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Abstract

AlkylGuanidino Ureas, from a Serendipitous Discovery to a Rational Design: Innovative Membrane-Active Antibacterial Agents †

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The relentless and global rise of bacterial resistance is undoubtedly one of the most worrisome Public Health issues. Currently available antibiotics are becoming increasingly ineffective for the treatment of infections caused by extensively-drug resistant (XDR) strains, prompting a challenging discovery and development of novel antibiotics, including with either innovative chemical scaffolds or Modes of Action (MoAs).

We recently reported the serendipitous discovery of AlkylGuanidino Ureas (AGUs), amphipathic compounds exerting a potent and broad-spectrum bactericidal activity [1–4]. Briefly, a bis-guanidino amine (**1**, Figure 1) [5] was found to spontaneously generate a multi-component mixture including oligomers through a hypothesized carbon dioxide capture. A multidisciplinary approach of in-depth MS studies, design, and synthesis led to the identification of tetrasubstituted guanidino urea (**2**, Figure 1) exhibiting Minimal Inhibitory Concentration (MIC) values ranging from 0.5 to 16 µg/mL on both Gram-positive and Gram-negative bacterial species, including on antibiotic-resistant clinical isolates with XDR phenotypes [2]. We subsequently designed and synthesized a library of analogues of **2** by modifying the length of the alkyl spacers and the *N*-guanidino substitutions, allowing a better understanding of some interesting structure-activity relationships (SARs) of AGUs [3].

Further insight into the AGUs MoA was obtained through the rational design of AGU derivatives via a molecular simplification approach, one of which (**3**, Figure 1) emerged among others for its potent antibacterial activity (MICs range 0.5–16 µg/mL). In addition, we developed a modified Parallel Artificial Membrane Permeability Assay (PAMPA) by using bacterial phospholipids-endowed bilayers and poorly permeable probes to assess the ability of AGUs to disrupt the bilayers and affect their permeability. Furthermore, molecular dynamics on simulated bacterial bilayers highlighted the strong interaction of AGUs with the membranes in a "carpet-like" manner. However, *in cellulo* assays with propidium iodide

and SYTO 9 validated the model-based experiments and confirmed the AGUs membrane-targeting MoA [6]. In summary, the AGUs class has proven to be worthy of interest in the Med Chem frame for its innovative chemical structure and potent antibacterial activity and could serve as a basis for the optimization of new, much-needed, antibacterials.

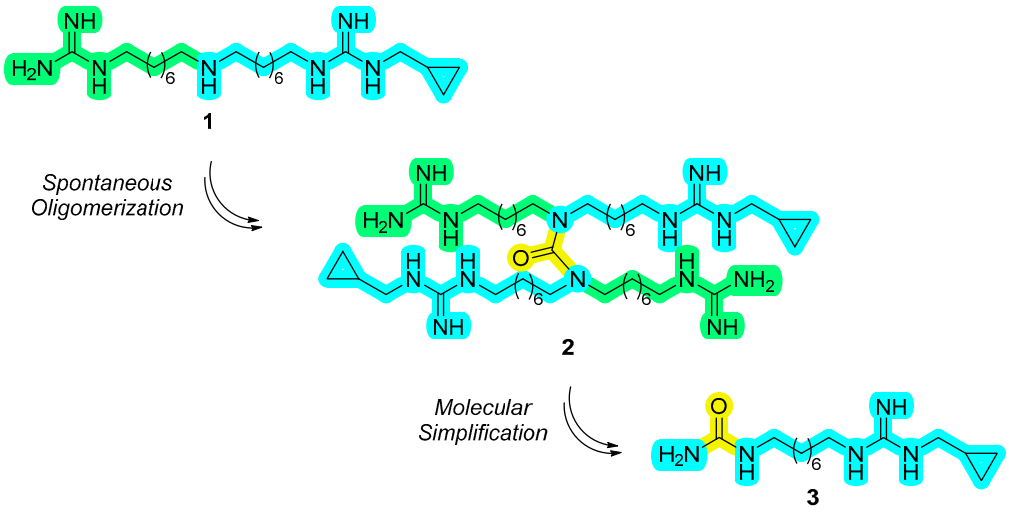

Figure 1. Representative compounds of the AGUs class: monomer **1**, symmetric dimer **2**, and simplified urea **3**. Fragments of the compounds are highlighted in different colors: in cyan the cyclopropylmethyl guanidino arm, in green the unsubstituted guanidino arm, and in yellow the carbonyl group composing the urea function. The arrows indicate the complex advancement of the research project on AGUs.

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