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Adenosine Deaminase-like Protein 1 (ADAL1) Catalyzes Removal of Different Alkyl Groups from N^6- or O^6-substituted Purine or 2-Aminopurine Nucleoside Monophosphates

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N^6-Methyl-AMP/dAMP aminohydrolase has been shown to be involved in the metabolism of pharmacologically important N^6-substituted purine nucleosides and 5′-monophosphate prodrugs thereof. Such compounds include abacavir, an approved anti-HIV agent, and GS-9219, a cytotoxic agent that targets lymphoid cells that is currently in early phase clinical trials. Currently, there are several O^6-substituted guanosine-5′-monophosphate prodrugs in clinical or preclinical development as anti-HCV agents including PSI-352938, a 3′,5′-cyclic phosphate prodrug with an O^6-ethyl substitution on the guanine and PSI-353661 and INX-189 phosphoramidate prodrugs containing O^6-methyl guanine. These compounds require removal of the O^6-alkyl group from the guanine base prior to metabolism to the active 5′-triphosphate. Therefore we assessed the ability of purified recombinant human N^6-methyl-AMP aminohydrolase to use O^6-substituted purine 5′-monophosphates as substrates. Human N^6-methyl-AMP/dAMP aminohydrolase was cloned, using primers described by Schinkmanova et al. (Collect. Czech. Chem. Commun., 2008), and overexpressed in *E. coli*. Mass spectroscopic analysis followed by amino acid sequence analyses indicated that the protein was adenosine deaminase like protein isoform 1 (ADAL1). Furthermore, activity and molecular weight profiles indicated that N^6-methyl-AMP/dAMP aminohydrolase and ADAL1 were indeed the same enzyme. An extensive structure-activity relationship study showed that ADAL1 was able to catalyze removal of different alkyl groups from not only N^6-substituted purine or 2-aminopurine nucleoside monophosphates but also from O^6-substituted purine nucleotides. The ADAL1 activity was susceptible to modifications in the phosphate moiety but not to changes in the sugar moiety. Overall, our data indicated that ADAL1 specifically acts at the 6-position of purine and 2-aminopurine nucleoside monophosphates.

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Inhibition of Human Cytomegalovirus Replication by Tricin (4′,5,7-Trihydroxy-3′,5′-dimethoxyflavone)

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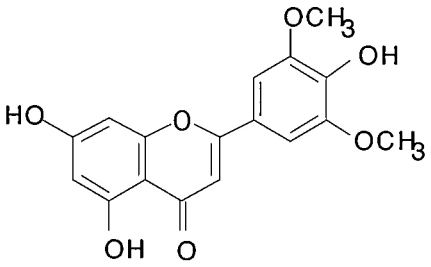
Human cytomegalovirus (HCMV) persists as a lifelong latent infection. However, HCMV is frequently activated in immunocompromised individuals, such as patients with AIDS or organ transplants, thereby causing severe morbidity and eventual mortality. Symptomatic HCMV infection has been treated with ganciclovir (GCV), but the appearance of GCV-resistant viruses is a recurrent problem in the treatment of immunocompromised patients with HCMV infection. Although PFA and CDV have been used in combination with GCV for the treatment of GCV-resistant HCMV, these treatments are not always successful. Therefore, effective new anti-HCMV agents and regimens need to be developed.

In this study, we show that the tricin (4′,5,7-trihydroxy-3′,5′-dimethoxyflavone), a derivative from Sasa albo-marginata, have anti-HCMV properties in the human embryonic fibroblast cell line MRC-5. On plaque assay, tricin showed dose-dependent inhibitory properties from 0.05 to 1.2 μM, but tricin had no virucidal effects on cell-free HCMV. Treatment with tricin 1 h before, or 1 h or 3 h after viral infection significantly suppressed HCMV replication. Moreover, tricin inhibited the expression of immediate early (IE) mRNA and DNA polymerase (UL54) mRNA in HCMV-infected cells. Western blot analysis also demonstrated that tricin decreased the expression of IE antigen (especially IE2) and cyclooxygenase 2 (COX-2) expression in HCMV-infected cells. In the presence of tricin, prostaglandin E2 (PGE_2) protein accumulation by HCMV infection was completely inhibited. These results suggest that tricin is a novel compound with potential COX inhibitor-dependent anti-HCMV activity.



Structure of tricin (4′, 5, 7−trihydroxy-3′, 5′ − dimethoxyflavone)

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Identification of Bicyclic Sulfone Inhibitors of HHV-6 Targeting the HHV-6 U77 Helicase

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We previously reported on the promising anti-HHV-6 activity of bicyclic sulfone derivatives (Naesens et al., 2006. Antiviral Res. 72, 60). We now examined the structure-activity relationship of a series of newly synthesized structural analogues. Their anti-HHV-6-activity was determined in HHV-6A (GS)-infected HSB-2 cells and HHV-6B (Z29)-infected MOLT-3 human T-lymphoblast cells, using a microscopic CPE reduction assay and real-time PCR quantitation of viral DNA. Some of the novel compounds were superior to the original compound, both in antiviral activity and selectivity. This strong inhibitory effect on HHV-6 replication was confirmed in HHV-6-infected fresh human cord blood lymphocytes. In order to identify the antiviral target, a resistance study was performed in which HHV-6A (GS) was serially passed in the presence of increasing concentrations of one bicyclic sulfone compound. After eight virus passages, a mutant virus was obtained with strong resistance to the bicyclic sulfones (antiviral EC_{50} values >300 μM), while its sensitivity to foscarnet and cidofovir was the same as that of control virus. DNA sequencing on the resistant virus revealed an isoleucine to methionine substitution at position 318 of the HHV-6 U77 helicase. Protein alignment showed that the Ile-318 residue in HHV-6 U77 is identical in both HHV-6 A and B variants and lies adjacent to motif IV, which is highly conserved among the herpesviruses and is required for DNA helicase activity. The I318M substitution selected by the bicyclic sulfones lies in an HHV-6 U77 region that aligns