidates for future therapeutics, particularly if selectivity over host cells can be achieved.[6–14]

In light of this need, our research group has recently developed a range of AMP-influenced mimetics which are based on a foldamer scaffold, under the presumption that control over antimicrobial properties could be obtained by fine-tuning the molecules' charge, amphiphilicity and conformation.[8,10–13,15–19] Previous efforts have looked at the influence of foldamer length and conformation on membrane interaction,[15–19] but as yet, the effects of charge and thereby amphiphilicity have not been studied against bacterial membranes by us. Herein, we outline recent efforts in this area and discuss the significance of these molecular properties on the ability of the compounds to lyse membranes composed of lipids extracted from *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), with the aim of developing a new class of antibacterial agent.

## 2. Results and discussion

It is well established that *N*-unsubstituted diarylureas exhibit a distinct difference in conformation when compared to their fully *N*-substituted counterpart, both in solution and the solid state (Fig. 1). For instance, upon full methylation, *N*,*N*′-diphenylurea changes from the *trans*,*trans*-conformation to the *cis*,*cis*-conformation, as shown.[20–23]

In such a case the conformation can be determined by either $^{1}$H NMR (solution state conformation), as evidenced by a diagnostic upfield shift in the aromatic signals, or by obtaining the X-ray crystal structures to determine the conformation in the solid state.[24]

The consequence of achieving such conformational control by simple *N*-substitution has been studied in a number of applications, not least: for facilitating conformational communication via stereogenic axes;[25] for controlling oligourea helicity;[26,27] for designing promising anticancer and anti-bacterial agents;[15–17,28] as a molecular splint;[29] for carrying out a so-called 'impossible' macrocyclisation;[30] and for the development of fluorescent sensors.[31]

Another way to potentially exploit this conformational switch is to prepare and evaluate compounds, which as a result, differ in

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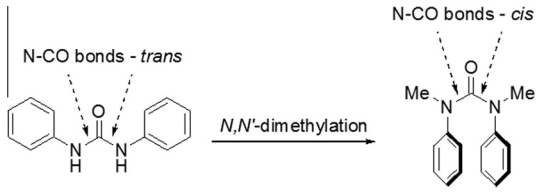



**Figure 1.** Conformational change induced in diphenylureas upon N-methylation.

their molecular dimensions and functionalities, such that activity can be studied and apportioned to the individual properties of interest in both the *trans* and *cis* forms, in this case their ability to lyse bacterial membranes; it is assumed that the p$K_a$ of the compounds being prepared, and thus their protonation state in the assay media, would be the same for both conformations.

In order to be able to test each property individually (conformation, charge and thereby amphiphilicity) a series of compounds were designed and prepared which exist in two discrete and stable conformations depending upon their N-methylation status, as outlined in Scheme 1 and Figure 2.

The compounds in Figure 2 were chosen to enable a direct comparison to be made between the level of membrane lysis achieved between compounds in the same conformation state, but differing protonation levels (series **1**–**3** and **4–7**) vs. compounds in the same protonation state, but differing conformation (**1** vs **4**, **2** vs **5** and **3** vs **6**). Compound **7** was a hybrid-type structure with both N–Me and N–H functionality and a protonation site.

Tables 1 and 2 show the concentration-dependent lysis of compounds **1**–**7** against membrane extracts from *S. aureus* and *E. coli*, respectively, over a treatment period of 1 h, in a calcein-release assay. The relatively weak maximum levels of lysis obtained, even at the highest concentrations, suggests that the compound-membrane interactions are not optimised against the membranes being studied. These results were confirmed in minimum inhibitory concentration (MIC) studies against cultures of both bacterial strains (Table 3), whereby relatively high values were observed. However, it should be noted that these are small, individual monomer molecules interacting with relatively large phospholipid membranes which are usually disrupted by large aggregated oligomers. Herein attempts have been made to identify key features for membrane interaction and disruption which will be taken forward into larger oligomers in future work.

Nonetheless, modest levels of membrane lysis are observed at the lowest concentration studied (Tables 1 and 2), where the highest levels are given by **7** (~40%) against *S. aureus* and **7** (~24%) against *E. coli*. Importantly, the variety of lysis levels obtained against both strains is indicative of some selectivity being observed with the different compounds, which differ in their charge, shape and amphiphilicity, against theses membranes, suggesting that once optimised, this class of molecule could be developed as pathogen-selective antimicrobial agents.

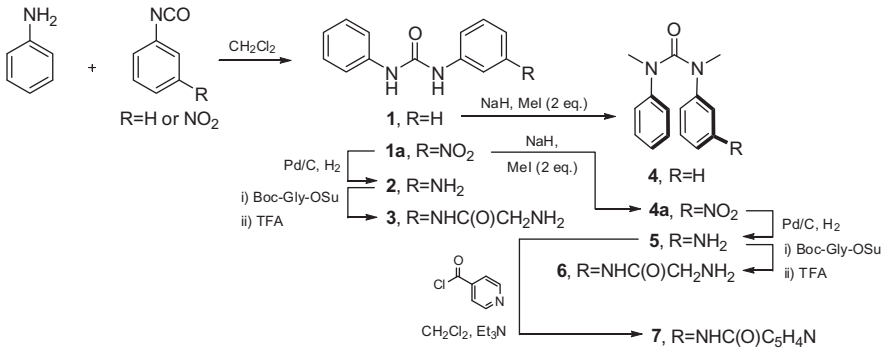
## 3. Comparisons: Structure and lysis

Against *S. aureus* the *trans*,*trans*-compounds **1**, **2** and **3** are more membrane lytic than the *cis*,*cis*-isomers **4**, **5** and **6** at almost all concentrations studied, with **3** being the most potent compound overall. Presumably, this is the case because this amine is the most ionised at the pH used (pH 7.4, Fig. 2), but that conformation or H-bonding must play a role too, since these are the main structural differences between compounds **3** and the less active analogue, **6**. That said, compound **7**, a *cis*,*cis*-diarylurea-NH-amide, which is less completely protonated at pH 7.4, is better still by several fold than all the *trans*,*trans*-compounds suggesting that more complicated factors are at play.

Interestingly, in the context of pathogen-selectivity, against *E. coli* the pattern is different, with **1** being the best of the *trans*, *trans*-analogues (despite being neutral at pH 7.4), although the overall levels of lysis are slightly lower than the same compounds against *S. aureus*. Conversely, in all cases, the *cis*,*cis*-compounds (**5** and **6**) tend to be more active than the *trans*,*trans*-compounds (**2** and **3**), with compound **6** being the best diarylurea overall by several fold, and comparable to compound **7**.

From the data in Tables 1 and 2 it appears as though lysis does correlate with the pKa of the nitrogen which is protonated for compounds **1**–**6**, such that the glycine derivatives **3** and **6** (99.9% positively charged) are consistently the best lytic compounds against both membrane types. The membrane of *S. aureus* is mainly composed of the negatively charged dimyristoylphosphatidylglycerol (DMPG) lipid, whilst the membrane of *E. coli* is mainly the negatively charged DMPG and the zwitterionic (neutral) dimyristoylphosphatidylethanolamine (DMPE) lipid.[32] As such, with the membrane's overall negative charge, it is expected that the positively charged compounds **3** and **6** would be the best at interacting with the membranes and ultimately lysing them at a critical concentration (albeit relatively high with these low molecular weight, un-optimised compounds). In addition, hydrophobic features to penetrate the lipid layer of the membrane are important confirming that amphiphilic molecules as a whole are required.[8]

Interestingly, compound **4** is poor against both strains of bacteria, as would be expected for a neutral, weakly amphiphilic compound with no H-bond donor capability, but **1** sits right in the middle of **2** and **3** in terms of is lytic ability against both *E. coli* and *S. aureus*. Presumably, this is due, in-part at least, to its capacity to H-bond both to itself and aggregate at the membrane surface, thus disrupting the membranes' electrical balance, and subse-




**Scheme 1.** Synthesis of the test compounds.