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Antisense Oligonucleotide-Mediated Inhibition of Hepatitis C Virus Gene Expression in Transformed Hepatocytes. *K.P. Anderson[1], V. Brown-Driver[1], M.C. Fox[1], R.F. Azad[1], S. Furusako[2], H. Sasmor[1], and R.C. Hanecak[1]. ISIS Pharmaceuticals, Carlsbad, CA USA[1], and Mochida Pharmaceutical Co., Tokyo, JAPAN[2]*

Translation of hepatitis C virus (HCV) RNA is directed by an internal ribosome entry site (IRES) which is located within conserved nucleotide sequences at the 5' end of HCV RNA. Antisense oligonucleotides complementary to sequences thought to be important for IRES function were evaluated for inhibition of HCV gene expression in a transformed hepatocyte cell line containing integrated genomic DNA expressing HCV RNA. Several phosphorothioate-linked oligodeoxyribonucleotides caused concentration-dependent reductions in HCV RNA and protein levels in treated cells consistent with RNase H mediated cleavage of RNA at the site of oligonucleotide hybridization. A phosphodiester-linked, 2'-methoxyethoxy modified oligonucleotide complementary to the polyprotein initiation AUG reduced HCV core protein levels in treated cells with no reduction in HCV RNA levels demonstrating a non-RNase H-mediated mechanism. These antisense oligonucleotides will provide useful tools for characterization of HCV RNA sequences important for efficient translation of HCV RNA, and are candidates for development as antiviral drugs.

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3D MODELING OF HCV PROTEASE AND COMPUTER SCREENING OF ITS INHIBITORS

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Based on the amino acid sequence of HCV protease and the homology for a serine protease, the 3D model of the protease was constructed. This model was refined by molecular mechanics or molecular dynamics with some distance constraints between the serine protease and the substrate sequence model of Ac-Cys-Cys-Ser-OCH3. The calculated model could explained the substrate specificity, which HCV protease hydrolyzed C-terminal amide bond of Cys in the substrate -Cys-Cys-Ser- sequence. The computer screening was applied with this model by using 'DOCK' program. We searched the active candidates on the 3D structure database of small molecules, which were generated from approximately 2,500 compounds in our chemical library. In this method, the complementality between a cavity and an inhibitor was evaluated by a contact or shape-fitting score. Consequently, some candidates, which were evaluated with the high score in the computer screening, were proved the inhibitory activity against HCV protease. One of these active compounds was thiazolidine derivative, which were synthesized and offered by Yamanouchi Pharmaceutical Co.,Ltd. In conclusion, we expect that this method can be useful for a preliminary screening of enzyme inhibitors.

Thiazolidine Derivatives