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BMS-200475, a Novel Carbocyclic 2'-Deoxyguanosine Analog with Potent and Selective Anti-Hepatitis B Virus Activity *in Vitro*. G.S. BISACCHI,* J.E. SUNDEEN, W.A. SLUSARCHYK, K. RINEHART, S. INNAIMO, S. CHAO, G. JACOBS, M.G. YOUNG, O. KOCY, Z. MERCHANT, P. EGLI, P. LAPOINTE, J. P. DARIS, A. MARTEL, R. COLONNO, R. ZAHLER. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ.

A current need exists for new potent and selective inhibitors of human hepatitis B virus (HBV). BMS-200475, a novel carbocyclic analog of 2'-deoxyguanosine, is a potent inhibitor of HBV (ED_{50}=3nM) with relatively low cytotoxicity (CC_{50}=30μM) as assayed in HepG2.2.15 cells. BMS-200475 also has selective activity against HBV compared to other viruses (*in vitro* ED_{50}s >10μM against human immunodeficiency virus, influenza virus, human cytomegalovirus, varicella zoster virus, and herpes simplex virus type I). An asymmetric synthesis affords BMS-200475 in good overall yield with high optical purity. For biochemical studies, the 5'-triphosphate of BMS-200475 as well as BMS-200475 radiolabelled at the 5'-position were prepared. The enantiomer of BMS-200475 was prepared and found to have poor HBV activity. Also, the adenine, thymine, and 5-iodouracil nucleoside base analogs of BMS-200475 display markedly diminished activities against HBV relative to BMS-200475.

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BMS-200,475, a New Anti-Hepatitis B Compound; Metabolic Studies and Comparison with 3TC, Penciclovir, and Lobucavir. G. Yamanaka*, T.M. Wilson, S.F. Innaimo, and R.J. Colonno. Bristol-Myers Squibb Pharm. Res. Inst., Wallingford, CT

BMS-200,475, a methylene-cyclopentane analog of deoxyguanosine, has potent antiviral activity against hepatitis B virus (ED_{50} = 3.7 nM, CC_{50} = 30 μM). In metabolic studies using human hepatoma cells, BMS-200,475 was specifically taken up and phosphorylated to its mono-, di- and triphosphate esters. In HepG2 cells, the uptake of nucleoside was linear between 1 and 25 μM, but intracellular triphosphate accumulated most efficiently in the low μM range. At 1 μM added drug, [^{3}H]BMS-200,475 triphosphate accounted for 60-80% of the intracellular nucleoside label, and accumulated to ~7 pmol/10^6 cells (7 μM). The half-life was ~15 h. When compared to three other nucleoside analogs reported to have activity against hepatitis B virus, BMS-200,475 was most efficiently phosphorylated to the triphosphate. Following a 3 day incubation at 25 μM added drug, the levels of intracellular triphosphate in HepG2 cells were, 17.9, 10.3, 1.2, and 0.29 pmol/10^6 cells, for BMS-200,475, 3TC, penciclovir, and lobucavir, respectively. This efficient phosphorylation of BMS-200,475, especially at low concentrations, is one reason for its high potency against Hepatitis B.

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Arabinogalactan (9kDa)-araAMP, a Novel Liver-targeting Conjugate Inhibits Selectively HBV Replication *in vitro*. L. Cui[1], A. Faraj[1], A. Alaoui[1], E. Groman[2], J. Rutkowski[2], L. Josephson[2], and J-P. Sommadossi[1]. Department of Pharmacology and Toxicology, Division of Clinical Pharmacology, The Liver Center, University of Alabama at Birmingham, Birmingham, Alabama 35294[1]; Advanced Magnetics, Inc., Cambridge, Massachusetts 02138[2].

Arabinogalactan(9kDa)-9-β-D-arabinofuranosyladenine-5'-monophosphate [AG (9kDa)-araAMP] was 25-fold more active than the parent compound 9-β-D-arabinofuranosyladenine (araA) in decreasing the intracellular replicative intermediate hepatitis B virus DNA level in HBV-transfected 2.2.15 cells with an EC_{50} of 2 μM. The putative active 5'-triphosphate metabolite, 9-β-D-arabinofuranosyladenine-5'-triphosphate (araATP), exhibited a strong IC_{50} of 5 μM toward woodchuck hepatitis virus DNA polymerase, consistent with the anti-HBV activity of these derivatives. AG (9kDa)-araAMP inhibited HepG2 cell proliferation by only 16% at a concentration as high as 822 μM, and no effects were observed in the presence of 8.22 μM and 82.2 μM of the drug. Lactate production, mitochondrial DNA content, and mitochondrial morphology were not affected by AG (9kDa)-araAMP at concentrations between 8.22 μM and 822 μM. In contrast, both araA and its *in vivo* metabolite, 9-β-D-arabinofuranosylhypoxanthine (araH), induced substantial dose-dependent effects on cell growth, lactate production, and mtDNA steady-state levels at concentrations of 8.22 μM and 82.2 μM. Slight loss of cristae within the mitochondria was also observed in cells incubated with araH. In summary, AG (9kDa)-araAMP exhibits a potent and selective anti-HBV activity *in vitro*, and the lack of toxic effects on host cell mitochondrial functions supports its further development.

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Lamivudine and Penciclovir Act Synergistically as Inhibitors of *In Vitro* Hepadnaviral Replication Penciclovir T. Shaw, D. Colledge and S.A. Locarnini. Victorian Infectious Diseases Reference Laboratory, Fairfield, Victoria, 3078, AUSTRALIA.

Lamivudine ([-]-β-L-2',3'-dideoxy-3'-thiacytidine; 3TC) and penciclovir (9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine; PCV) are potent inhibitors of hepatitis B virus (HBV) replication. Both drugs have entered phase III clinical trials for treatment of chronic HBV infection. Lamivudine and penciclovir are deoxycytidine and deoxyguanosine analogs respectively and their modes of action and how they interact are matters of both theoretical and practical interest. We compared the antiviral activities of 3TC and PCV alone and in combination in primary duck hepatocyte (PDH) cultures derived from ducklings congenitally infected with the duck hepatitis B virus (DHBV). 3TC and PCV inhibited DHBV replication to a comparable extent when used alone (50% inhibitory concentrations with 95% confidence intervals were 0.55 (0.50 - 0.59) μM for 3TC and 0.35 (0.27 - 0.43) μM for PCV. In combination, the two nucleoside analogs acted synergistically over a wide range of clinically relevant concentrations. Synergy between PCV and 3TC was also observed in acutely- infected cells and in "washout" experiments designed to assess the persistence of antiviral activity after drug removal. Furthermore, the combination was more effective in reducing the normally recalcitrant viral covalently closed circular (CCC) DNA form of DHBV than either drug alone. These results suggest that combinations of 3TC and PCV may act synergistically against HBV in vivo.