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Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles

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ABSTRACT

A library of 28 small cationic 1,4-substituted 1,2,3-triazoles was prepared for studies of antimicrobial activity. The structures addressed the pharmacophore model of small antimicrobial peptides and an amphipathic motif found in marine antimicrobials. Eight compounds showed promising antimicrobial activity, of which the most potent compound **10b** displayed minimum inhibitory concentrations of 4–8 μg/mL against *Streptococcus agalacticae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. The simple syntheses and low degree of functionalization make these 1,4-substituted 1,2,3-triazoles interesting for further optimizations.

Antimicrobial resistance to conventional antibiotic treatment is rapidly increasing, combined with lackluster efforts to develop novel classes of antibiotics by major pharmaceutical companies.[1–3] Infections caused by multi resistant bacteria is therefore one of the fastest growing medical threats to modern society.[4] Disturbingly, resistant bacteria have existed since the discovery of the first antibiotics. In recent years the race between growing resistance and progress of new antibiotics has intensified in favor of the bacteria. Unfortunately, no antibiotic has yet passed clinical trials for which there has not been reported cases of resistance.[5,6]

Most antibiotics applied today work through specific interactions with key intra- and extra-cellular targets in bacteria, and in a highly specific manner.[7] Due to the high target specificity, uncritical use of antibiotics easily selects for mutated bacteria to proliferate. A well known mechanism of resistance is expression of beta-lactamases that metabolizes beta-lactam based antibiotics.[8] An expanding field within antibiotic research in academia focuses on structures working through less specific mechanisms, like interactions with the bacterial cell membrane and non-specific interactions with intracellular targets.[9–12] The interest in these mechanisms of action comes from antimicrobial peptides (AMPs), that are important constituents of innate immunity in most living organisms. AMPs have a net positive charge (+2 to +9), consist of 12–50 residues, and fold into secondary structures with bactericidal properties.[13] These amphipathic structures, having a positively charged hydrophilic face and a lipophilic face, interact with anionic phospholipids on the surface of bacterial cell membranes. This is followed by membrane permeabilization by the lipophilic residues, leading to cell membrane disruption and ultimately cell lysis.[14,15]

Even though AMPs are considered to be highly active therapeutic compounds, there are some major issues in utilizing them on a large scale. Important drawbacks include low oral bio-availability, low metabolic stability, high manufacturer costs, and lack of patient-friendly administration methods aside from topical treatments.[16] Due to these obstacles, only a small number of antimicrobial agents utilized today are AMPs.[17] A way to circumvent the practical challenges associated with AMPs is to make smaller peptides and scaffold-based peptidomimetics that maintain the antimicrobial activity, but have improved pharmacokinetic properties. This has been demonstrated by Strøm et al., who have synthesized small beta-peptidomimetic structures (MW < 650) with high activity against a variety of resistant bacteria and with potential for *per oral* administration.[18,19] Recently, the group of Strøm[20] has reported a series of small cationic aminobenzamides (example shown as **E23** in Fig. 1) that mimic amphipathic structures found in marine antimicrobials such as synoxazolidinone A[21] and ianthelline[22] and display a membranolytic effect resembling many AMPs. The focus of this work was to further develop such amphipathic structures addressing both small AMPs and marine antimicrobials, and optimize these for antimicrobial activity. The di-functionalized 1,2,3-triazole was chosen as the core

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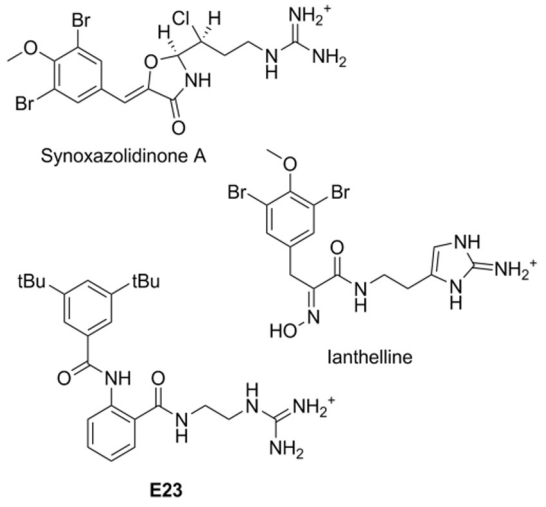
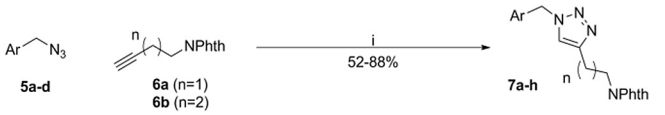


Fig. 1. Synoxazolidone A[21] (MRSA (MIC); 10 μg/mL), Ianthelline[22] (MRSA (MIC); 20 μg/mL), and **E23**; a natural product mimic by Strøm et al.[20] (MRSA (MIC); 4 μg/mL).

scaffold, due to the known biochemical properties of this type of structures.[23,24] Of importance was that triazoles are bioisosteres of amide bonds, which are more stable against proteolytic degradation than amides in AMPs.[25–27] The study included initial synthesis of 24 compounds to investigate the effects of varying between four lipophilic groups and three cationic groups, and including chain length variations. These results were followed up by synthesis of four optimized compounds based on the results from the initial series of di-functionalized 1,2,3-triazoles.

In order to synthesize a collection of disubstituted 1,2,3-triazole amphiphiles with the desired lipophilic- and cationic hydrophilic functionalities, the "click" chemistry protocol developed by Sharpless[28] and Meldal[29] was chosen. By using different catalysts for the "click" chemistry step, 1,2,3-triazoles with different substitution patterns can be prepared, i.e., 1,4-substitution when using copper (I) and 1,5-substitution when using ruthenium(II)-catalysis.[30] The



Scheme 1. (i) $CuSO_4{\cdot}5H_2O$ (5 mol %), Sodium ascorbate (10 mol%), Benzoic acid (10 mol%), tBuOH:H_2O (1:2), rt, 10 min – over night (Ar; **5a** = Ph, **5b** = naphthyl, **5c** = 3,5-di-*t*-Bu-Ph and **5d** = 3,5-CF_3-Ph).

1,4-substitution pattern was chosen here, due to the fact that the copper(I) catalysts used in these reactions are water insensitive (unlike their ruthenium counterparts), excluding the need for working under inert conditions. Thus, the first target compounds given in Fig. 2 (**1a**–**4f**) were prepared in order to screen the effects of different lipophilic aromatic groups and hydrophilic cationic nitrogen groups.

The "click" chemistry protocol requires two coupling partners carrying an azide and a terminal alkyne. It was found most convenient to insert the azide on the lipophilic moiety and the terminal alkyne on the nitrogen carrying functionality. The azides (**5a-d**, shown in Scheme 1) were synthesized from the respective commercially available bromides and alcohols, by well-established reactions (details are shown in the supporting information).[31–34] The alkynes carrying a handle for N-functionalization, were prepared from 3-butyn-1-ol and 1-chloropent-4-yne respectively, under Mitsunobu- or Finkelstein modified Gabriel-conditions (details shown in the supporting information).[35,36] This yielded **6a** and **6b** (shown in Scheme 1) with the same masked N-functionality and a difference of one methylene group in the carbon chain.

The alkynes (**6a** and **6b**) and azides (**5a-d**) were then combined to form [1,4]-1,2,3-triazoles (**7a-h**) using copper catalyzed "click"-chemistry conditions as shown in Scheme 1.[37] Thus, by using four different azides and two lipophiles, eight different "core" 1,2,3-triazoles ready for N-functionalization (**7a-h**) were prepared (see Scheme 1).

Three different cationic groups were evaluated; a primary amine (**a** and **d**), a tertiary amine (**b** and **e**) and a guanidine group (**c** and **f**) as shown in Scheme 2. The interest for the primary amine and the guanidine came from the functionalities found in AMPs,

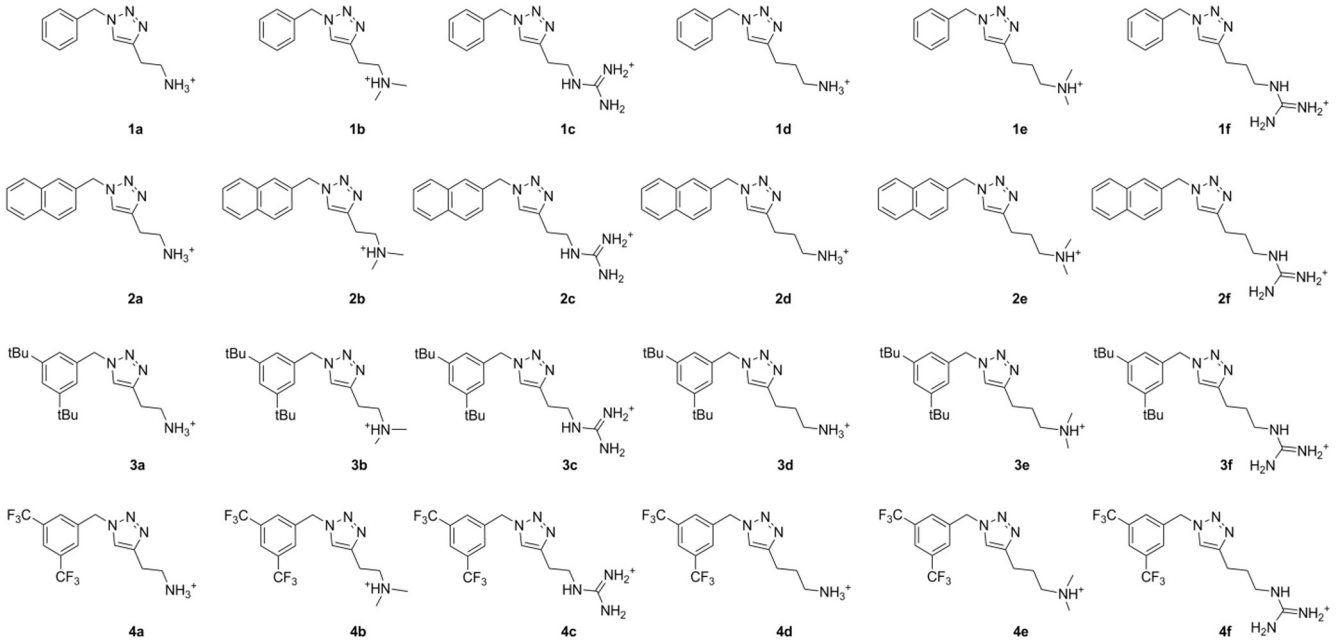


Fig. 2. Initial target [1,4]-1,2,3-triazoles **1a**–**4f** to be screened for antimicrobial effects. Counter-ion: Cl^-.