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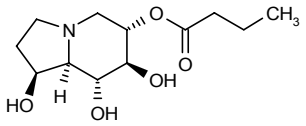
Celgosivir

Prop INN; USAN

α-Glucosidase Inhibitor
Anti-Hepatitis C Virus Drug

MX-3253
MBI-3253
MDL-28574

6-*O*-Butanoylcastanospermine
(1*S*,6*S*,7*S*,8*R*,8a*R*)-6-Butanoyloxyoctahydroindolizine-1,7,8-triol
Butanoic acid [1*S*-(1α,6β,7α,8β,8aβ)]-octahydro-1,7,8-trihydroxy-6-indolizinyl ester



$C_{12}H_{21}NO_5$
Mol wt: 259.2989
CAS: 121104-96-9
CAS: 141117-12-6 (as hydrochloride)
EN: 157972

Abstract

Despite advances in antiviral therapeutics, hepatitis C virus (HCV) infection continues to be a major worldwide health concern. In the search for newer agents with novel mechanisms of action, such as compounds which target virus-specific enzymes, inhibition of α-glucosidase I is considered an attractive anti-HCV strategy since this enzyme is involved in the biosynthesis of glycoproteins that, when expressed on the viral surface, are essential for virus-host interactions. The naturally occurring iminosugar castanospermine is an α-glucosidase I inhibitor with marked antiviral activity against a number of viruses. Unfortunately, the agent also inhibits intestinal sucrases and causes osmotic diarrhea. In contrast, celgosivir, the 6-*O*-butanoyl derivative of castanospermine, is a relatively inactive inhibitor of intestinal sucrase and appears to be nontoxic to the gastrointestinal tract. It possesses antiviral activity that is 30-fold greater than the parent compound, its active metabolite. Celgosivir has displayed potent antiviral activity *in vitro* and *in vivo* against several viruses, including HIV-1, herpes simplex virus (HSV), bovine viral diarrhea virus (BVDV) and HCV, and the agent was chosen for further development as a treatment for HCV infection. The antiviral efficacy and safety of celgosivir were demonstrated in clinical trials in HIV-1-infected patients and it is currently undergoing phase II development for the treatment of HCV infection.

Synthesis

Celgosivir can be prepared by several related ways:

1) Reaction of castanospermine (I) with butyryl chloride (II) in pyridine at 0 ºC, followed by TLC chromatography purification (1). Scheme 1.

2) Reaction of castanospermine (I) with dibutyltin oxide in refluxing methanol, followed by *in situ* treatment with butyryl chloride (II) and triethylamine (2). Scheme 1.

3) Acylation of castanospermine (I) with benzoyl chloride and pyridine at 0 ºC gives the 6,7-di-*O*-benzoyl derivative (III), which is treated with 2-methoxypropene (IV) and a catalytic amount of TsOH in DME to yield 6,7-di-*O*-benzoyl-1,8-*O*-isopropylidenecastanospermine (V). Removal of the benzoyl-protecting groups of (V) by means of sodium methoxide in methanol affords the free acetonide (VI), which is selectively monoacylated with butyryl chloride (II) in THF to provide the 6-*O*-butyryl acetonide (VII). Finally, this compound is treated with HCl in ethanol (3). Scheme 2.

4) Condensation of castanospermine (I) with 2,2,2-trichloroethyl butyrate (VIII) in pyridine catalyzed by crude porcine pancreatic lipase to give a mixture of the 6-*O*- and 7-*O*-butyryl derivatives that are separated by radial silica gel chromatography (4). Scheme 1.

5) Reaction of castanospermine (I) with bis(tributyltin)oxide in refluxing xylene to give a stannyl complex, which, without isolation, is acylated with butyryl chloride (II) at –17 ºC and finally treated with anhydrous HCl in ethanol (5, 6). Scheme 1.

Introduction

Hepatitis C virus (HCV) infection continues to be a major worldwide health concern, with approximately 170 million people chronically infected with HCV and about 3-4 million individuals newly infected every year. HCV is predominantly responsible for most cases of acute hepatitis

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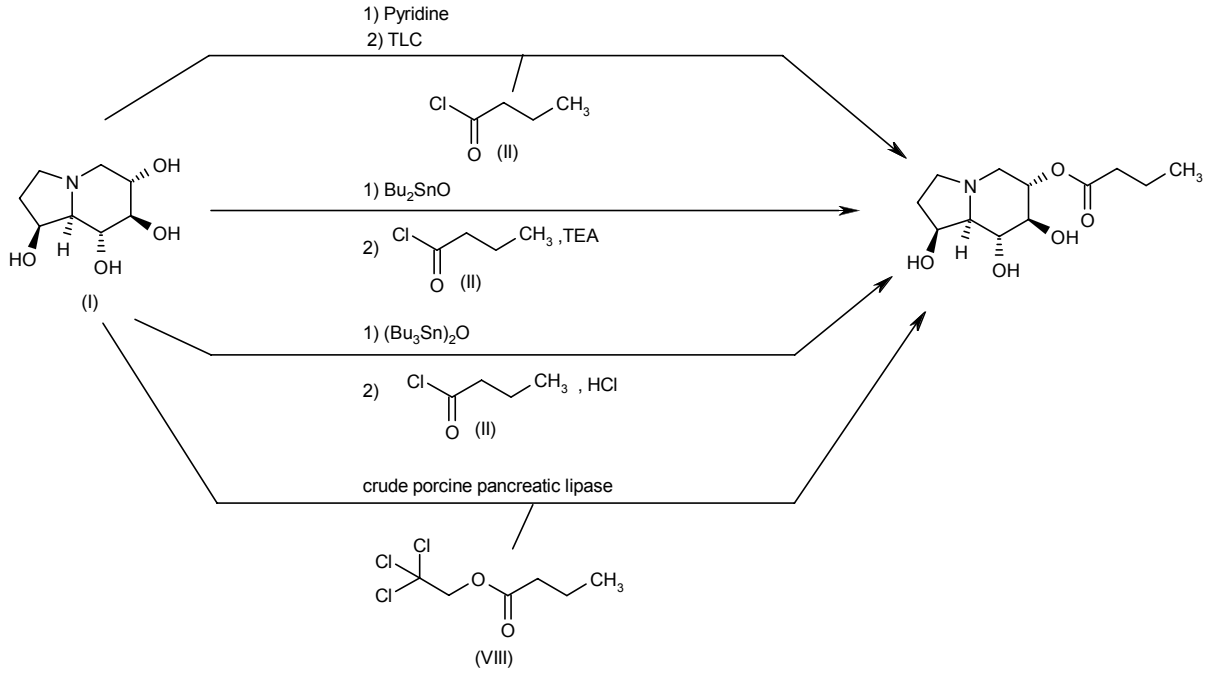
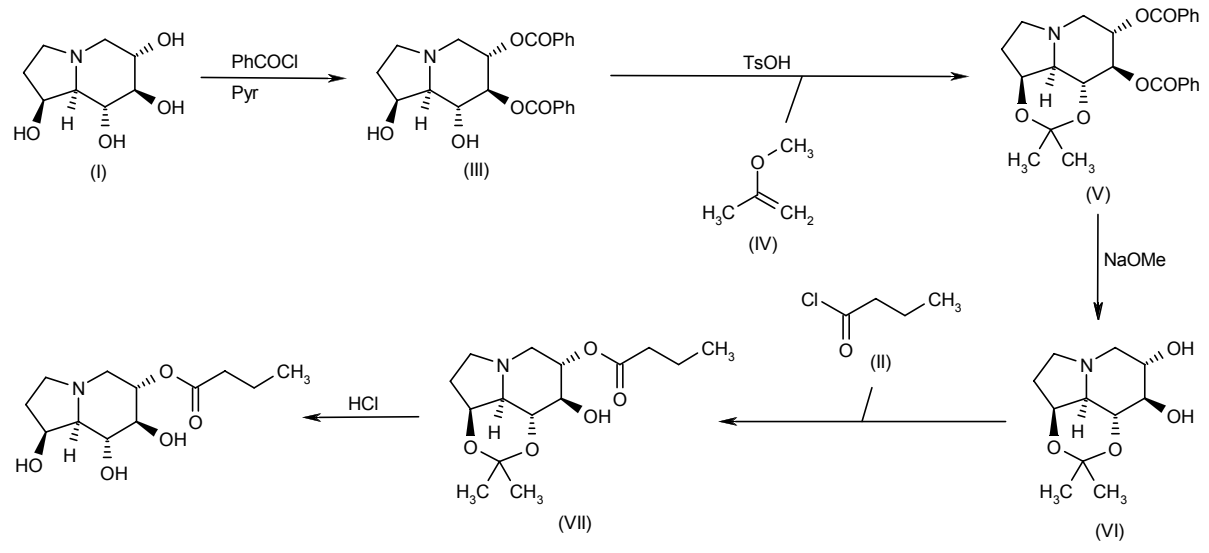
Scheme 1: Synthesis of Celgosivir



Scheme 2: Synthesis of Celgosivir



and chronic liver disease (*e.g.*, cirrhosis and liver cancer). Approximately 80% of all newly infected individuals develop chronic HCV infection. Of those individuals chronically infected, 10-20% and 1-5% develop cirrhosis and liver cancer, respectively. Despite advances in antiviral therapeutics, including interferons, interleukins, immunomodulators and therapeutic vaccines, which has enabled the rapid clearance of HCV in the majority of patients, these agents are not always effective in curing the disease and can be associated with numerous side effects. New

treatment options are therefore being developed, including viral enzyme inhibitors which target virus-specific enzymes such as proteases, helicases and polymerases. However, efforts to identify and screen novel effective enzyme inhibitors have been difficult, since HCV does not appear to replicate efficiently or reinfect cultured cells. Researchers therefore use other members of the Flaviviridae family that do easily replicate *in vitro*, and several anti-HCV enzyme inhibitors have been identified and are currently under active development, as shown in Table I.

Naturally occurring iminosugars, at submicromolar concentrations, have been shown to inhibit endoplasmic reticulum (ER) α-glucosidase I, which is involved in the biosynthesis of glycoproteins. Glycoproteins interact with calnexin or calrectulin (CNX/CRT) in the ER, an interaction which is essential for their proper folding, export from the ER and function. This process of CNX/CRT-mediated folding is crucial for replication in various enveloped viruses, such as HIV-1 and herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), since the glycoproteins expressed on the surfaces of these viruses are critical for virus-host interactions. α-Glucosidase I inhibitors such as castanospermine and deoxynojirimycin (DNJ) have been shown to inhibit this process, with detrimental effects to the virus. Thus, α-glucosidase inhibitors may be effective against HCV infection (7-14).

Castanospermine is a naturally occurring, water-soluble alkaloid iminosugar derived from the Moreton Bay chestnut tree (*Castanospermum australe*) which has shown particular promise as an antiviral agent. Unfortunately, castanospermine, like DNJ, is associated with osmotic diarrhea since it also inhibits intestinal sucrases. In contrast, celgosivir (MX-3253, formerly MBI-3253 and MDL-28574), the 6-*O*-butanoyl derivative of castanospermine, has increased lipophilicity and is a relatively inactive inhibitor of intestinal sucrase, and the release of castanospermine, its active metabolite, via gut esterases occurs slowly. Celgosivir appears to be nontoxic to the gastrointestinal tract and possesses antiviral activity 30-fold greater than the parent compound. Celgosivir has displayed potent antiviral activity *in vitro* and *in vivo* against several viruses, including HIV-1, HSV-1 and HSV-2 and bovine viral diarrhea virus (BVDV) (12, 15, 16). The agent was therefore chosen for further development as an antiviral and displayed efficacy and safety in studies conducted in HIV-1-infected patients (see below). Clinical trials are now in progress assessing its potential as an anti-HCV therapy (17).

Pharmacological Actions

Experiments using JM and H9 cells infected with an HIV-1 isolate (GB8) showed that celgosivir potently inhibited syncytium formation (IC_{50} = 0.89-1.1 μM), with activity 30-fold greater than that of castanospermine (IC_{50} = 39 μM). However, it was less effective than the parent compound in inhibiting purified α-glucosidase I (IC_{50} = 1.27 and 0.12 μM, respectively), but it was significantly more potent in causing accumulation of glucosylated oligosaccharides in HIV-infected cells, which was attributed to enhanced cellular uptake due to to its greater lipophilicity. Celgosivir was shown to inhibit cell adhesion and cell-to-cell spread of HIV-1. Pretreatment of HIV-permissive $CD4^+$ cells with the agent markedly decreased their ability to bind to chronically HIV-1-infected H9 cells, ultimately reducing virus production. The suppressive effects of celgosivir on cell adhesion and syncytium formation may be due to its ability to inhibit the cell-surface marker LFA-1 (CD18/CD11a), which was demonstrated both *in vitro* in JM cells and *in vivo* in human and murine cells following oral administration to xenochimeric (hu-PBL-SCID/beige) or normal mice; the agent had no significant effects on the mitogenic response of uninfected human mononuclear leukocytes or other cell-surface markers on JM cells, such as CD4, CD8, CD2, CD25, CD45, CD3, HLA-DR or CD54 (12, 18-20).

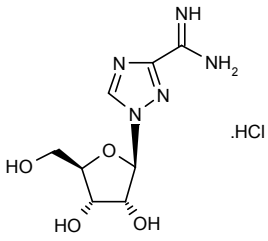
Celgosivir was shown to act synergistically when combined with the non-nucleoside reverse transcriptase inhibitor (NNRTI) MKC-442 in cell viability experiments using HIV-1-infected T-cell lines (C8166, MT-4 and JM). Marked synergistic effects were also observed when the nucleoside analogue zidovudine (AZT) was added to the 2-drug combination (21). Synergistic activity was also observed in cell viability assays using HIV-1_{RF}-infected MT-4 cells and in p24 reduction assays using HIV-1_{RF}-infected H9 cells when celgosivir was combined with AZT, didanosine (ddI), zalcitabine (ddC), nevirapine or saquinavir. Synergism was even more marked with triple-drug combinations. Combination indices for celgosivir ranged from 0.35 to 0.44 when the agent was combined with AZT and nevirapine, and from 0.34 to 0.67 when combined with AZT and saquinavir. No adverse effects on cell division were observed with any of the drug combinations (22).

The antiviral efficacy of celgosivir against HSV-1 was demonstrated in *in vivo* experiments using a murine zosteriform rash model. Treatment of HSV-1-infected mice with the agent (200 mg/kg p.o. starting 3 days prior to or at the time of infection) resulted in significant delays in lesion development and a decrease in brain viral load. The agent was rapidly taken up by brain tissue and continued administration resulted in maintenance of high brain levels of the compound. Pretreatment was more effective than treatment starting at the time of infection (approximate 100-fold *vs*. 10-fold decreases in brain viral load, respectively) (23).

Several *in vitro* experiments using Madin-Darby bovine kidney cells (MDBK) infected with the HCV surrogate virus BVDV revealed the potential efficacy of celgosivir as a treatment for HCV infection. Treatment with celgosivir or castanospermine inhibited the number of plaques released from infected cells. Celgosivir was more potent than castanospermine, *N*-nonyl-DNJ and *N*-butyl-DNJ in both plaque (IC_{50} = 16 μM *vs.* 110, 105 and > 250 μM, respectively) and cytopathic (IC_{50} = 47 μM vs. 367, 74 and 550 μM, respectively) assays. Celgosivir exhibited low host toxicity (CC_{50} > 1000 μM); toxicity was only observed with *N*-nonyl-DNJ. Synergistic antiviral effects were observed when celgosivir was combined with interferon alfa or ribavirin, indicating that it may potentiate the

Table I: Anti-HCV enzyme inhibitors undergoing active development (from Prous Science Integrity)®.

Drug	Mechanism of action	Source	Phase
1. Viramidine hydrochloride	IMP dehydrogenase inhibitor	Valeant	Phase III
2. Celgosivir	α-Glucosidase inhibitor	Migenix	Phase II
3. Merimepodib	IMP dehydrogenase inhibitor	Vertex	Phase II
4. IDN-6556	Caspase inhibitor	Idun Pharmaceuticals (Pfizer)	Phase II
5. JTK-003*	RNA-directed RNA polymerase (NS5B) inhibitor	Japan Tobacco	Phase II
6. Ciluprevir	HCV NS3 protease inhibitor	Boehringer Ingelheim	Phase II
7. Valopicitabine	RNA-directed RNA polymerase (NS5B) inhibitor	Idenix	Phase II
8. R-1626*	Polymerase inhibitor	Roche	Phase I
9. HCV-796*	RNA-directed RNA polymerase (NS5B) inhibitor	ViroPharma; Wyeth	Phase I
10. VX-950	HCV NS3 protease inhibitor	Vertex	Phase I
11. T-1106	RNA-directed RNA polymerase (NS5B) inhibitor	Toyama	Preclinical
12. BCL-2125/XTL-2125*	RNA-directed RNA polymerase (NS5B) inhibitor	B&C Biopharm; XTL Biopharmaceuticals	Preclinical
13. BCL-2329/XTL-2329*	RNA-directed RNA polymerase (NS5B) inhibitor	B&C Biopharm; XTL Biopharmaceuticals	Preclinical



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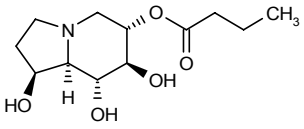

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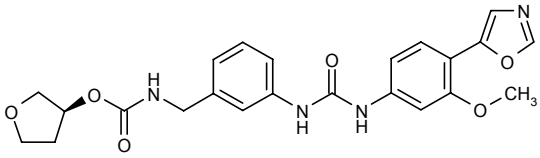

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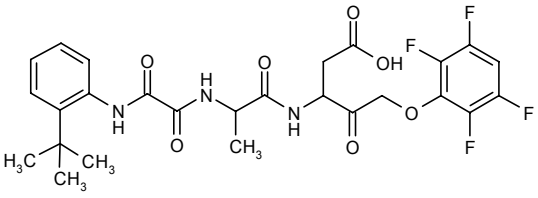

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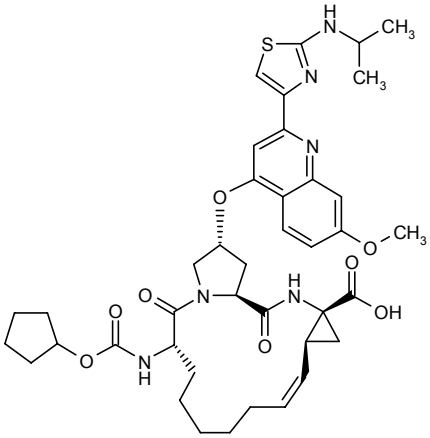

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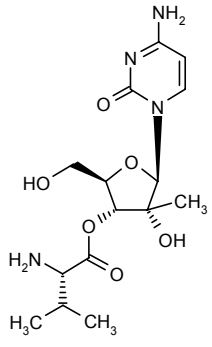

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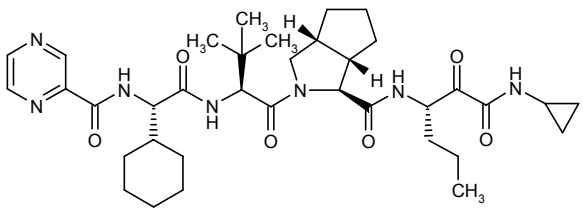

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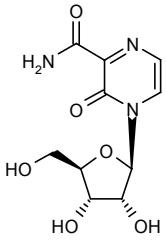

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*Chemical structure not yet detected.

effects of interferon alfa or ribavirin in the treatment of HCV infections (24, 25).

Comparable results were obtained in a similar study where EC_{50} values for celgosivir and castanospermine for inhibiting virus release in a single-cycle assay (multiplicity of infection [MOI] approximately 1) were 2 and 19.4 μM, respectively. EC_{50} values for blocking the cytopathic effects of the virus in multiple-cycle assays (MOI = 0.001, 0.01 and 0.1) were 5.3, 7.1 and 14.9 μM for celgosivir, respectively, and 56.5, 61.5 and 177 μM for castanospermine, respectively. Regrowth times (time for 1 x $\log_{10}$ regrowth) following pretreatment for 24 h with 11, 33 and 100 μM celgosivir were 9, 5 and 8 h posttreatment, respectively, as compared to 12, 10 and 18 h, respectively, for ribavirin and 4 h for untreated infected cells. These results suggest that while ribavirin targets early viral replication, celgosivir targets late replication. Minimal cytotoxicity (CC_{50} > 1000 μM) was observed for both celgosivir and castanospermine against noninfected human hepatocytes. It was proposed that celgosivir, via inhibition of proper glycosylation of E1, E2 and envelope glycoproteins, hinders normal viral assembly and targets a late stage of BVDV replication (26).

Metabolism and Pharmacokinetics

The uptake and metabolism of [^{14}C]-labeled celgosivir and castanospermine were examined *in vitro* using JM-1 and B16F10 cells and *in vivo* in mice. Celgosivir was more readily taken up by cells as compared to castanospermine, with an approximately 30-50-fold higher amount of radioactivity detected in cells treated with the former as compared to the latter agent. Celgosivir was rapidly converted to the parent compound once taken up by cells. Although plasma levels of castanospermine were 5-10-fold higher in mice orally administered celgosivir (25 mg/kg) as compared to castanospermine-treated (16 mg/kg) animals, levels were equivalent in mice treated with either agent i.v. This suggests that celgosivir is rapidly converted to castanospermine in blood. Repeated dosing with celgosivir resulted in higher levels of the compound in blood and tissues (27).

The pharmacokinetics of celgosivir and castanospermine were examined in rats (10 mg/kg i.v.; 25 mg/kg p.o.) and dogs (3 mg/kg i.v.; 10 mg/kg p.o.). Celgosivir was barely detected in plasma following administration, suggesting rapid and extensive metabolism to castanospermine. Following oral administration, at least 94% of the celgosivir dose was absorbed and excreted in urine. The majority of radioactivity was detected in urine within the 24 h after oral or i.v. dosing and more than 92% was identified as castanospermine. Systemic availability of castanospermine was significantly enhanced with oral celgosivir dosing since the C_{max} and AUC values of castanospermine in rats treated with celgosivir were 6 and 2 times higher, respectively, as compared to values obtained following oral castanospermine (16 mg/kg) (28).

The effects of the antidiarrheal agent loperamide (35 mg/kg p.o.) on the pharmacokinetics of celgosivir (35 mg/kg p.o.) were examined in both normal rats and rats with castor oil-induced diarrhea. C_{max}, t_{max} and AUC values for castanospermine obtained in celgosivir-treated normal rats (8.8 ± 1.15 μg/ml, 0.44 ± 0.01 h and 10.5 μg.h/ml, respectively) were similar to values obtained for rats pretreated with loperamide (6.3 ± 2.33 μg/ml, 0.47 ± 0.05 h and 9.5 μg.h/ml, respectively), indicating that loperamide had no significant effect on the pharmacokinetics of celgosivir. C_{max} and AUC values for castanospermine in diarrhea-induced rats were decreased by 54% and 44%, respectively, as compared to normal rats. These results suggest that concomitant administration of loperamide with celgosivir to reduce the possible gastrointestinal effects of the latter may be an effective and safe therapy and may prevent the reduction in systemic exposure seen in patients with diarrhea (29) (see Table II).

Table II: Pharmacokinetics of oral celgosivir in rats and in HIV-positive patients administered once-daily oral doses for 14 days (from Prous Science Integrity®).

Dose	AUC (ng.h/ml)	C_{max} (ng/ml)	t_{max} (h)	C_{min} (ng/ml)	$t_{1/2}$ (h)
Rats					
35 mg/kg	10,500	8760	0.44		
35 mg/kg *fasted*	7200	5280	0.32		
35 mg/kg *diarrhea*	5900	4030	0.44		
35 mg/kg + loperamide	9500	6300	0.47		
HIV-positive patients					
10 mg	1000	266		8	13
20 mg	1750	520		11	27
40 mg	3610	1070		23	30
80 mg	6530	1720		29	16
160 mg	13,300	3730		70	21
240 mg	21,500	5760		110	16
360 mg	32,100	7310		179	15
450 mg	39,800	10,500		191	14

AUC, area under the concentration-time curve; C_{max}, peak plasma concentration; t_{max}, time to peak plasma concentration; C_{min}, trough plasma concentration; $t_{1/2}$, elimination half-life. Data from Refs. 24 and 25.

Table III: Clinical studies of celgosivir (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
HIV infection		Celgosivir [oral solution], p.o. o.d. x 14 d Celgosivir [capsules], p.o. b.i.d. x 14 d Celgosivir, 300 mg p.o. b.i.d. x 8 wks	75	The maximum tolerated dose (MTD) of celgosivir oral solution was 400 mg once daily in HIV-infected patients; similar tolerability was found with twice-daily doses of 240 and 300 mg. Grade 3 toxicity led to early termination in patients given the capsule formulation	26
HIV infection	Randomized Double-blind Multicenter Pooled/meta-analysis	Celgosivir, 50 mg o.d. x 12 wks→ Celgosivir, 50 mg o.d. + Zidovudine, 200 mg t.i.d. x 12 wks Celgosivir, 150 mg o.d. x 12 wks → Celgosivir, 150 mg o.d. + Zidovudine, 200 mg t.i.d. x 12 wks Celgosivir, 300 mg o.d. x 12 wks → Celgosivir, 300 mg o.d. + Zidovudine, 200 mg t.i.d. x 12 wks Celgosivir, 400 mg o.d. x 12 wks → Celgosivir, 400 mg o.d. + Zidovudine, 200 mg t.i.d. x 12 wks Placebo x 12 wks →Zidovudine, 200 mg t.i.d. x 12 wks% Zidovudine, 200 mg t.i.d. x 24 wks	400	Celgosivir alone or combined with zidovudine was safe and induced no serious adverse events in HIV-infected patients with baseline $CD4^+$ T-cell counts of 100-500 cells/μl	27-29

The pharmacokinetics of single (10-160 mg p.o.) and multiple (10-450 mg/day p.o. for 14 days) doses of celgosivir (oral solution) were examined in a phase I trial in asymptomatic HIV-positive patients. Following single doses, castanospermine was rapidly detected in plasma, with peak concentrations achieved at 1-2 h postdosing. Both single-dose and steady-state castanospermine pharmacokinetics were linear. Increases in C_{max} and AUC were proportional over the dose range studied and the terminal $t_{1/2}$ after single doses of 80 and 160 mg/kg was 18 h. Increases in C_{min}, C_{max} and AUC values at steady state following multiple doses were generally dose-proportional and steady-state apparent oral clearance and $t_{1/2}$ values were constant across the dose range (30) (see Table II).

Clinical Studies

The safety and tolerability of celgosivir (oral solution once or twice daily for 14 days; capsules 300 mg b.i.d. for 8 weeks) were reported from a phase I study involving a total of 75 asymptomatic HIV-positive patients ($CD4^+$ cell counts = 399/mm^3) who had not received antiviral therapy for the preceding 30 days. No clinically significant changes in laboratory parameters were observed. Adverse effects were predominantly gastrointestinal and included grade 2 loose stools, diarrhea and flatulence during the first week. These adverse effects were grade 3 and recurrent at oral solution doses of 450 mg/day and 240 and 300 mg b.i.d. It was concluded that the maximum tolerated dose (MTD) for the oral solution was 400 mg/day and an increase in daily intake of the solution may be tolerable up to 300 mg b.i.d. Treatment with the capsule formulation administered to a total of 14 patients was terminated early at 14 weeks due to recurrent grade 3 elevations in transaminases, creatinine kinase and lactate dehydrogenase (LDH). All toxicities were reversible following discontinuation. It was suggested that the poor tolerability of the capsule formulation may be related to bioavailability (31). The results from this and the following study are illustrated in Table III.

The efficacy and safety of celgosivir (50, 150, 300 or 400 mg p.o. once daily for 24 weeks) were examined in a multicenter, randomized, double-blind, placebo-controlled phase II trial in approximately 400 HIV-positive, asymptomatic or mildly symptomatic patients with baseline $CD4^+$ cell counts of 100-500 cells/μl. Following 12 weeks of celgosivir or placebo monotherapy, patients entered a combination treatment period where they were continued for another 12 weeks on blinded celgosivir or placebo and administered open-label AZT (200 mg t.i.d.); AZT-treated patients were continued on monotherapy for another 12 weeks. Celgosivir was well tolerated and not associated with serious adverse events. Adverse events were infrequent and the most common were generally mild to moderate flatulence and diarrhea. The discontinuation rates for patients with $CD4^+$ cell counts of 301-500 and 100-300 cells/μl at entry were 14% and 19%, respectively, due to voluntary withdrawal, noncompliance and gastrointestinal adverse events. Efficacy endpoints were plasma HIV-1 RNA levels, $CD4^+$ cell counts, clinical assessment and plasma p24 antigen. Good antiviral efficacy was observed in approximately one-third of the patients. Stable results were found in another one-third of the population while poor efficacy was seen in the remaining one-third (32-34).

Celgosivir is currently undergoing phase IIa development to examine its safety and efficacy as a treatment for chronic HCV infection. A phase IIb study is planned to

determine the efficacy of celgosivir in combination with the current gold standard (17).

Source

Migenix, Inc. (CA, US); acquired from Virogen, Ltd. (celgosivir was synthesized and originally studied at the former Marion Merrell Dow Research Institute Laboratories as an anti-HIV agent.)

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