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Manumycin from a new *Streptomyces* strain shows antagonistic effect against methicillin-resistant *Staphylococcus aureus* (MRSA)/vancomycin-resistant enterococci (VRE) strains from Korean Hospitals

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An antimicrobial compound, highly effective against multidrug-resistant (MDR) bacteria, purified from a *Streptomyces* strain was identified as manumycin. The minimal inhibitory concentrations (MICs) of manumycin against 8 different strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were ranged 2 to 32 µg/ml. Similarly, MICs of manumycin against 4 vancomycin-resistant enterococci (VRE) strains were ranged 8 to 32 µg/ml while it remained ineffective against 4 other VRE strains. Compared to vancomycin, manumycin provided slightly weaker activity against MRSA strains but stronger activity against 4 VRE strains. This is the first report of antagonistic effect of manumycin against MDR pathogens.

Key words: Manumycin, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE).

INTRODUCTION

Manumycin is a group of small and discrete class of antibiotics which consist of almost a dozen secondary metabolites produced exclusively by *Streptomyces* (Sattler et al., 1998). Manumycin was first reported by Buzzetti and coworkers in 1963. Its chemical structure has two unsaturated carbon chains, so called m-C7N and C5N unit, which are linked in meta-fashion to unique multifunctional six-membered ring. Manumycin-type compounds derived from the m-C7N unit vary in its stereochemistry and the nature of the oxygen substituent

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Abbreviations: MDR, Multi drug resistant; **MRSA,** methicillin resistant *Staphylococcus aureus*; **VRE,** vancomycin resistant enterococci; **NMR,** nuclear magnetic resonance; **COSY,** correlation spectroscopy; **TOCSY,** total correlation spectroscopy; **HMQC,** heteronuclear multiple-quantum correlation spectroscopy.

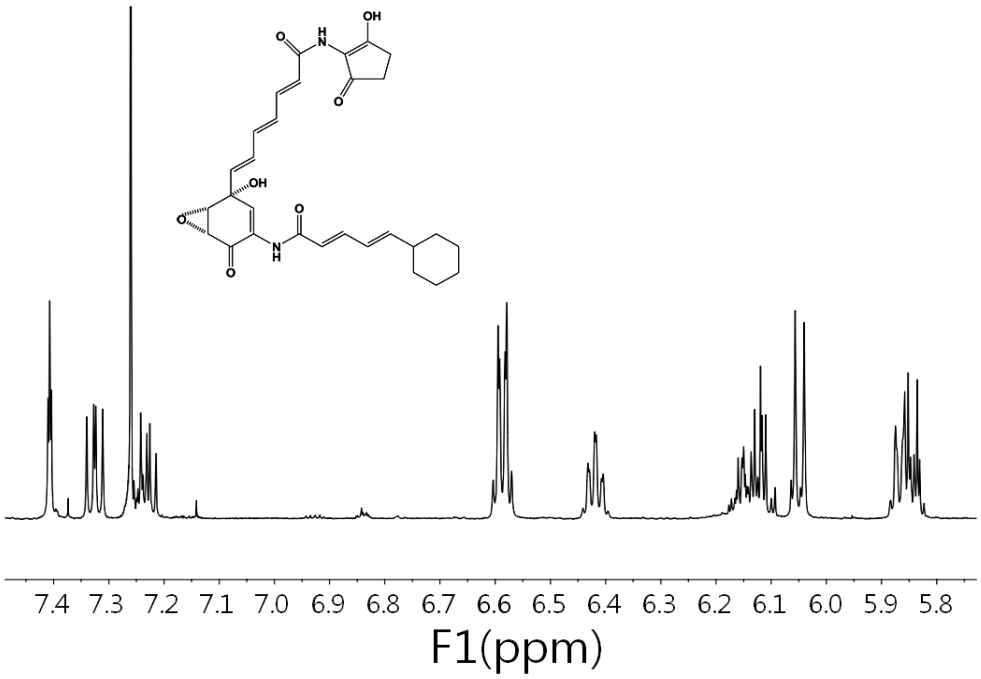

Figure 1. A typical ^{1}H-NMR spectrum obtained from purified manumycin; *Inset*, structure of manumycin. An active compound (Compound C3) was purified from culture broth of *Streptomyces* sp. CS392 (GenBank accession no. JN128646), according to our recent report (Cho et al., 2012). The compound was identified as manumycin based on NMR along with COSY, TOCSY and HMQC (detailed not shown).

(Hwang et al., 1996; Kohno et al., 1996; Sattler et al., 1998). Manumycin exhibits biological activity against Gram-positive bacteria, fungi and some insects (Hwang et al., 1996; Kohno et al., 1996; Sattler, 1998; Thiericke et al., 1987). In 1993, manumycin A was reported to inhibit Ras farnesyltransferase (FTase) (Hara et al., 1993). Manumycin was also reported to show antitumor activity *in vitro* and *in vivo* in nude mouse xenograft models (Ito et al., 1996; Xu et al., 2001). Manumycin A induces caspase-mediated apoptosis in human hepatoma HepG2 cell line (Zhou et al., 2003). Apart from these broad activities, there is no report dealing with the antimicrobial activity of manumycin against multi drug resistant (MDR) pathogens. The objective of this article is to report the potential of manumycin against hospital-acquired multidrug resistant pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). The study is also significant being the first report of antimicrobial activity of manumycin against MDR hospital acquired pathogens.

MATERIALS AND METHODS

The active compound (Compound C3) was purified from culture broth of *Streptomyces* sp. CS392 (GenBank accession no. JN128646), according to our recent report (Cho et al., 2012). The compound identification was carried out by using NMR, COSY, TOCSY and HMQC. For cytotoxic effect of the compound (identified as manumycin) against human cell lines, four types of human cancer cell lines (A549 from lung, HepG2 from liver, MCF-7 from breast, and MG-63 from bone) were from Korean Cell Line Bank, Seoul, Korea. Cells were seeded in a 96 well plate at 0.5×10^4 cell/well. Manumycin was added at 1-100 µg/ml. After 24 h incubation with or without manumycin, MTT solution (0.5 mg/mL) was added and the cells were incubated for 4 h at 37°C. After removing the supernatant, DMSO was added and read at 590 nm. The minimal inhibitory concentrations (MIC) of manumycin were determined by agar dilution method according to Mueller-Hinton-agar dilution method (Schreiber and Jacobs, 1995). After inoculation of test organisms in the agar plates containing various concentrations of drugs, results were observed after incubating them at 37°C for 18 h.

RESULTS AND DISCUSSION

In our recent study, we have purified 3 antimicrobial compounds (C1, C2 and C3) from *Streptomyces* sp. CS392 (Cho et al., 2012). In this study, the major compound 'C3' was identified as manumycin according to various structural parameters such as nuclear magnetic resonance (NMR), correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY) and heteronuclear multiple-quantum correlation spectroscopy (HMQC). A typical ^{1}H-NMR spectrum obtained from purified manumycin with its chemical structure is illustrated in Figure 1. We have investigated the antagonistic effects of

Table 1. MIC of manumycin against various pathogens[1].

Microorganism	Manumycin	Vancomycin
Staphylococcus aureus KCTC 1928	2	0.5
MRSA-693E	2	0.5
MRSA 4-5	2	1
MRSA 5-3	2	1
MRSA-B15	16	4
MRSA-S3	16	4
MRSA-S1	16	4
MRSA-P8	16	4
MRSA-U4	32	4
Enterococcus faecalis ATCC 29212	4	1
VRE-2	>65	>65
VRE-3	>65	>65
VRE-4	>65	>65
VRE-5	>65	>65
VRE-6	32	>65
VRE-82	8	>65
VRE-98	8	>65
VRE-89	16	>65

[1]MIC (µg/mL) value of manumycin were determined by agar dilution method according to Mueller-Hinton-agar dilution method (Schreiber and Jacobs, 1995). After inoculation of test organisms in the agar plates containing various concentrations of drugs, results were observed after incubating them at 37°C for 18 h.

manumycin against *S. aureus* and MRSA strains as well as *Enterococcus faecalis* and VRE strains. Manumycin did not show antimicrobial activity against drug sensitive Gram-negative pathogens such as *Alacligenes faecalis* ATCC 1004, *Salmonella typhimrium* KCTC 1925, *Pseudomonas aeruginosa* KCTC 1637 and *Escherrichia coli* KCTC 1923. Effect of manumycin against various strains in terms of MIC value is illustrated in Table 1. Growth of *S. aureus* KCTC 1928 as well as 3 MRSA strains, namely; MRSA-693E, MRSA 4-5, and MRSA 5 to 3, was inhibited by manumycin at 2 µg/ml. On the other hand, 4 other MRSA strains, namely; MRSA-S1, MRSA-S3, MRSA-B15 and MRSA-P8, were inhibited at 16 µg/ml and rest strain, namely; MRSA-U4, was suppressed at 32 µg/ml of manumycin. Compared to vancomycin with MIC of 4 µg/ml, manumycin remains weaker for 5 tested MRSA strains with MIC values of 16 to 32 µg/ml as mentioned above. However, effect of manumycin against rest 3 MRSA strains with MIC value of 2 µg/ml, namely; MRSA-693E, MRSA 4 to 5, and MRSA 5 to 3, was inferior to vancomycin, only slightly. This discrepancy in the MIC values for the similar types of pathogens may be attributed to the different nature of the strains. Furthermore, although vancomycin showed stronger antimicrobial activity than manumycin against non resistant *E. faecalis*, it did not show any effect against any of the tested VRE strains (Table 1). In contrast, manumycin showed antagonistic effect against 4 VRE strains, namely; VRE-82 and VRE-98 with MIC of 8 µg/ml, VRE-89 with16 µg/ml and VRE-6 with 32 µg/ml. Hence, manumycin shows broader antimicrobial spectra than that offered by vancomycin against MDR bacteria.

So far many attempts have been made to explore effective antimicrobial compounds against resistant bacteria. For example, anti-MRSA compounds such as marinopyrroles A and B from *Streptomyces* sp. CNQ-418 (MIC= ≤ 2 µM) (Hughes et al., 2008), MC21A and MC21B from *Pseudoalteromonas phenolica* (MIC=1-2 to 1-4 µg/mL) (Isnansetyo et al., 2003; Isnansetyo and Kamei, 2009), abyssomicin C from Verrucosispora AB-18-032 (MIC = 4 to 13 µg/mL) (Bister et al., 2004; Keller et al., 2007), lydicamycin from *Streptomyces lydicus* (MIC 6 µg/mL) (Furumai et al., 2002), and so on. Similarly, BE-43472B from *Streptomyces* sp. was reported to exhibit antimicrobial activity against MRSA (MIC = 0.11 to 0.45 µM) and VRE (MIC = 0.24 µM) (Socha et al., 2006) and 2,4-diacetylphloroglucinol (DAPG) isolated from *Pseudomonas* sp. AMSN exhibited antimicrobial activity against MRSA (MIC = 4 µg/mL) and VRE (MIC = 8 µg/mL) (Isnansetyo et al., 2003). Thus, the effect of manumycin against MRSA-693, MRSA 4-5 and MRSA 5-3 (MIC = 2 µg/mL) is stronger than that of DAPG (MIC = 4 µg/mL), lydicamycin (MIC = 6 µg/mL) and abyssomicin C (MIC = 4 to 13 µg/mL) whereas it is relatively weaker than that of BE-43472B (MIC 0.11 to 0.45 µM) and marinopyrroles A and B (MIC = ≤ 2 µM).

Moreover, anti-VRE activity of manumycin (MIC = 8 to 16 µg/mL for 3 VRE strains) was comparable with those

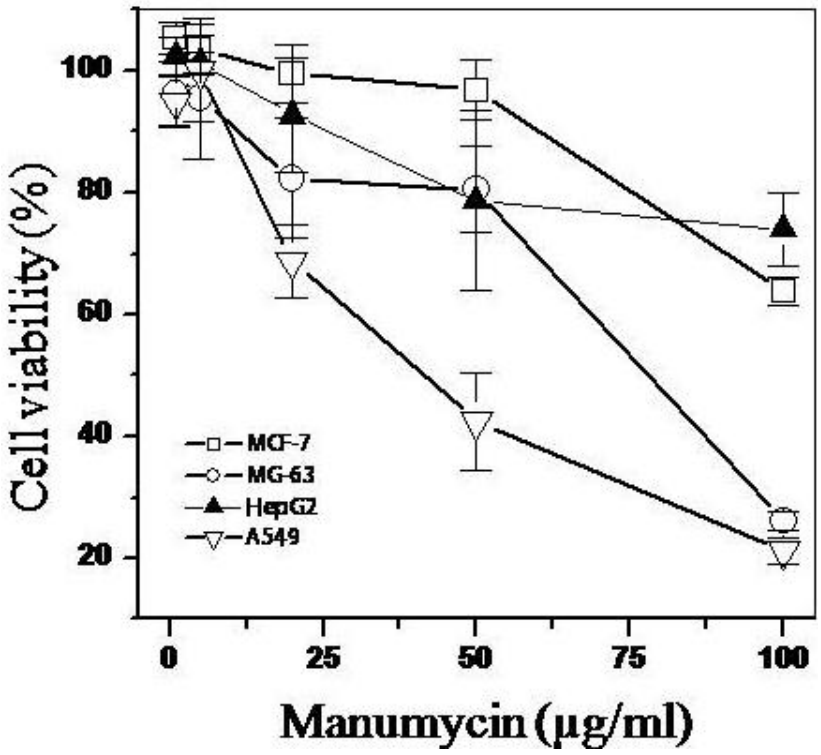

Figure 2. Cytotoxic effect of manumycin against human cell lines. Four types of human cancer cell lines (A549 from lung, HepG2 from liver, MCF-7 from breast, and MG-63 from bone) were from Korean Cell Line Bank, Seoul, Korea. Cells were seeded in a 96 well plate at 0.5×10^4 cell/well. Manumycin was added at 1 to 100 µg/ml. After 24 h incubation with or without manumycin, MTT solution (0.5 mg/mL) was added and the cells were incubated for 4 h at 37°C. After removing the supernatant, DMSO was added and read at 590 nm.

of DAPG (MIC = 8 µg/mL) but weaker than BE-43472B (MIC = 0.24 µM). As illustrated in Figure 2, manumycin with its effective antibacterial concentration (~20 µg/ml, Table 1), when tested for 24 h, did not pose toxic effect against MCF-7 and HepG2 cell lines. It posed ~20 and ~30% of toxicity against MG63 and A549 cell lines, respectively. Although a detailed study is needed, manumycin so far seems safe to use as antimicrobial drug on the basis of cell viability results.

Conclusion

The results show that manumycin isolated from *Streptomyces* sp. CS392 displayed antimicrobial activity against hospital acquired MDR pathogens such as MRSA and VRE strains. Compared to vancomycin manumycin displayed slightly weaker but wider range of antimicrobial spectrum. More detailed study should be done to elucidate its mode of antimicrobial action and antimicrobial effects either as a single antibiotic or in the combination with other commercial antibiotics (synergistic effect), which are our future goals.

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