Drugs of the Future 2007, 32(2): 137-143

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.02.1075008

Pradefovir Mesilate

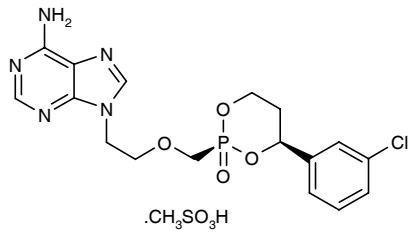
Treatment of Hepatitis B

Rec INNM

Pradefovir Mesylate (USAN)
MB-06866
MB-6866
Hepavir B
Remofovir mesylate (former name)

9-[2-[(2*R*,4*S*)-4-(3-Chlorophenyl)-2-oxido-1,3,2-dioxaphosphinan-2-ylmethoxy]ethyl]adenine mesylate

InChI=1/C17H19ClN5O4P.CH4O3S/c18-13-3-1-2-12(8-13)14-4-6-26-28(24,27-14)11-25-7-5-23-10-22-15-16(19)20-9-21-17(15)23;1-5(2,3)4/h1-3,8-10,14H,4-7,11H2,(H2,19,20,21);1H3,(H,2,3,4)/t14-,28+;/m0./s1



$C_{18}H_{23}ClN_5O_7PS$
Mol wt: 519.8972

CAS: 625095-61-6

CAS: 371778-91-5 (racemic free base)

CAS: 625095-60-5 (free base)

EN: 309134

Abstract

Adefovir dipivoxil is the oral prodrug of adefovir, an acyclic phosphonate analogue of adenine that is effective against hepatitis B virus (HBV) but poorly absorbed. Adefovir dipivoxil is mainly converted to active adefovir in plasma, and although it exhibits anti-HBV activity in patients, it is associated with dose-limiting renal toxicity and was approved only at suboptimal doses. Consequently, a novel series of phosphate and phosphonate prodrugs of adefovir called HepDirect™ prodrugs were designed that specifically target the liver and thus bypass the risk of nephrotoxicity. Pradefovir mesilate (previously known as MB-06886, Hepavir B and remofovir mesylate) emerged from this series as the lead compound. Pradefovir is activated via oxidation mediated by cytochrome P-450 (CYP) 3A4, which is predominantly expressed in the liver. The novel prodrug is highly stable in both plasma and tissues and demonstrated potent preclinical and clinical anti-HBV activity. Pradefovir is undergoing phase II development for the treatment of chronic hepatitis B.

Synthesis

Pradefovir mesilate can be synthesized as follows:

Treatment of adenosine (I) with ethylene carbonate (II) in the presence of NaOH in refluxing DMF gives 9-(2-hydroxyethyl)adenosine (III). Diethyl phosphite (IV) is then condensed with formaldehyde, followed by reaction with *p*-toluenesulfonyl chloride and Et_3N to afford diethyl (tosyloxymethyl)phosphonate (V). Subsequent condensation of tosylate (V) with (III) in the presence of *t*-BuONa gives the phosphonyloxymethoxyethyl adenosine (PMEA) diethyl ester (VI), which is hydrolyzed to the corresponding phosphonic acid adefovir (VII) by means of *in situ*-generated iodotrimethylsilane. The condensation of phosphonic acid (VII) with 1(*S*)-(3-chlorophenyl)-1,3-propanediol (VIII) utilizing DCC/pyridine leads to the cyclic phosphonate (IX) as a roughly equimolecular mixture of diastereoisomers, which can be chromatographically separated to furnish the title *cis*-isomer pradefovir. In an improved procedure, activation of adefovir (VII) with oxalyl chloride and *N,N*-diethylformamide generates the formamidine-protected phosphonyl dichloridate (X), which upon condensation with diol (VIII) at low temperature gives the cyclic phosphonate (XI) as a mixture in which the desired *cis*-diastereoisomer predominates. After hydrolysis of the formamidine (XI) with ethanolic AcOH, recrystallization as the corresponding mesylate salt provides the title adefovir prodrug in high diastereomeric excess (1-5). Scheme 1.

The optically pure diol intermediate (VIII) can be obtained by several procedures. Aldol condensation of 3-chlorobenzaldehyde (XII) with the lithium enolate of ethyl acetate gives the hydroxy ester (XIII), which is reduced to racemic 1-(3-chlorophenyl)-1,3-propanediol

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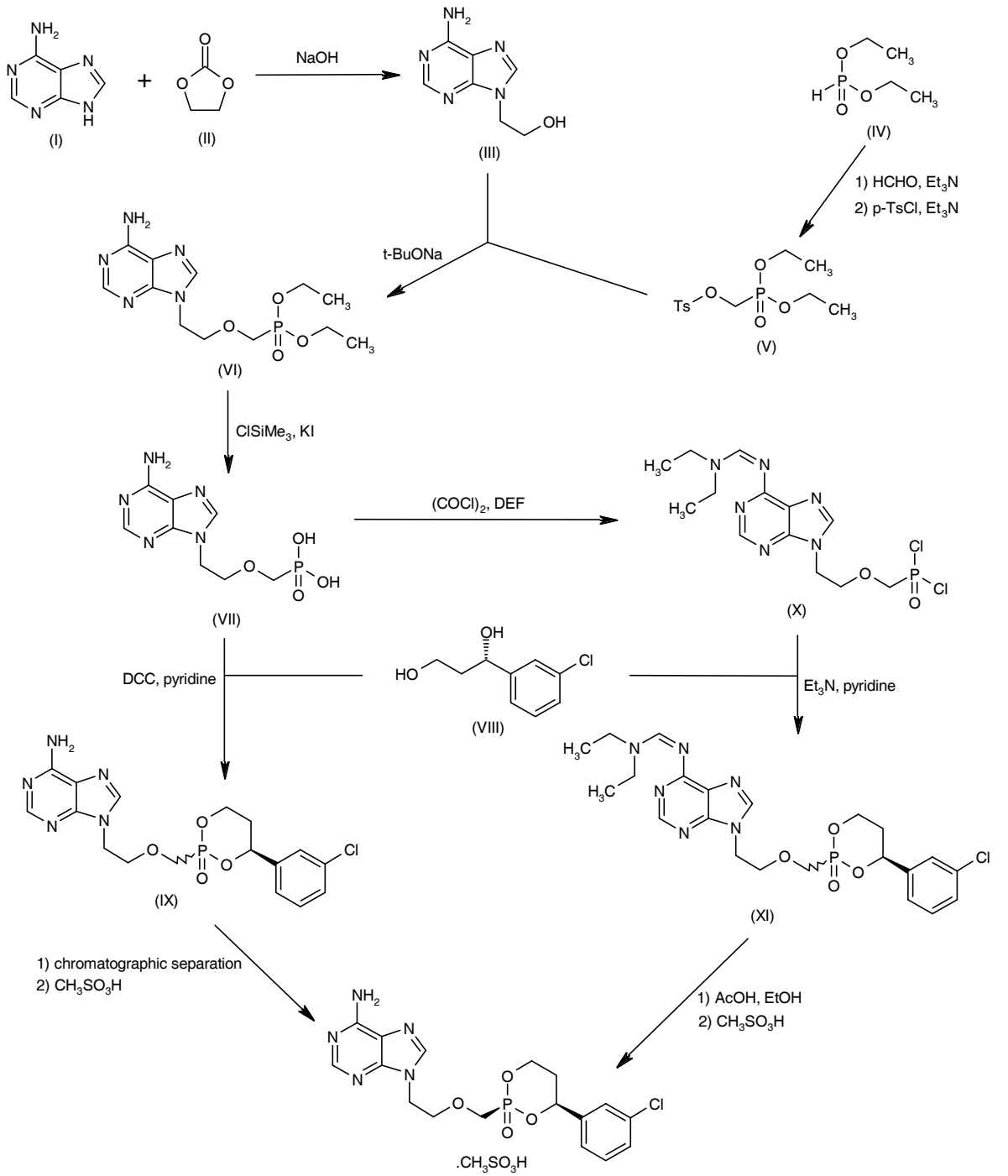
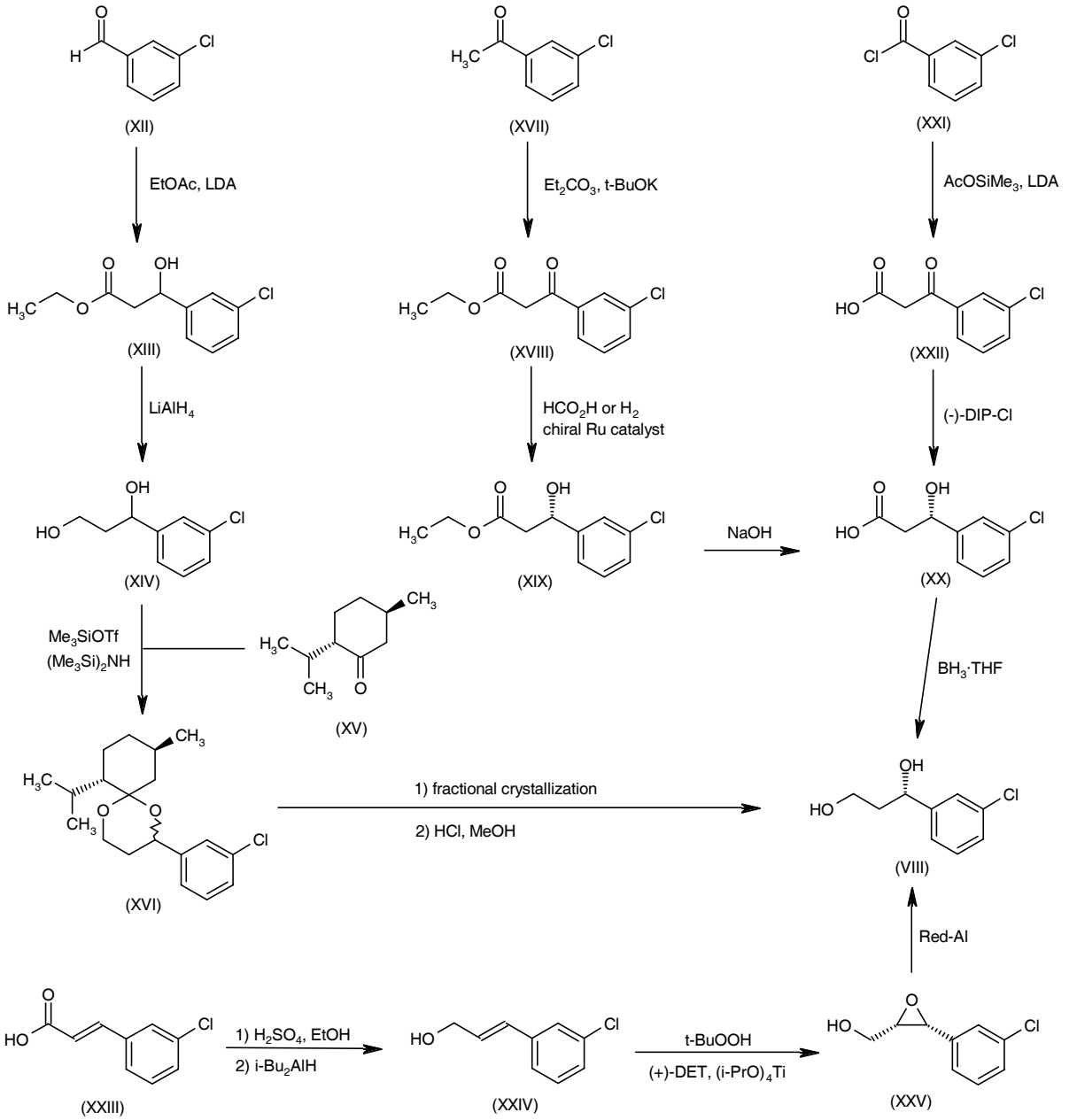
Scheme 1: Synthesis of Pradefovir Mesilate



(XIV) by means of $LiAlH_4$ in Et_2O. Ketalization of diol (XIV) with (–)-menthone (XV) employing trimethylsilyl triflate and hexamethyldisilazane produces the spiro ketal (XVI) as a diastereomeric mixture, from which the target (*S*)-diol (VIII) is obtained by fractional crystallization, followed by acidic ketal hydrolysis (1-3). In a different strategy utilizing an enantioselective reduction, 3'-chloroacetophenone (XVII) is condensed with diethyl carbonate by means of *t*-BuOK in THF to yield the keto ester (XVIII), which is selectively reduced to the (*S*)-hydroxy ester (XIX) by catalytic hydrogenation over a chiral ruthenium catalyst. After saponification of ester (XIX), the chiral hydroxy acid obtained (XX) is reduced to (VIII) with borane in THF. Similarly, the (*S*)-hydroxy acid (XX) can be produced by

Scheme 2: Synthesis of Intermediate (VIII)



condensation of 3-chlorobenzoyl chloride (XXI) with the lithium enolate of trimethylsilyl acetate, followed by acidic desilylation to yield the keto acid (XXII), which is then reduced to (XX) utilizing (–)-*B*-chlorodiisopinocampheylborane (DIP-Cl) in cold CH_2Cl_2 (1-5). In a further method, 3-chlorocinnamic acid (XXIII) is esterified with H_2SO_4/EtOH, followed by reduction with DIBAL to provide the cinnamyl alcohol (XXIV), which undergoes Sharpless asymmetric epoxidation to (XXV) with *t*-butyl hydroperoxide in the presence of (+)-diethyl tartrate. Reductive cleavage of the chiral epoxide obtained (XXV) by means of Red-Al then furnishes the target 1(*S*)-(3-chlorophenyl)-1,3-propanediol (VIII) (1, 2). Scheme 2.

Background

The human hepatitis B virus (HBV) is a partially double-stranded, relaxed circular enveloped DNA virus of approximately 3,200 base pairs which belongs to the Hepadnaviridae family. Chronic infection with HBV is a major healthcare problem, with an estimated 400 million individuals affected worldwide, including at least 1.25 mil-

lion diagnosed in the U.S. HBV-infected individuals are at a high risk for mortality and morbidity from cirrhosis, hepatic decomposition and hepatocellular carcinoma and it is estimated that HBV-related disease is responsible for 1-2 million deaths per year worldwide (6-9).

Conventional therapies for chronic HBV infection include monotherapy with s.c. interferon alfa (IFN-α2b), the oral nucleoside analogue lamivudine or the oral nucleotide analogue adefovir dipivoxil; two related agents, entecavir and telbivudine, were recently approved in 2005 and 2006, respectively. Although these therapies have been shown to improve the management of HBV infection, they are limited by the associated low rate of sustained off-therapy responses (*i.e.*, < 50% of the patients), the high rate of adverse events and the emergence of resistance. Thus, researchers continue to search for new treatments with increased potency, more favorable safety profiles and a lower risk of resistance development (6, 10-15).

Adefovir (PMEA) is an acyclic phosphonate analogue of adenine that is effective against HBV following cellular kinase- or 5-phosphoribosyl-1-pyrophosphate synthetase-mediated phosphorylation to adefovir diphosphate. Adefovir inhibits HBV DNA polymerase (reverse transcriptase) via competition with its endogenous substrate deoxyadenosine triphosphate and causes DNA chain termination subsequent to its incorporation into viral DNA. However, it has low oral bioavailability due to poor intestinal permeability. An oral prodrug of adefovir, adefovir dipivoxil (Hepsera™; Gilead), was designed which exhibited efficacy in reducing serum HBV DNA and alanine aminotransaminase (ALT) levels in hepatitis B e antigen (HBeAg)-positive and -negative patients. Unfortunately, adefovir dipivoxil is associated with dose-limiting renal toxicity and the U.S. Food and Drug Administration (FDA) approved dosing at only suboptimal levels of 10 mg/day (6, 16-20).

Researchers therefore continue to search for safer, more potent anti-HBV agents and prodrugs for adefovir. One strategy for enhancing the potency and reducing the toxicity of an agent is to target it to specific organs. This can be achieved through the use of drug carriers such as antibodies, endogenous and synthetic polymers and labeled liposomes which target drugs to blood vessels of specific tissues. However, these carriers do not always allow delivery to extravascular sites, and thus potency is not optimized. Another strategic option is the use of low-molecular-weight prodrugs which are widely distributed throughout the body but are cleaved to the active parent compound intracellularly by an organ-specific enzyme. A series of phosphate and phosphonate prodrugs called HepDirect™ prodrugs were designed by coupling adefovir with both enantiopure and racemic substituted 1,3-propanediols to optimize the activation and byproduct properties, and by including a ring substituent rendering the compound sensitive to cytochrome P-450 (CYP) isozyme-mediated oxidative cleavage. CYP isozymes are predominantly expressed in the parenchymal cells of the liver and these prodrugs would therefore target the liver, bypassing the risk of renal toxicity. Pradefovir mesilate (previously known as Hepavir B, remofovir mesylate and MB-06886) emerged from this series as the lead compound. Pradefovir is activated via CYP3A4-mediated oxidation and exhibited high plasma and tissue stability. Pradefovir could enhance the therapeutic index of adefovir and was therefore selected for further development for the treatment of chronic HBV infection (3, 5, 21-25).

Preclinical Pharmacology

The activation of pradefovir to adefovir was examined using subcellular microsomal fractions of rat and human microsomes. Cleavage of pradefovir occurred via CYP3A-catalyzed oxidation, yielding an intermediate ring-opened monoacid which underwent a β-elimination reaction, converting it to adefovir and an aryl vinyl ketone. The K_m, V_{max} and intrinsic clearance rate (Cl_{int}) for the agent were 25 ± 7 μM, 1.2 ± 0.1 nmol/min/mg protein and 48.1 ± 8 μl/min/mg protein, respectively, in rat microsomes, and 160 ± 9 μM, 1.8 ± 0.03 nmol/min/mg protein and 11.3 ± 0.4 μl/min/mg protein, respectively, in human microsomes. Activation of pradefovir was also tested in isolated rat hepatocytes by measuring nucleoside triphosphate levels. The C_{max} and $AUC_{0\text{-}4h}$ values obtained for pradefovir and adefovir were 379 ± 131 pmol/kg *vs.* 41 ± 6 pmol/kg, respectively, and 756 ± 291 pmol.h/mg *vs.* 87 ± 12 pmol.h/mg, respectively (5, 22).

When tested against a panel of human recombinant CYP enzymes incubated in human microsomes, pradefovir was found to be activated to adefovir only by CYP3A4. Conversion to adefovir occurred with a K_m, V_{max} and Cl_{int} of 60 μM, 228 pmol/min/mg protein and about 359 ml/min, respectively. Activation of the prodrug was significantly inhibited by addition of the selective CYP3A4 inhibitor ketoconazole and a specific CYP3A-inhibitory monoclonal antibody. Moreover, pradefovir (0.2, 2 and 20 μM) did not exhibit direct or mechanism-based inhibition of CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP2E1 or CYP1A2. In addition, pradefovir (10 μg/ml) was not an inducer of CYP1A2 or CYP3A4/5 in primary human hepatocyte cultures. Studies using portal vein-cannulated rats treated with multiple doses of pradefovir (300 mg/kg/day p.o. for 8 days) revealed that the liver was the site of metabolic activation to adefovir; the intestine was not significantly involved. In addition, multiple dosing did not induce any CYP enzymes (22, 26-28).

Tissue and plasma metabolite profiles were analyzed following oral administration of pradefovir (equivalent to 30 mg/kg adefovir) to mice and rats. Results indicated a 4.5- and 7.5-fold greater exposure of adefovir-related metabolites (adefovir monophosphate and diphosphate, respectively) to the liver as compared to the kidney and intestine (21).

Further studies in rats demonstrated the liver-targeted efficacy of pradefovir. Analysis of whole-body autoradiograms showed that radioactivity in the liver following single oral doses (30 mg/kg) of [^{14}C]-pradefovir was 15 times higher than that seen with [^{14}C]-adefovir dipivoxil; esti-

mated liver radioactivity $AUC_{0\text{-}24h}$ values for adefovir dipivoxil and pradefovir were 889 and 14,012 nCi.h/g, respectively. In contrast, kidney radioactivity following [^{14}C]-pradefovir dosing was only about one-third that observed with [^{14}C]-adefovir dipivoxil (kidney radioactivity $AUC_{0\text{-}24h}$ = 3783 nCi.h/g *vs.* 10,264 nCi.h/g). Similar results were obtained in cynomolgus monkeys administered single oral doses (4 mg/kg) of the labeled prodrugs. Levels of radioactivity after dosing with [^{14}C]-pradefovir were 60 times higher in the liver and two-thirds lower in the kidney as compared to levels observed following [^{14}C]-adefovir dipivoxil. Results from liver uptake experiments in portal vein-cannulated rats and monkeys receiving a single oral dose of pradefovir (30 mg/kg) indicated that no adefovir was detectable in portal plasma early after dosing. This suggests that intestinal CYP3A4 is not involved in pradefovir conversion to adefovir. Portal/systemic extraction ratios in both species were high, further indicating excellent liver targeting of pradefovir. No significant safety concerns were seen in rats and monkeys after oral dosing for 28 days at levels of 30 mg/kg and up to 60 mg/kg, respectively. However, renal toxicity was observed at doses of 100 mg/kg or higher (29).

Pharmacokinetics and Metabolism

The single-dose pharmacokinetics and metabolism of i.v. and p.o. [^{14}C]-pradefovir (30 mg/kg) were determined in rats and cynomolgus monkeys. Rates for oral absorption and bioavailability were 29.7% and 5.42%, respectively, in rats and 65.6% and 19.4%, respectively, in monkeys. Extensive conversion to adefovir and other metabolites was observed after oral dosing in both species, and adefovir and metabolite A were the major metabolites detected in urine in both rats and monkeys following i.v. and p.o. dosing. The elimination $t_{1/2}$ for the prodrug was 0.7 h in both species after i.v. dosing and total body clearance and apparent volume of distribution in rats and monkeys were 5.85 and 2.6 l/h/kg, respectively, and 5.99 and 2.7 l/kg, respectively. Excretion of total radioactivity in urine accounted for 61.8% and 12.9% of the i.v. and p.o. doses, respectively, in rats; these rates were 43.3% and 34.9%, respectively, in monkeys. Biliary excretion of the drug was suggested since fecal excretion of radioactivity after i.v. doses accounted for 37.5% of the dose in rats and 17.4% of the dose in monkeys (30).

The pharmacokinetics of pradefovir (10, 30 or 60 mg p.o.) and adefovir were determined in a double-blind, placebo-controlled study conducted in 23 healthy male volunteers. Single escalating does of pradefovir were generally well tolerated. The prodrug was rapidly absorbed, with C_{max}, $AUC_{0\text{-}96h}$ and $AUC_{0\text{-}\infty}$ values for both prodrug and adefovir increasing with pradefovir dose. The adefovir:pradefovir ratio for both $AUC_{0\text{-}96h}$ and $AUC_{0\text{-}\infty}$ ranged from 1.4 to 1.8. Renal clearance of pradefovir (18-31 l/h) increased with pradefovir dose and was greater than glomerular filtration rate (125 ml/min or 7.5 l/h). Metabolic clearance was suspected to be responsible for most of the clearance of pradefovir since the fraction of total body clearance due to renal clearance was very low, ranging from 0.045 to 0.083. Food intake was shown to have no effect on pradefovir (30 mg/kg) or adefovir exposure, although the rate of systemic availability of adefovir may be reduced (31, 32).

Safety

A study in rats compared kidney and liver gene expression following 8-day oral dosing with either pradefovir (300 mg/kg/day) or adefovir dipivoxil (40 mg/kg/day). A marked increase of more than 20-fold in mRNA levels of multidrug resistance gene (*Mdr1b*) and 3-5-fold increases in the cell cycle genes cyclin B, cyclin G and *GADD153* were observed in the kidney following dosing with both prodrugs. Pradefovir also induced renal expression of genes encoding stearyl-CoA desaturase and fatty acid synthase by 25-fold or more. In contrast, adefovir dipivoxil downregulated more than 50 genes in the kidney, including *CMOAT* (canalicular multispecific organic anion transporter; 3-fold) which is essential for adefovir excretion, and several drug-metabolizing enzymes (*e.g.*, CYP1A, CYP2A, CYP2B, CYP2C, CYP3A, GST and UDPGT; 5-40-fold). With the exception of pradefovir which downregulated hepatic expression of the gene for stearyl-CoA desaturase by 9.6-fold and upregulated *Mdr1b* by more than 40-fold, both prodrugs had very little affect on gene expression in the liver. Overall, these findings point to substantially less potential for nephrotoxicity with pradefovir compared to adefovir dipivoxil (33).

Clinical Studies

The safety, tolerability and pharmacokinetics of pradefovir (5, 10, 30 and 60 mg/day p.o. for 28 days) were examined in a placebo-controlled, dose-escalation study in 40 patients with chronic HBV infection. Treatment was well tolerated, with no unexpected adverse events reported. The majority of adverse events were mild and the incidence was similar in both pradefovir and placebo groups; headache was the most common. The agent was rapidly absorbed and converted to adefovir (median t_{max} approximately 1 h for pradefovir and adefovir). The C_{max} and AUC values for both the prodrug and adefovir increased with dose. The $t_{1/2}$ for pradefovir was 4-14 h as compared to 29-39 h for adefovir on day 28. All doses produced clinically significant decreases in serum HBV DNA. The median reductions in these levels from baseline were 2.13, 2.60, 2.53 and 2.97 $\log_{10}$ copies/ml for the respective pradefovir doses (34).

The safety, tolerability and pharmacokinetics of pradefovir (5, 10, 20 and 30 mg/day p.o. for 28 days) were examined in a placebo-controlled trial in 45 adult Asian patients with chronic HBV infection. Pradefovir was well tolerated, with no unexpected adverse events seen. The incidence of adverse events was similar in active treatment and placebo groups and the most common was upper respiratory infection (30%). The prodrug was rapidly absorbed and converted to adefovir, with C_{max} and AUC

values increasing with dose; $t_{1/2}$ values for pradefovir and adefovir were 6.8-11 and 43-53 h, respectively. All doses effectively reduced serum HBV DNA levels, with median reductions of 1.64, 2.48, 2.72 and 2.66 log_{10} copies/ml for the respective pradefovir doses (35).

A multicenter, randomized, open-label, parallel-group study conducted in 244 adult Asian patients with compensated HBV infection (mean baseline HBV DNA = 7.9-8.2 log_{10} copies/ml; median baseline ALT = 2-2.8 x ULN) compared the safety, tolerability, pharmacokinetics and pharmacodynamics of pradefovir (5, 10, 20 and 30 mg/day p.o. for 48 weeks) with adefovir dipivoxil (10 mg/day p.o. for 48 weeks). All patients were adefovir dipivoxil-naïve but most had received prior IFN or other antiviral therapy. A total of 242 patients received more than 1 dose of the treatment drugs. Pradefovir was concluded to be safe and well tolerated. The majority of adverse events reported were mild and similar rates were obtained for both pradefovir and adefovir dipivoxil groups. The most common adverse events reported in the highest dose pradefovir group were diarrhea, dyspepsia, nasopharyngitis, upper respiratory tract infection and headache. One patient receiving 10 mg pradefovir developed a serious case of treatment-related hepatoma. Pradefovir was rapidly absorbed and converted to adefovir. C_{max} and AUC values for both prodrug and adefovir increased with dose at week 24; negligible accumulation for both pradefovir and adefovir was detected between 12 and 24 weeks. The AUC values for adefovir in patients receiving 30 mg pradefovir were about 75% those for patients administered adefovir dipivoxil at week 24. At 48 weeks, significantly greater median reductions in serum HBV DNA were observed with pradefovir doses of 10, 20 and 30 mg as compared to adefovir dipivoxil and the low pradefovir dose (4.84 ± 0.16, 4.89 ± 0.18 and 5.54 ± 0.15 log_{10} copies/ml, respectively, *vs.* 4.19 ± 0.20 and 4.09 ± 0.22 log_{10} copies/ml, respectively). The percentage of patients with HBV DNA levels below 400 copies/ml was 45%, 63%, 56% and 71% for the respective pradefovir dose groups as compared to 36% for the group receiving adefovir dipivoxil. Analysis of the relationship between reductions in serum HBV DNA and adefovir AUC values at 24 weeks revealed that the dose of 30 mg pradefovir achieved 98% of E_{max} (36-38).

Pradefovir is presently in phase II clinical trials for the treatment of chronic HBV infection (39, 40).

Sources

Metabasis Therapeutics, Inc. (US); licensed to Valeant Pharmaceuticals International and just recently to Schering-Plough Corp.

References

1. Kopcho, J.J., Matelich, M.C., Reddy, K.R., Ugarkar, B.G. (Metabasis Therapeutics, Inc.). *Process for preparation of cyclic prodrugs of PMEA and PMPA*. JP 2005525422, US 2003225277, WO 2003095665.

2. Erion, M.D., Kopcho, J.J., Matelich, M.C., Reddy, K.R. (Metabasis Therapeutics, Inc.). *Novel phosphonic acid based prodrugs of PMEA and its analogues*. EP 1532157, US 2003229225, WO 2004037161.

3. Reddy, K.R., Matelich, M.C., Ugarkar, B.G. et al. *HepDirect™ prodrugs of adefovir: Design, synthesis and optimization*. 227th ACS Natl Meet (March 28-April 1, Anaheim) 2004, Abst MEDI- 27.

4. Martin, K. (Metabasis Therapeutics, Inc.). *Lewis acid mediated synthesis of cyclic esters*. CA 2565966, EP 1753762, US 2005282782, WO 2005123729.

5. Erion, M.D., Reddy, K.R., Boyer, S.H. et al. *Design, synthesis, and characterization of a series of cytochrome P_{450} 3A-activated prodrugs (HepDirect prodrugs) useful for targeting phospho(on)-ate-based drugs to the liver*. J Am Chem Soc 2004, 126(16): 5154-63.

6. Prous Science Disease Briefings: *Hepatitis B* (online publication). Updated 2007.

7. Hepatitis B Foundation. *What is hepatitis B? Statistics*.

8. Lavanchy, D. *Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures*. J Viral Hepat 2004, 11(2): 97-107.

9. Ganem, D., Prince, A.M. *Hepatitis B virus infection - Natural history and clinical consequences*. New Engl J Med 2004, 350(11): 1118-29.

10. Lok, A.S. *New treatment of chronic hepatitis B*. Semin Liver Dis 2004, 24(Suppl. 1): 77-82.

11. Marcellin, P. *Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B*. New Engl J Med 2003, 348(9): 808-16.

12. Santantonio, T., Mazzola, M., Iacovazzi, T., Miglietta, A., Guastadisegni, A., Pastore, G. *Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine*. J Hepatol 2000, 32(2): 300-6.

13. Wong, D.K. et al. *Effect of alpha-interferon treatment in patients with hepatitis B e positive chronic hepatitis B. A meta analysis*. Ann Intern Med 1993, 119(4): 312-23.

14. Graul, A., Castañer, J. *BMS-200475*. Drugs Fut 1999, 24(11): 1173-7.

15. Sorbera, L.A., Castañer, J., Castañer, R.M., Bayés, M. *Telbivudine*. Drugs Fut 2003, 28(9): 870-9.

16. Balzarini, J., Hao, A., Herdewijn, P., Johns, D.G., De Clercq, R. *Intracellular metabolism and mechanism of antiretrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound*. Proc Natl Acad Sci USA 1991, 88(4): 1499-503.

17. Cundy, K.C., Shaw, J.P., Lee, W.A. *Oral, subcutaneous, and intramuscular bioavailabilities of the antiviral nucleotide analog 9-(2-phosphonylmethoxyethyl)adenine in cynomolgus monkeys*. Antimicrob Agents Chemother 1994, 38(2): 365-8.

18. Cundy, K.C., Barditch-Crovo, P., Walker, R.E., Collier, A.C., Ebeling, D., Toole, J., Jaffe, H.S. *Clinical pharmacokinetics of adefovir in human immunodeficiency virus type I-infected patients*. Antimicrob Agents Chemother 1995, 39(11): 2401-5.

19. Heathcote, E.J., Jeffers, L., Perrillo, R. et al. *Serum HBV DNA suppression and seroconversion following long-term ade-*

fovir dipivoxil therapy in chronic hepatitis B patients. Hepatology 2001, 34(4, Part 2): Abst 577.

20. http://www.fda.gov/ohrms/dockets/ac/02/briefing/3885B1_01_Gilead.pdf

21. Erion, M.D., van Poelje, P.D., MacKenna, D.A. et al. *Liver-targeted drug delivery using HepDirect prodrugs*. J Pharmacol Exp Ther 2005, 312(2): 554-60.

22. van Poelje, P.D., Colby, T.J., Fujitaki, J.M. et al. *MB6866 (Hepavir B), a HepDirect™ prodrug of adefovir: Mechanism of activation and liver targeting*. 54th Annu Meet Am Assoc Study Liver Dis (Oct 24-28, Boston) 2003, Abst 1143.

23. Reddy, K.R., Gomez-Galeno, J.E., Lemus, R.H., Colby, T.J., van Poelje, P.D., Erion, M.D. *MB06886 (Hepavir B), a HepDirect™ prodrug of adefovir: Mechanism of activation and liver targeting*. 227th ACS Natl Meet (March 28-April 1, Anaheim) 2004, Abst MEDI-28.

24. Reddy, K.R., Ugarkar, B.G., Matelich, M.C. et al. *Hepavir B (MB06866): A novel hepatitis B antiviral therapy using the HepDirect™ prodrug technology for targeting adefovir to the liver*. 227th ACS Natl Meet (March 28-April 1, Anaheim) 2004, MEDI-342.

25. Erion, M.D., Colby, T.J., Reddy, K.R. et al. *HepDirect™ prodrugs A novel strategy for targeting drugs to the liver*. 53rd Annu Meet Am Assoc Study Liver Dis (Nov 1-5, Boston) 2002, Abst 551.

26. Lin, C.-C., Fang, C., Benetton, S., Xu, G.-F., Yeh, L.-T. *Metabolic activation of pradefovir by CYP3A4 and its potential as an inhibitor or inducer*. Antimicrob Agents Chemother 2006, 50(9): 2926-31.

27. Srivastava, P., Li, C., Fang, C., Turk, R., Lin, C.-C. *Evaluation of potential of cytochrome P450-mediated drug interactions with Hepavir B and adefovir dipivoxil*. Drug Metab Rev 2003, 35(Suppl. 2): Abst 352.

28. Lin, C.-C., Fang, C., Benetton, S., Yeh, L.-T. *Pradefovir is a substrate, but neither an inhibitor nor an inducer for cytochrome P450*. 56th Annu Meet Am Assoc Study Liver Dis (Nov 11-15, San Francisco) 2005, Abst 811.

29. Lin, C.-C., Yeh, L.-T., Vitarella, D., Hong, Z., Erion, M.D. *Remofovir mesylate: A prodrug of PMEA with improved liver-targeting and safety in rats and monkeys*. Antiviral Chem Chemother 2004, 15(6): 307-16.

30. Lin, C.-C., Xu, C., Zhu, N., Lourenco, D., Yeh, L.-T. *Single-dose pharmacokinetics and metabolism of [^{14}C]remofovir in rats and cynomolgus monkeys*. Antimicrob Agents Chemother 2005, 49(3): 925-30.

31. Lin, C.-C., Xu, C., Teng, A., Yeh, L.-T., Peterson, J. *Pharmacokinetics of pradefovir and PMEA in healthy volunteers after oral dosing of pradefovir*. J Clin Pharmacol 2005, 45(11): 1250-8.

32. Xu, C.R., Teng, A., Lin, C.-C., Yeh, L.-T. *Absence of food effect on the extent of exposure of pradefovir in healthy adult volunteers*. 35th Annu Meet Am Coll Clin Pharmacol (ACCP) (Sept 17-19, Cambridge) 2006, Abst 60.

33. Fang, C., Lim, C., Srivastava, P., Lau, J.Y., Lin, C. *Liver-targeting prodrug of PMEA induces a much more favorable kidney and liver toxicological gene expression in rats compared to BisPOM-PMEA*. 42nd Annu Meet Soc Toxicol (March 9-13, Salt Lake City) 2003, Abst 1274.

34. Lau, D., Nguyen, T., Tong, M. et al. *Safety, tolerability, pharmacokinetics and pharmacodynamics of remofovir in chronic HBV patients in US and Canada following daily dosing for 28 days*. 40th Annu Meet Eur Assoc Study Liver (April 13-17, Paris) 2005, Abst 74.

35. Lin, C.-C., Chao, Y.-C., Lai, M.-Y. et al. *Safety, tolerance, pharmacokinetics and pharmacodynamics of remofovir, a liver-targeting prodrug of PMEA in HBV patients following daily dosing for 28 days*. Hepatology [55th Annu Meet Am Assoc Study Liver Dis (Oct 29-Nov 2, Boston) 2004] 2004, 40(4, Suppl. 1): Abst 1141.

36. Lin, C., Xu, C., Yeh, L., Sullivan-Bolyai, J., Xu, Y. *Pharmacokinetics and pharmacodynamics of pradefovir, a liver-targeting prodrug of PMEA in HBV patients*. 41st Annu Meet Eur Assoc Study Liver (April 26-30, Vienna) 2006, Abst 36.

37. Lee, K.S., Lim, S.G., Chuang, W.L. et al. *Safety, tolerability and antiviral activity of pradefovir mesylate in patients with chronic hepatitis B virus infection: 48-Week analysis of a phase 2 study*. 41st Annu Meet Eur Assoc Study Liver (April 26-30, Vienna) 2006, Abst 741.

38. Sullivan-Tolyai, J., Lim, S.G., Lee, K.W. et al. *Safety, tolerability, antiviral activity, and pharmacokinetics of pradefovir mesylate in patients with chronic hepatitis B virus infection: 24-Week interim analysis of a phase 2 study*. 56th Annu Meet Am Assoc Study Liver Dis (Nov 11-15, San Francisco) 2005, Abst LB07.

39. *Dose-ranging study of pradefovir patients with compensated hepatitis B (NCT00230503)*. ClinicalTrials.gov Web site, February 6, 2007.

40. *Open-label treatment extension study for patients who complete study RNA200103-201 (NCT00230490)*. ClinicalTrials.gov Web site, February 6, 2007.

Additional References

Lin, C.-C., Teng, A., Yeh, L.-T., Vitarella, D., Hong, Z., Erion, M. *Development of Hepavir B, a prodrug of PMEA with excellent liver-targeting properties*. J Hepatol 2004, 40(Suppl. 1): Abst 374.

Lin, C.-C., Yeh, L.-T., Xu, C., Liu, Y., Zhu, N. *Absorption, metabolism and excretion of [14C]Hepavir B and [^{14}C]Hepsera in rats and monkeys*. 54th Annu Meet Am Assoc Study Liver Dis (Oct 24-28, Boston) 2003, Abst 1193.