

# Dipeptides having Ferulic Acid Moiety for Antiviral

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Ferulic acid and its derivatives show several bioactivity as anticancer, antifungus, and antioxidase. We synthesized the dipeptides with ferulic acid moiety for the antiviral agents. The starting material was 5-phenyl-2-oxazolone via the cyclization of hippuric acid by the treatment of dicyclohexylcarbodiimide (DCC), and then oxazolone reacted with 4-hydroxy-3-methoxy benzaldehyde (vanilin) in the presence of acetic anhydride to give 4-hydroxy-3-methoxybenzylidene-oxazolone derivarive, which was easily transformed to the ring opening products such as benzylideneglycyl-amino acids containing ferulic acid moiety by the reaction of amino acid sodium salts.

The synthesized dipeptides having ferulic acid moiety were tested for the inhibition of HIV-1.

Since a virus depends on its host cell for many functions of virus replication, it is difficult to inhibit virus multiplication without at the same time affecting the host cell itself. Because of this, the spectacular medical successes following the discovery of anti-bacterial agents have not been followed by similar success in the search for specific antiviral agents. A few antiviral compounds are successful in controlling virus infection in laboratory situations and certain of these have been used in restricted clinical cases; but no substance has yet been found with more than limited practical use. The chemical inhibition of virus action is known to occur at the stages of virus replication: for example, Kethoxal for free Influenza virus (FLUV); Amantazine for FLUV and Benzyloxycarbonyl peptide for Measles at the stage of the entry of Nucleic acid; Benzimidazole and Guanidine for Poliovirus, Fluorodeoxyuridine, Iododeoxyuridine, and Acyclovir for Herpesvirus (HSV), and Azidothymidine for Human immunodeficiency virus (HIV) at the stage of the Nucleic acid replication; Isatin thiosemicarbazone for Smallpox virus at the stage of the late protein

synthesis. At the stage of the adsorption and the release, however, none of the therapeutic antiviral agents are known.

The glycoprotein, designed F (fusion polypeptide), is involved in virus penetration, through fusion of viral and cell membranes, and in virus-induced cell fusion and hemolysis, activities which are activated by a host protease to yield two disulfide-linked polypeptides, F1 and F2.

The proteolytic cleavage that activates these biological properties of the virus generates a new N-terminus on the F1 polypeptide, which is the polypeptide whose C-terminus is associated with the lipid bilayer of the viral membrane.<sup>1)</sup> The following lines of evidence suggested that the structure of the new N-terminus is important for the expression of the biological activities of the F protein, each of which involves membrane fusion: (1) as indicated above, the expression of the activities is dependent on the cleavage; (2) the new N-terminal region is extremely hydrophobic, raising the possibility that it could be involved in interactions with the target cell membrane<sup>2)</sup>; (3) the amino acid sequence in this region is highly conserved among different paramyxoviruses; (4) in a mutant of Sendai virus which is cleaved and activated by a

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protease different from that which activates the wild type virus, the N-terminal amino acid sequence of F1 is the same as that of the wild type; i.e., Phe-Phe-Gly-providing further evidence for the requirement for a specific amino acid sequence in that region for biological activity; (5) finally, we noted a similarity between this N-terminal amino acid sequence of F1 and that of an oligopeptide, benzyloxycarbonyl-D-Phe-L-Phe-L-(nitro) Arg was found in 1968 to inhibit plaque formation by measles virus. Subsequently Norrby<sup>3)</sup> showed that this oligopeptide, and some others with similar observations, inhibited measles virus penetration and virus-induced cell fusion and hemolysis, and we made similar observations with another paramyxovirus, SV5. Because of the indication that the N-terminal region of F1 was involved in the biological activities of the protein, and with the hypothesis that it might be possible to inhibit these activities of the protein, we synthesized a pyrimidine base with peptides which resembled this region of F1 polypeptide, and investigated their ability to inhibit the replication of several different paramyxoviruses. The hemagglutinin protein of myxoviruses is also cleaved by a protease supplied by the host cell or present in the extracellular fluid, e.g., serum plasmin,<sup>4)</sup> and although this cleavage has no effect on hemagglutination, it activates the infectivity of the virus.<sup>5)</sup> As in the case of paramyxoviruses, the cleavage of the HA protein of influenza virus yields two disulfide-linked subunits, HA1 and HA2, and a new N-terminus is generated on the HA2 subunit, the C-terminus of which is embedded in the membrane.<sup>6)</sup> Thus, there is a structural and functional analogy between the paramyxovirus F protein and the myxovirus HA protein, an analogy that is strengthened by the fact that the sequences of the first nine amino acids of the N-terminus of the HA2 polypeptide of several influenza virus strains resemble that of the F1 polypeptide of paramyxoviruses, except that in influenza virus there is an N-terminal glycine that precedes phenylalanine, which is the N-terminus of the

F1 polypeptide. Because of these structural and functional similarities between the HA and F proteins, dipeptides having ferulic acid moiety were synthesized which resembled the N-terminus of the HIV polypeptide and tested for their inhibitory activity.

## Materials and Methods

*Cells.* MT-4, a T4 lymphocyte line carrying human T-cell lymphotropic type I (HTLV-1) and HUT-78, a T4 lymphocyte line not carrying HTLV-1, were used for the anti-HIV assays. The cells were mycoplasma-negative. The cell lines used for the cytostatic assays, namely, Molt/4F and CEM, have been described elsewhere.

*Viruses.* HIV type 1 (HIV-1) was obtained from the culture supernatant of HUT-78 cell line persistently infected with HTLV-III<sub>B</sub>. The reported method for preparing [5-<sup>3</sup>H] uridine-labelled HIV-1 particles was applied.

*Anti-HIV Assays.* Activity of the compounds against HIV-1 replication was based on the inhibition of virus-induced cytopathogenicity, determined by trypan blue exclusion. MT-4 cells were suspended at  $3 \times 10^6$  cells/ml and infected with HIV at 1000 CCID<sub>50</sub>/ml. Immediately after infection, 100  $\mu$ l of the cell suspension was brought into each well of flat bottomed microtiter tray containing various concentration of the test compounds. After 5 days incubation at 37 °C, the number of viable cells was determined microscopically in a hemacytometer by trypan blue exclusion. Anti-HIV activity was also determined by monitoring viral antigen expression in HUT-78 cells at day 12 after HIV-1 infection. Indirect immunofluorescence, using a polyclonal antibody as probe, and laser flow cytofluorographic analysis, were used for the determination of antigen-positive cells. HUT-78 cells were infected with HIV-1 at a multiplicity of infection of 0.4, and three quarters of the culture medium containing appropriate concentrations of the compounds were replenished every 4<sup>th</sup> days.

*Compounds.* Starting material, 5-phenyl-2-

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oxazolone (1), was obtained by the cyclization of hippuric acid with the treatment of dicyclohexylcarbodiimide (DCC). Oxazolone (1) reacted with vanillin in the presence of acetic anhydride to afford 4-(4-hydroxy-3-methoxy-benzylidene)-5-phenyl-2-oxazolone (2) in good yield. The obtained compound (2) has the ferulic acid moiety and the structure of intramolecular active ester. Compound (2) was treated with sodium salt of several amino acids to give the desired dipeptides having ferulic acid moiety. Using amino acids were hydrophobic amino acids such as Gly, Ala, Val, Leu, Ile, Phe, DPhe, and Pro.

*Results.* All drugs were tested for antiviral activity against HIV-1. None of all compounds were cytotoxic for host cells as  $CC_{50}$  100  $\mu$ g/ml.

Unfortunately, antiviral active drugs were not found in this research.

References

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