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Original article

Unraveling the structure–activity relationship of tomatidine, a steroid alkaloid with unique antibiotic properties against persistent forms of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is responsible for difficult-to-treat and relapsing infections and constitutes one of the most problematic pathogens due to its multiple resistances to clinically available antibiotics. Additionally, the ability of *S. aureus* to develop small-colony variants is associated with a reduced susceptibility to aminoglycoside antibiotics and *in vivo* persistence. We have recently demonstrated that tomatidine, a steroid alkaloid isolated from tomato plants, possesses anti-virulence activity against normal strains of *S. aureus* as well as the ability to potentiate the effect of aminoglycoside antibiotics. In addition, tomatidine has shown antibiotic activity against small-colony variants of *S. aureus*. We herein report the first study of the structure–activity relationship of tomatidine against *S. aureus*.

1. Introduction

Antibiotic resistance is becoming an alarming health problem compounded by the lack of new therapeutic options to fight resistant pathogens [1,2]. Since the first synthetic antibiotics were introduced nearly 80 years ago, several scaffolds have become new classes of antibiotics [3,4]. As an adaptive response to this arsenal, pathogens have acquired efficient resistance mechanisms [5–7], leading eventually to multi-resistant strains [3,8]. The development of new antibacterial agents has not paralleled the development of resistance [3,4,9], and drug approvals in the antibiotic field have diminished by half in the past 20 years [10]. Additionally, there has been a lack of new chemical scaffolds among new drugs. Indeed, between 1930 and 1970, 20 different classes of antimicrobial agents were discovered, including widely used classes such as the penicillins, aminoglycosides and tetracyclines [3,5]. In the thirty years that followed, the discovery of new classes came to a standstill [4,9]. As a result, most of the recently introduced antibiotics are still based on scaffolds discovered or designed 60 years ago, which increases the likelihood of resistance development [3,9].

Among highly resistant pathogens, *Staphylococcus aureus* (*S. aureus*) has evolved methicillin-resistant (MRSA) and often multi-resistant strains. Since 2002, we have witnessed the emergence of strains resistant to vancomycin, which is generally considered the last resort antibiotic for this pathogen [7,11]. MRSA is responsible for many hospital- and community-acquired infections and is often associated with recurring and difficult-to-treat infections, which increase morbidity and mortality in both humans and animals [5,12].

Among the mechanisms that allow *S. aureus* to cause resistance and persistent infections and reduce its susceptibility to antibiotics, this pathogen often presents itself in the form of respiratory-deficient small-colony variants (SCVs) [13]. SCVs suffer from a deficient electron transport chain and a weaker proton-motive

Abbreviations: S. aureus, *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SCV, small-colony variant; MIC, minimal inhibitory concentration; SAR, structure–activity relationship.

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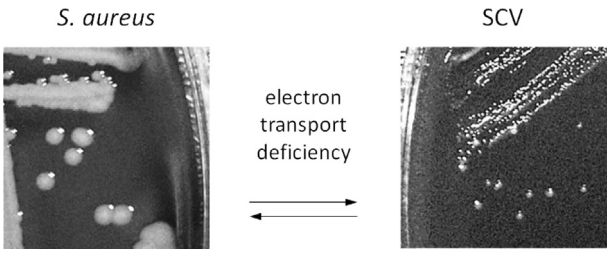

Fig. 1. Characteristics of normal and small colony variants of *S. aureus*.

force, which concertedly impede bacterial growth while increasing resistance to antibiotics such as aminoglycosides [12,14–16]. As opposed to normal strains (Fig. 1), SCVs possess characteristics that contribute to prevent acute infections, but allow *in vivo* persistence [14,15,17].

S. aureus has the ability to switch between the normal and SCV phenotypes. While normal strains exhibit virulence, fast growth and dissemination and are responsible for acute infections, SCVs are associated with colonization, biofilm production and persistent infections [16]. They possess a deficient electron transport chain, which modifies the proton-motive force (PMF) and affects susceptibility to aminoglycoside (AMG) antibiotics.

Tomatidine (**1**, Fig. 2), the aglycone version of tomatine (**2**), is an important antimicrobial defense metabolite of many solanaceous plants and possesses potential anticancer [18–21], chemosensitizer [22], anti-Leishmania [23], anti-hyperlipidemic [24], and anti-inflammatory properties [25]. Apart from its action on eukaryotic organisms, our group has recently demonstrated that tomatidine possesses anti-virulence activity against normal strains of *S. aureus* without impairing growth, while also displaying potent antibiotic activity against SCVs [12,14]. Most importantly, tomatidine has demonstrated its ability to potentiate the bactericidal activity of aminoglycoside antibiotics against the normal phenotype of *S. aureus* [12]. An example of such potentiating effect is observed when tomatidine is used in combination with gentamicin, which improves the minimal inhibitory concentration (MIC) of gentamicin by 8-fold (0.5 μg/mL to 0.06 μg/mL in the absence or presence of tomatidine, respectively). The antibacterial mechanism of action, structure–activity relationship (SAR) and target of tomatidine remain to be elucidated.

In order to better understand the antibacterial properties of tomatidine and explore its SAR, we undertook the synthesis of structural analogs. Our initial goal was to firstly understand the SAR of tomatidine, both as a potentiator of the antibiotic action of gentamicin and as a growth inhibitor of *S. aureus* SCVs. The results of these efforts are reported below.

2. Chemistry

Tomatidine **1** (Fig. 2) is a steroid alkaloid structurally characterized by 6 rings, 12 stereogenic centers, a 3β-hydroxyl group and spiro-fused E, F rings in the form of an aminoketal. It was isolated by hydrolysis of its glycosylated analog tomatine **2** [26]. The structure of tomatidine was elucidated via a combination of infrared spectroscopy, elemental analysis and X-ray crystallography [13,27]. In the absence of an identified cellular target, our initial objective was to elucidate SAR, based on the initial hypothesis that the steroidal A–D rings act as a rigid scaffold to orient pharmacophores defined by the 3β-hydroxyl group on ring A and the spiroaminoketal group on rings E, F. Accordingly, our attention focused on the two extremities of the molecule. An important question from the outset was whether the closed or the open form of the aminoketal is responsible for biological activity. Indeed, the aminoketal can be opened by hydrolysis to reveal a hydroxyl function and a tethered amino and keto functional groups on ring D [28,29].

Analogs bearing modifications on ring A were prepared as indicated in Scheme 1. The aminoketal moiety was first protected as a formamide [30] by *N*,*O*-double formylation of tomatidine hydrochloride **3** with acetic formic anhydride, followed by chemoselective deprotection of the formate ester in mild basic conditions to yield *N*-formyltomatidine **4** in quantitative yield. Compound **4** underwent a Mitsunobu inversion with acetic acid [31], followed by protective groups acidolysis to afford 3α-hydroxyl tomatidine **6** in high yields [32]. Palladium-catalyzed *O*-allylation of *N*-formyltomatidine **4** delivered *O*-allyltomatidine **8** in good yield after deformylation [33]. Direct oxidation of the C3 alcohol of compound **4** using Dess–Martin periodinane [34] delivered 3-ketotomatidine **9**, which was subsequently deprotected to yield **10** by acidic hydrolysis [32]. Finally, starting from 3-ketotomatidine intermediate **9**, amino analogs **12**, **14**, **16** and **18** were generated via reductive amination [35] followed by deprotection in low to moderate yields.

In order to better understand the SAR of the aminoketal moiety on rings E, F and ascertain whether a closed or open form of rings E and F constitute the active form of the molecule, we generated analogs modified with open rings E and/or F via two complementary approaches.

Firstly, tomatidine was used as starting materials to generate open chain derivatives (Scheme 2). Opening of ring E was performed via catalytic hydrogenation of the spiroaminoketal moiety on platinum oxide [36] to yield piperidine derivative **19**. On the other hand, dihydrofuran **22** was obtained by acetylation of the spiroaminoketal moiety of **3** to give intermediate **20**, followed by acid-catalyzed elimination then ester hydrolysis [37–39]. Subsequent reduction delivered the corresponding tetrahydrofuran **23** with no control of stereochemistry on the newly created stereogenic centers. Acetylation of tomatidine hydrochloride **3** also yielded triacetylated compound **24**, which was opened in acidic conditions to give intermediate **25** [37–39] then reduced and deprotected to afford triol **27**. Unfortunately, all attempts to hydrolyze the terminal acetamide of **27** resulted in product decomposition.

The second approach in the synthesis of analogs modified on rings E and F started from commercially available pregnenolone acetate **28**, which bears the same A–D ring system and stereochemistry as tomatidine (Scheme 3). Hydrogenation and ester hydrolysis [40] followed by ^{t}BDMS protection of the hydroxyl group

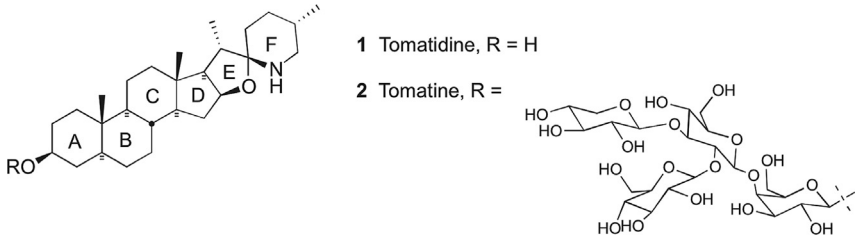

Fig. 2. The structures of tomatidine and tomatine.