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CODONOPSININE DERIVATIVES AS POTENTIAL INHIBITORY AGENTS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial-related and economically-relevant Gram-positive bacterial pathogen that has been known to display multidrug-resistance properties towards a wide range of structurally-unrelated antibiotics and antimicrobial agents. On the other hand, codonopsinine is a plant-based pyrrolidine alkaloid which is known to display remarkable antibiotic, hypotensive and low cytotoxic activity. Previously, 30 codonopsinine derivatives with electron rich functional groups were synthetically produced. In this study, selected microbiological and pre-clinical assays were carried out to investigate the possibility of using codonopsinine derivatives as potential new inhibitory agents against MRSA and methicillin-sensitive *S. aureus* (MSSA) isolates. In the MIC assay, only two novel compounds, MFM501 and MFM514, showed good inhibitory activity with MIC values between 7.81 to 31.3 µg/ml against 38 MRSA and 13 MSSA isolates. The MBC/MIC ratio exhibited that both active compounds has a bacteriostatic effect against MRSA and MSSA isolates. In the time-kill studies, MFM501 showed a time-dependent killing action while MFM514 displayed a concentration-dependent killing effect. MFM514 also exhibited a faster bacteriostatic action than MFM501. Additionally, SEM analysis suggests that both active derivatives may exert its inhibitory activity via bacterial lysis and/or cytoplasmic membrane disruptions which resulted in the various irregular, distorted, shrunken and larger shapes of the treated MRSA cells. In the *in vitro* cytotoxic assay, both active molecules showed low cytotoxic activity with IC_{50} value of > 625 µg/ml against three non-cancerous mammalian cells. Following that, oral acute toxicity study revealed that the estimated LD_{50} value for MFM501 was > 300 mg/kg and < 2000 mg/kg (Category 4) while MFM514 exhibited less toxicity with an estimated LD_{50} value of > 2000 mg/kg and < 5000 mg/kg (Category 5). Finally, in the mouse protection assay, the ED_{50} values for MFM501 and MFM514 were calculated at 87.16 mg/kg and 29.39 mg/kg dosage, respectively, while both active codonopsinine derivatives exhibited a dose-dependent mice protection trend. This study showed that both active pyrrolidone compounds have the potential to be developed further as clinically active, safe and efficacious anti-MRSA agents. Since the dire need for new antibacterial agents are literally crucial for the survival of mankind, it is hoped that this study will serve as a proof-of concept paper to interested investors and/or healthcare-related companies for further R&D endeavour and/or commercial applications.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial-related, Gram-positive bacteria that has been known to display multidrug-resistance properties towards a wide range of structurally-unrelated antibiotics and antimicrobial agents (Johari et al., 2015). Recently, novel strains have also emerged outside of hospital settings, aptly named as community-acquired (CA) MRSA and livestock-associated (LA) MRSA (Stefani et. al., 2012; Monecke et al., 2011).

Nevertheless, MRSA is still regarded as a major healthcare-associated threat, and one of the most common antibiotic-resistant and economically relevant pathogen in the world as exemplified in Figure 1.1 (Centers for Disease Control and Prevention [CDC], 2015a; CDC, 2015b; Smyth et al., 2010; Oluwatuyi, Kaatz & Gibbons, 2004). MRSA infections or hospital-acquired (HA)-MRSA infections have been a bane in the healthcare community since it was reported in 1961, only two years after methicillin was introduced into clinical practice (Sakoulas & Moellering, 2008; Chambers & DeLeo, 2009).

Although epidemiological data from separate studies were often not comparable owing to differences in study design and populations sampled, a decreasing rate of HA-MRSA infections have been detected all over the world (Chen & Huang, 2014; David, Cadilla, Boyle-Vavra & Daum, 2014). Recent studies have strongly suggest that these declining trends were due to the replacement of current HA-MRSA clones with CA-MRSA strains (Chen & Huang, 2014; David, Cadilla, Boyle-Vavra & Daum, 2014; D'Agata, Webb, Horn, Moellering & Ruan, 2009).

Nonetheless, high MRSA infections (>50%) were still reported in several European and Asian countries such as Malta (51.8%), Romania (64.5%), Vietnam (74.1%). South Korea (77.6%) and Sri Lanka (86.5%) (European Centre for Disease Prevention and Control [ECDC], 2013; Lai et al., 2014). In Malaysia, HA-MRSA has been isolated from patients in Hospital Kuala Lumpur (HKL) as early as 1978, nineteen years after its first appearance in the United Kingdom (UK) (Hanifah, Hiramatsu &