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Herpes Thymidine Kinase Inhibitors: Resolution and Enantioselective Synthesis of L-653,180 and Water-soluble Prodrugs

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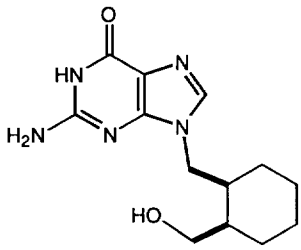

[1S,2R] enantiomer of L-653,180

Herpes simplex virus coded thymidine kinase (HSV-TK) has been shown to be important in the efficient reactivation of latent herpes infection. HSV-TK inhibitors have been shown to be effective in reducing rates of spontaneous virus reactivation in animal models. Racemic (±)-9-[[(*Z*)-2-(hydroxymethyl)cyclohexyl]-methyl]guanine [L-653,180] was discovered to be a potent, selective nonsubstrate inhibitor of HSV TK. Published studies with L-653,180 have demonstrated encouraging results in various *in vitro* studies, but results *in vivo* showed marginal effects on rates of spontaneous reactivation, which were attributed to limiting pharmacokinetic parameters and dosing problems associated with insolubitliy. Various prodrug derivatives of L-653,180 have been prepared, which were designed to overcome these limitations including the O-phosphoryl derivative and the 2,6-diamino- and 2-amino-purine analogs. Plasma pharmacokinetics and enzymatic activation were studied as a means to identify the most promising candidate for future studies. Racemic L-653,180 was also resolved by chiral chromatography. Absolute stereochemistry of the enantiomers was determined by conversion of chiral intermediates to chiral fused cyclohexyl lactones of known stereochemistry. An enantioselective synthesis of the more active L-653,180 [1S,2R] stereoisomer was also devised employing a porcine pancreatic lipase hydrolysis of a prochiral intermediate.

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HSV-Thymidine Kinase: a molecular target for transfection imaging in gene therapy of cancer. L.I. Wiebe, E.E. Knaus and K.W. Morin. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.

In contrast to cancer chemotherapy, antiviral chemotherapy has benefited from the identification of highly selective molecular targets. Differences in substrate specifications for virus-encoded enzymes and cellular enzymes have been exploited to obtain antiviral activity with minimal cytotoxicity in non-infected cells. Through our interests in scintigraphic imaging of focal Herpes Simplex virus (HSV) infections in the brain, we have developed several radioiodinated pyrimidine nucleosides, including (E)-5-(2-iodovinyl)-2'-deoxyuridine (IVDU), (E)-5-(2-iodovinyl)-2'-deoxyuridine (IVFRU) and their corresponding, brain-targeted 3'-O-(1-methyl-1,4-dihydropyridyl-3-carbonyl)- chemical delivery system (CDS) derivatives. IVFRU has antiviral potency similar to IVDU and has the advantage of improved stability against pyrimidine phosphorylase-catalysed phosphorolysis *in vivo*. The CDS provides substantially increased lipophilicity for improved diffusion across the blood-brain-barrier. The molecular target for these agents is virus-encoded thymidine kinase (TK). The HSV-TK gene is currently under investigation as a suicide gene in gene therapy of cancer. Expression of HSV-TK renders the tumor cells possessing this gene sensitive to antiviral therapy, creating a highly specific molecular target. Low toxicity chemotherapy can be achieved, as long as gene transfection efficiency and HSV-TK expression are high and as long as no non-target cells become transfected. IVFRU and IVFRU-CDS have been studied *in vitro* and *in vivo* using both murine mammary carcinoma and murine sarcoma cell lines that are insensitive (wild type) to these antiviral agents, and corresponding cell lines that have been rendered sensitive by HSV-TK gene transfection. Planar scintigraphic studies in mice bearing transplanted tumors of both cell lines indicate selective uptake of the radioiodinated antivirals. Detailed biodistribution data will be presented in support of the potential clinical utility of this technique to evaluate gene therapy using viral genes.