

論文の内容の要旨

論文題目 : **Identification of novel hepatitis C virus NS3 helicase inhibitors from marine organisms**

(C型肝炎ウイルスのNS3ヘリケースを阻害する海洋天然物由来新規化合物の同定)

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The hepatitis C virus (HCV) is a major cause of liver failure and hepatocellular cancer, with about 170 million people chronically infected worldwide. The virus, which belongs to the family *Flaviviridae*, has a single-stranded RNA genome of positive polarity. The genome of HCV that codes for a unique polyprotein of approximately 3000 amino acids, directly translated by host cell machinery into a single precursor polyprotein that is processed by enzymatic cleavage into 10 proteins of diverse function. The proteins encoded by HCV have been extensively studied as drug targets. Among the nonstructural proteins, NS3 is a multifunctional protein with serine protease and helicase activity at N and C-terminal, respectively. The HCV NS3 helicase rearranges nucleic acid duplexes in a reaction fueled by ATP hydrolysis, making it a well validated target for the development of direct acting antiviral therapy. Telaprevir and boceprevir are two NS3 protease drugs that were approved by the FDA for use as a triple therapy in

combination with pegylated interferon and ribavirin for the treatment of chronic HCV genotype 1 infected patients. This triple therapy improved the sustained virologic response up to 70-80% depending on the genotypes. However, the emergence of viral resistance and adverse effects associated with this new therapy is still the main concerns in HCV research. No vaccine is available for hepatitis C and no NS3 helicase inhibitors have been entered in clinical trials. These encourage developing new NS3 helicase inhibitors that could be used with the NS3 protease inhibitors as antiviral agents.

Natural products are potential sources of structurally diverse chemical compounds with various biological activities. Among natural sources, marine organisms harbor highly diverse microorganisms that are major sources of secondary metabolites with unique chemical structures. Therefore, marine organisms are expected to be potential chemical sources of starting materials for driving new drug development. The aim of this project was to isolate the potential NS3 helicase inhibitors from marine organisms. Three novel NS3 helicase inhibitors: manoalide, psammaplin A, and polybrominated diphenyl ether (PBDE) were identified from marine sponge using a high-throughput screening photoinduced electron transfer (PET) system. PET system showed that manoalide, psammaplin A, and PBDE decreased the NS3 helicase activity with IC_{50} values of 56, 17, and 107 μ M, respectively. A radioisotope-labeled RNA helicase assay indicated that the NS3 RNA helicase activity was inhibited by manoalide with an IC_{50} of 15 μ M, which validates the PET system. The inhibitory effects of these inhibitors on the NS3 ATPase activity were examined, which have been shown that they attenuated the NS3 ATPase activity in a dose-dependent manner. Biochemical kinetic analysis demonstrated that both manoalide and psammaplin A do not affect the apparent K_m values of NS3 ATPase activity, suggesting that they act as noncompetitive inhibitors. The formation of

NS3-RNA complex was inhibited by manoalide at a concentration close to its IC₅₀ for ATPase inhibition, whereas psammaplin A, and PBDE inhibited the complex formation in a concentration dependent manner. The direct interaction between NS3 and inhibitors documented that they might be bound to the NS3 helicase and modulate to change its structure. The structure-activity relationship of PBDE against HCV ATPase revealed that biphenyl ring, bromine, and phenolic hydroxyl group on benzene backbone might be a basic scaffold for the inhibitory potency. Most importantly, both psammaplin A and PBDE showed the inhibitory effects on viral replication using replicon cell lines with EC₅₀ values of 6.1 and 3.3 μM, respectively.