European Journal of Medicinal Chemistry 125 (2017) 492–499

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Structure-activity relationships and optimization of acyclic acylphloroglucinol analogues as novel antimicrobial agents

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
17 September 2016
Accepted 18 September 2016
Available online 19 September 2016

Keywords:
Anti-MRSA
Acylphloroglucinol
Mechanism
Structure-activity relationship

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious threat to global public health, because it exhibits resistance to existing antibiotics and therefore high rates of morbidity and mortality. In this study, twenty-one natural product-based acylphloroglucinol congeners were synthesized, which possessed different side chains. Antibacterial screening against MRSA strains revealed that acyl moiety tailoring is a prerequisite for the antibacterial activity. Moreover, the lipophilicity, rather than the magnitude of the hydrophobic acyl tail dominates variability in activity potency. Compound **11j** was identified as a promising lead for the generation of new anti-MRSA drug development. It was discovered by optimization of the side chain length in light of the potency, the breadth of the antibacterial spectrum, the rate of bactericidal action, as well as the membrane selectivity. Compound **11j** exerted profound *in vitro* antibacterial activity against the MRSA strain (JCSC 2172), and its MIC was 3-4 orders of magnitude lower than that of vancomycin. A preliminary mode of action study of compound **11j** at the biophysical and morphology levels disclosed that the mechanism underlying its anti-MRSA activity included membrane depolarization and, to a lesser extent, membrane disruption and cell lysis.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogenic bacterium responsible for serious infections due to its high rates of morbidity and mortality [1,2]. Infection by MRSA is considered to be one of the most intractable infectious diseases because of its emerging resistance to existing antibiotics. It is resistant to both the β-lactam classes of antibiotics and the macrolide, fluoroquinolone, aminoglycoside, tetracycline, and lincosamide classes of antibiotics with numerous resistance mechanisms [3,4]. As a result, the nosocomial MRSA is considered as a predominant pathogenic bacterium, and it created a crisis in public health systems especially in developed countries [4]. Thus, the discovery of new therapeutic agents that are effective against the bacteria evolving multidrug-resistant pathogens is crucial for the future maintenance of public health.

In a continuation to our efforts to search for active antibacterial lead compounds from Chinese medicinal plants and their chemical derivatives, we recently reported that rhodomyrtone (**1**), a natural acylphloroglucinol isolated from the traditional Chinese medicinal plant *Rhodomyrtus tomentosa* (Aiton) Hassk. (Myrtaceae) shows significant activity against a wide range of Gram-positive pathogenic bacteria, including MRSA, with low MIC values [5,6]. The previous pharmacological evaluations revealed that **1** might be an excellent antibiotic candidate. It exhibited desirable antibacterial properties, including a wide spectrum of activity, rapid bactericidal action, avoidance of antibiotic resistance, and novel mechanisms of action [7–11]. However, the development of rhodomyrtone suffered from bottlenecks, including a paucity of natural supply and the difficulty for total synthesis [6,12]. Recently, several natural acyclic acylphloroglucinol compounds structurally similar to rhodomyrtone, such as callistenone C (**2**) [13] and

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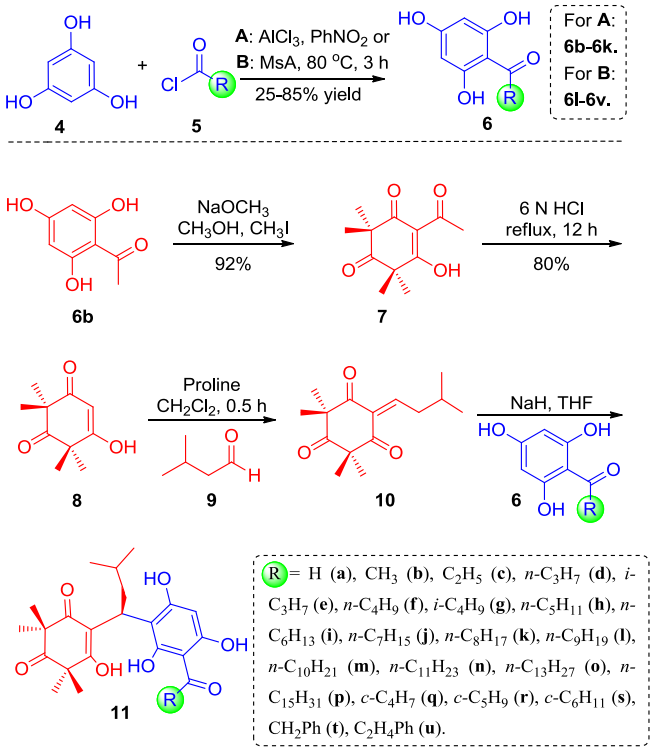
http://dx.doi.org/10.1016/j.ejmech.2016.09.054
0223-5234/

semimyrtucommulone (**3**) [14] (Scheme 1), were reported and showed potent antibacterial activities. With the inspiration of this discovery, efforts towards optimization through a total synthetic approach accompanied by a full exploration of the structure-activity relationships (SAR) of acyclic acylphloroglucinols were conducted and reported herein.

It is well-accepted that biophysical processes play a critical role in the antimicrobial activity of amphipathic molecules [15]. Therefore, tuning the amphiphilic properties by changing the molecular dimensions, as well as the lipophilicity to modulate the biophysical balance should have a critical impact on the biological activities of the modified molecules. Both callistenone C (**2**) and semimyrtucommulone (**3**) are apparently amphipathic metabolites with a 'head and tail' connection. Therefore, structure modification, in anticipation of profound and diverse changes in biological activity, can be readily accessed by targeting either the head (phloroglucinol and β-triketone) or/and tail (isobutyl and isobutyryl) moieties through synthesis. Accordingly, a few acyclic acylphloroglucinol analogues were prepared by replacing their isobutyryl tails with other substituent groups of different lengths and spatial characteristics, in order to manipulate amphiphilic properties and further modulate structure-activity relationships.

In the present study, twenty-one acyclic acylphloroglucinol congeners were synthesized, and their antibacterial potencies were evaluated against standard *Staphylococcus aureus* (SA, ATCC 6538) and methicillin-resistant *S. aureus* (MRSA, JCSC 2172) strains. Significant antibacterial activities were observed for most of the acylphloroglucinol analogues. In particular, the compounds **11i-11n** exhibited antibacterial potencies higher than that of the positive control substance vancomycin. Furthermore, the bactericidal spectrum of compounds **11i-11p** versus a panel of Gram-positive pathogens, as well as their selectivity over normal cells was also elaborated. Herein, the details of the total synthesis of the acyclic acylphloroglucinols and their antibacterial evaluations and mechanistal studies were presented. Lead optimization was also completed, based on an extensive structure-activity relationship (SAR) study.



Scheme 2. Syntheses of acylphloroglucinol analogues.

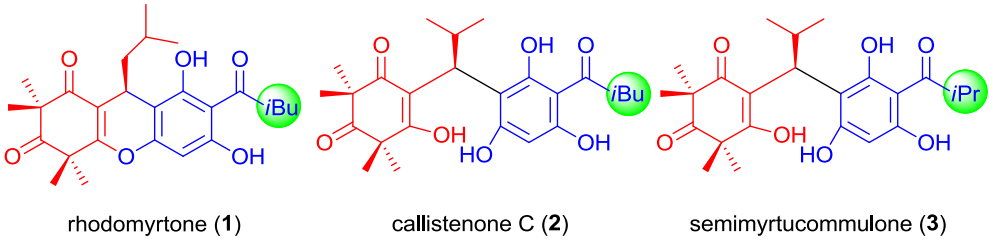
2. Results and discussion

2.1. Chemistry

The synthetic route for the series of acyclic acylphloroglucinol analogues **11** employed previously reported procedures with improved reaction conditions, as outlined in Scheme 2 [12,15]. Syncarpic acid **8** was synthesized using commercially available phloroglucinol **4** and acyl chloride **5**. Briefly, the acylphloroglucinol **6b** could be readily obtained from phloroglucinol **4** using standard aluminum trichloride-catalyzed Friedel-Crafts acylation, compound **6b** was then subjected to selective *C*-tetramethylation with MeI, which afforded the desired β-triketone **7** in excellent yield. Acid-catalyzed retro-Claisen condensation provided **8** smoothly in 80% yield. Afterwards, the key intermediate **10** was successfully accessed through a Knoevenagel condensation. The crucial coupling reaction of **10** and the acylphloroglucinols **6** was accomplished through base-catalyzed Michael addition, which resulted in the acyclic acylphloroglucinols **11** in good yields, ranging from 30% to 80%. The structures of all new compounds were unambiguously confirmed by HRMS, ^{1}H NMR, and ^{13}C NMR spectral measurements.

2.2. Biological evaluation

The minimum inhibitory concentrations (MIC) against MRSA cells of the acyclic acylphloroglucinol analogues **11**, using vancomycin as the positive control, were measured using the microdilution susceptibility assay in Mueller-Hinton broth (MHB) following CLSI guidelines. The highest concentration used for testing the acylphloroglucinol analogues **11** was 32 μg/mL due to their potent antibacterial activity. Results from the anti-MRSA screening demonstrated a clear structure-activity relationship wherein the lipophilic ability of the acyl group tails had a significant influence on the potency of bacterial growth inhibition (Table 1). As a comparison, the synthesized compounds **11** were also evaluated for their antibacterial activity against a standard, drug-susceptible *S. aureus* (ATCC 6538).



Scheme 1. Structures of selected natural acylphloroglucinols.