Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of novel hamamelitannin analogues as potentiators for vancomycin in the treatment of biofilm related *Staphylococcus aureus* infections

Arno Vermote [a], Gilles Brackman [b], Martijn D. P. Risseeuw [a], Tom Coenye [b], Serge Van Calenbergh [a,*]

[a] Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium
[b] Laboratory for Pharmaceutical Microbiology, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 8 June 2016
Revised 25 July 2016
Accepted 26 July 2016
Available online xxxx

Keywords:
Hamamelitannin analogues
Antibiotic potentiators
Quorum sensing
Staphylococcus aureus
Biofilms

ABSTRACT

Staphylococcus aureus is a frequent cause of biofilm-related infections. Bacterial cells within a biofilm are protected from attack by the immune system and conventional antibiotics often fail to penetrate the biofilm matrix. The discovery of hamamelitannin as a potentiator for antibiotics, recently led to the design of a more drug-like lead. In the present study, we want to gain further insight into the structure–activity relationship (S.A.R.) of the 5-position of the molecule, by preparing a library of 21 hamamelitannin analogues.

1. Introduction

For a long time, bacteria have been solely regarded as simple, free living, planktonic micro-organisms. Today, however, it is generally accepted that microbes often live in communities.[1] As bacteria grow on biotic as well as abiotic surfaces, they tend to form structurally complex and dynamic systems, known as biofilms. Biofilms are sessile assemblages of cells attached to a surface and enclosed in an adhesive and self-produced extracellular matrix.[2] The latter is typically composed of polysaccharides, proteins and extracellular DNA (eDNA). The phenotype of certain bacteria subpopulations in the biofilm (especially apparent for so-called 'persisters'), together with the limited penetration of antimicrobial drugs through the matrix are two main reasons for the capacity of biofilm bacteria to escape the effect of antibiotics. Moreover, cells within a biofilm are protected from attack by the immune system.[3,4] Bacterial biofilms are implicated in many medical conditions, including periodontal disease, tuberculosis, respiratory tract infections in cystic fibrosis patients and staphylococcal wound infections. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that is a human commensal organism, but may turn into a versatile and dangerous pathogen causing infections that are difficult to eradicate.[5,6] Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are notorious for their resistance to a wide range of antibiotics.

For the last decades, antibacterial research and development has focused on drugs that kill bacteria or inhibit their growth by interfering with essential cellular processes. However, conventional antibiotics inherently impose selective pressure on bacteria and cause resistance, which constitutes a complex global public health challenge. Inadequate investment in antibiotic research and a poorly filled antibiotics pipeline add urgency to the situation.[7,8]

We decided to explore agents which potentiate the effect of existing antibiotics. Bacterial biofilm formation and virulence are associated with quorum sensing (QS) mechanisms. QS is a cell-to-cell communication system by which bacteria sense population density and control gene expression. The natural product hamamelitannin (2′,5-di-*O*-galloyl-D-hamamelose, HAM, **1**, Fig. 1) increases the susceptibility of *S. aureus* towards a wide range of antibiotics by affecting peptidoglycan thickness and eDNA release through the QS receptor TraP.[9] Structural optimization of HAM led to bisbenzamide **2** and lead compound **3** (Fig. 1). The latter increases the effect of antibiotics in two in vivo infection models, in which it is superior to the parent natural product (HAM, **1**).[10] In the present study, we want to gain further insight into the structure–activity relationship (S.A.R.) of the 5-position of **3**, by preparing a library of 21 derivatives.

* Corresponding author.
E-mail address: serge.vancalenbergh@ugent.be (S. Van Calenbergh).

http://dx.doi.org/10.1016/j.bmc.2016.07.058
0968-0896/

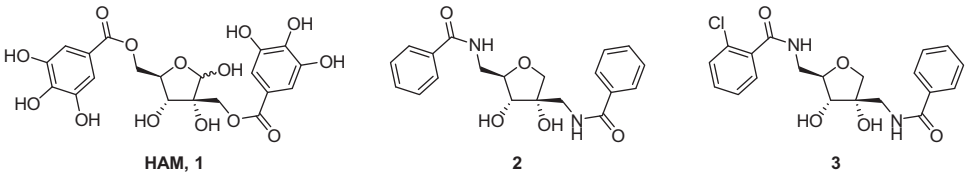

Figure 1. Structure of hamamelitannin (**1**), a more drug-like bisbenzamide derivative **2** and lead compound **3**.

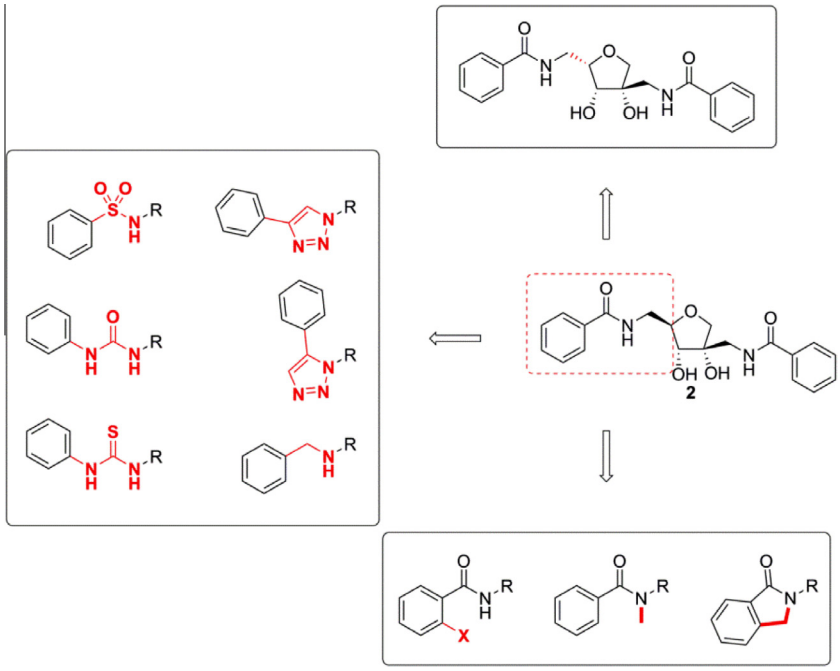

Figure 2. Overview of the structure variations described in this study.

The structure variation focuses on three aspects (Fig. 2). First, we wanted to investigate the stereochemical requirements at C_4 by synthesizing the C_4 epimer of bisbenzamide **2**. In order to investigate the role of the 5-amido group, we explored alternative nitrogen-based moieties to link the aromatic group to the central scaffold. Finally, we wanted to corroborate the hypothesis that *ortho* substitution of the 5-phenyl ring increases activity.

2. Chemistry

The commercially available lactone **4** was converted to its 4-epimer following the method of Batra et al.[11] (Scheme 1), which involves intramolecular opening of an intermediate epoxide in a 5-*exo*-tet process. Successful inversion of configuration at C_4 was apparent from NMR analysis of the protected lyxonolactone **5**. In the ^{1}H NMR spectrum, the H_4 signal appears at 4.65 ppm as a ddd, which couples to H_3, H_{5a} and H_{5b} in the COSY spectrum, while the H_4 signal of the starting material appears as a triplet at 4.59 ppm. The H_3–C_3–C_4–H_4 dihedral angle of nearly 90° is responsible for the seemingly reduced multiplicity of the H_4 signal in the starting material. Correspondingly, in ribonolactone **4**, H_3 appears as a doublet, where one would expect a higher multiplicity (dd) (Supporting info). Compound **5** was converted to 2-*C*-branched-chain derivative **7** according to the method of Simone et al.[12] Borohydride reduction, tosylate-promoted cyclisation and subsequent substitution with sodium azide gave intermediate **9**. Reduction of this bis-azide with trimethylphosphine, EDC-mediated coupling of the resulting amine with benzoic acid and removal of the acetonide protecting group gave the desired 4-epimer **11**.

Antimicrobial potentiator **3**, identified in a previous study, comprises two different aromatic moieties, linked via amides to the central scaffold. The known amine **13**[10] served as a valuable precursor for the synthesis of sulfonamides (**19a**–**i**), urea (**20a**) and thiourea (**20b**) derivatives (Scheme 2). Reductive amination of **13** with benzaldehyde and $NaBH_4$ gave benzylamine **21**. The triazole regioisomers **22** and **23** were synthesized via a copper- or ruthenium-catalyzed 1,3-dipolar cycloaddition reaction on the azide of precursor **12**.

Phthalimide **24**, known from a previous study,[10] served as a versatile and orthogonally protected intermediate for the synthesis of **33**–**35** (Scheme 3). For the selective methylation of the 5-benzamide, we started with a one-pot reduction of azide **24** and acylation of the resulting amine, yielding benzamide **25**. Removal of the phthalimide with ethanolic hydrazine gave primary amine **26**, which was subjected to diazotransfer (**27**). After methylation, intermediate **28** was converted to final compound **33** via known procedures. HAM derivatives **34a**–**d** were obtained via EDC-mediated acylation of **13** with the appropriate benzoic acid. Radical bromination of commercially available methyl 2-methylbenzoate, subsequent treatment of bromide **31** with amine **13** and acidic hydrolysis of the acetonide in **32** gave isoindolinone **35**, which can be considered as a rigidified 5-benzamide analogue.

3. Results and discussion

The minimum inhibitory concentrations (MIC) of all final compounds against *S. aureus* Mu50 were higher than 500 μM, which rules out a direct effect on growth (data not shown). Subsequently,