Development of a new flavanone derivative that exhibits strong antibiotic activity against drug resistant bacteria

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1. Overview of the research

We have developed a new flavanone derivative that exhibits strong antibiotic activity against drug-resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA), which cause great problems at medical treatment sites as the cause of opportunistic and hospital-acquired infections.

This flavanone derivative simultaneously inhibits the microbe's DNA gyrase and topoisomerase IV, and until now there were no reports of a compound that can simultaneously inhibit both enzymes.

Based on its antibacterial mechanism, we think it will be more difficult for bacteria to develop resistance to it than has been the case with traditional antibiotics.

Going forward, we want to develop a structure for even more highly active compounds and prepare for the pharmacokinetics and toxicity tests needed for clinical application.

2. Superiority of the developed technology

Much has been written about the relationship between flavanone derivatives and antibacterial activity as well as the mechanisms for that activity. However, there have been no reports to date about flavanone derivatives similar to the compound in this application that have antibacterial activity against vancomycin-resistant Staphylococcus aureus (VRSA). Not only is the compound of this application effective against methicillin-resistant Staphylococcus aureus (MRSA), which is currently a large problem at medical treatment sites, but it also exhibits excellent antibacterial activity against VRSA, which is an even more serious problem than MRSA. Moreover, a major characteristic of the compound is that it simultaneously inhibits the microbe's DNA gyrase and topoisomerase IV. In other words, it is a dual inhibitor. Both of DNA gyrase and topoisomerase IV are vital enzymes for DNA replication in microbes, and are familiar as the target molecules of the quinolone antibiotics. Typically, antibiotics exhibit activity by inhibiting one of these two targets, and there have been no reports until now of a compound such as the compound in this application that simultaneously inhibits both targets. In addition, from the viewpoint of ease of development of drug resistance, it should be more difficult to block the activity at two locations than at one location. Therefore, it is likely that it will be more difficult

to develop drug resistance to this compound than to traditional antibiotics. This may be a key to winning the current game of "cat and mouse" where new antibiotics are introduced to kill drug-resistant strains but then drug resistance to the new antibiotic soon appears.

Originally, domestic patent applications for this compound were made. However, the above characteristics have been recognized, and currently international patents are also pending.

3. Marketability and future of this technology

The appearance of MRSA, VRSA and other multiple drug-resistant microbes seriously threatens the effectiveness and safety of medical treatments, and there is always need for development of the effective antibiotic. Because infections of drug-resistant bacteria are occurring in every region of the world, there is an endless demand for antibiotics that can treat intractable microbial infections such as MRSA, VRSA and multi-drug resistant bacteria. Therefore, the economic benefits when this research is complete will be huge. Also, because bacteria resistant to this compound will not easily develop as stated above, its usable period before appearance of resistance (time on the market) should be long. Regarding patents, international patents are pending, and we would like to make full use of them.

4. Objective and necessity of this experimental research

One task that we want to carry out in this experimental research is the search for even more highly active compounds. It is important in the development of synthesized pharmaceuticals to create compounds that are easier to synthesize and that have stronger activity. Moreover, because most of the experiments thus far conducted in this research have been in vitro (experiments conducted under artificially created conditions, such as in a test tube), we would like to proceed to in vivo experiments (experiments conducted within a living organism, such as in animals) in the future. During the period for which we have applied, we would like to study the toxicity of this compound when administered to mice, and investigate the ability of the compound to transfer into the blood and its pharmacokinetics (how the compound is absorbed in the body, dispersed into tissue, metabolized, and is eliminated) after being administered to mice. By clarifying these properties, we believe that it will be possible to develop a compound with more practical usefulness as an antibiotic.

5. Methods and procedures of the research

Through the research to date, it is known that changing the sugar portion of the flavanone derivative from galactose to glucose results in a wider antimicrobial spectrum (the antimicrobial spectrum is the range of types of microbes whose reproduction the antimicrobial compound inhibits; the wider the spectrum, the more types of microbes the compound is effective against). Also, because the antimicrobial activity increases at the same time, we believe that there is a high possibility that changing the sugar portion to another sugar (such as sucrose) and changing the configuration of the binding of the sugar to the flavanone skeleton (for example, changing from a α-configuration to a β-configuration) increases activity. Based on these concepts, the

applicant (person responsible for the experimental research) Yoshito Zamami along with the participants in the experimental research, Kawamura and Katoh, plan to design and synthesize new flavanone derivatives. Once these compounds are synthesized, we would like to measure their antimicrobial activity and study their structure-activity relationship. This work will be done by Zamami, Kawamura and Katoh, and Choh, a participant in this research, will conduct a part of the measurement of antimicrobial activity.

Also, as stated above, we are planning to study the acute toxicology of the compound when administered to mice, and investigate the ability of the compound to transfer into the blood and its pharmacokinetics after being administered to mice. The acute toxicology test will be done by Choh. Zamami and Kawamura will investigate the ability of the compound to transition into the blood. Investigation of the pharmacokinetics will be divided among all of the research participants (absorption: Zamami, Kawamura; Tissue distribution: Zamami, Choh, Kawamura; Metabolism: Zamami, Choh; Elimination: Zamami). Furthermore, to investigate the metabolism of the compounds, we would like to conduct a more detailed study comparing the compound with various possible metabolites that are separately synthesized. This separate synthesis and comparison is to be conducted by Zamami, Choh and Katoh.

6. Targets of the experimental research

In the research results to date, the minimum inhibitory concentration (MIC) of the compound of the application against MRSA is 0.13 μg/mL. The antibacterial activity of vancomycin against the same MRSA is 0.25 μg/mL, so the compound has twice the activity of vancomycin against MRSA. However, the target of the research of this application is to find a compound that has at least 10 times the activity of vancomycin. Also, MIC of the compounds of this application against VRSA is 0.25 μg/mL MIC of vancomycin against the same VRSA is over 128 μg/mL). Through this research, we also want to find a compound that has an activity level about 10 times stronger against VRSA. Furthermore, we want to find a compound that has an acute toxicity (LD_{50}) of at least 100 mg/kg.

7. Target patents

Patent name	New flavanone derivative		
Patent application number	PCT/JP2010/062759	Application date	2010/7/29
Applicant	Okayama University		
Inventors	Yoshito Zamami, five others		
Summary	A new flavanone derivative that contains an acyl group and contains glucose, galactose or other sugar with as a substituent was found to have effect against various drug-resistant bacteria including MRSA, VISA, VSSA, VRE, and VRSA.		
Outline	Peptide compound including arginine residue 9 to arginine residue 13 and amino-acid sequence of sequence number 1.		

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