

INH-I001, a HIV Integrase Inhibitor with Potent In Vitro Anti-HIV Activity: Microsome and Cytosol Stability Studies, Cytochrome P450 Data and Pharmacokinetics



University of Georgia Center for Drug Discovery
 Room 320 R.C. Wilson Pharmacy Building
 Athens, GA 30602
 Phone: (706) 542-8293
 Fax: (706) 583-8283
 E-mail: vnair@rx.uga.edu

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Vasu Nair^{*1}, Xiaohui Ma¹, Catherine White¹, Shawn Blue¹, Guochen Chi¹, Joseph Patti²

¹Department of Pharmaceutical and Biomedical Sciences and the Center for Drug Discovery, University of Georgia, Athens, GA, USA; and ²Inhibitex, Inc., Alpharetta, GA, USA

Introduction

Enzymes of the *pol* gene of HIV have been identified as important viral targets for the discovery of anti-HIV therapeutic agents. While the viral enzymes, HIV reverse transcriptase and HIV protease, have been successfully targeted with a number of efficacious therapeutic agents, research efforts on drug discovery on the third enzyme of the *pol* gene, HIV integrase, have only recently produced favorable Phase III clinical efficacy and a FDA approved drug. Because the issues of toxicity and resistance are commonly encountered problems for all types of anti-HIV drugs, the discovery of new and different classes of integrase inhibitors remains a significant scientific challenge. This presentation will focus on the anti-HIV activity, metabolic stability, cytochrome P450 data and pharmacokinetics of a small molecule inhibitor of HIV-1 integrase, INH-I001/VN-III-083.

Methods

Anti-HIV Efficacy Evaluation in Human PBMCs.

INH-I001 and the control compound, AZT, were tested in a PBMC cell-based, microtiter anti-HIV assay against the clinical isolate, HIV-1₂₀₀ (NSI phenotype) and HIV-1₁₋₁₀₀ (SI phenotype). Parallel drug cytotoxicity studies (without virus) used an MTS (Promega) assay system. Following infection, the PBMC cultures were maintained for 7 days at 37 °C, 5% CO₂. After this period, cell-free supernatant samples were collected for analysis of RT activity and cells were stained with MTS to determine compound cytotoxicity (see *J. Med. Chem.* 2006, 49, 445-447).

Metabolism in Pooled Human Liver Fractions

Incubations contained 1.0 mg/ml protein in a final volume of 500 µl. The reaction solution (100 µl) was terminated with 100µl of ice-cold acetonitrile (ACN) at 0, 30, 60, 120, 180 min. Precipitated proteins were removed by centrifugation at 5,000g for 5 min at room temperature before analysis using HPLC detection of the compound and its metabolite(s). Cytochrome P450 inhibition studies were carried out as described in the literature (*Xenobiotica* 1998, 28, 1203-1253; *Pharmacol. Rep.* 2006, 58, 453-472; *Drug Metab. Dispos.* 1997, 25, 1130-1136).

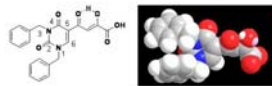
Caco-2 Permeability Studies

Caco-2 cells (clone HT837 from ATCC) were grown with DMEM supplemented with 10% FBS, 1% NEAA, 1% L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin at 5% CO₂, 37°C. After 80-90% confluence, Caco-2 cells, passage 40-50, were seeded on Transwell insert at a density of 6.5x10⁴ cells/cm² and cultured for 19-23 days. Apical to basolateral permeability was assessed in HBSS. Integrity of the monolayer was checked prior to and after the experiment by Lucifer yellow. Samples and controls were analyzed by reversed-phase HPLC.

Pharmacokinetic Studies in Rats

Oral doses of 5mg/kg, 10mg/kg and 12.5mg/kg were utilized in the studies. Serial blood samples (300 µl) were obtained for 8 hours. Blood was centrifuged for 5 minutes at 14,000 rpm to separate the plasma, which was stored at -20°C prior to analysis. Drug concentrations were determined by HPLC with UV detection. Pharmacokinetic parameters were determined with WINNONLIN using compartmental and non-compartmental methods.

Major Tautomeric Form of Integrase Inhibitor, INH-I001 (VN-III-083), and the Conformation of its Anion



C₂₂H₂₄N₄O₆ Molecular Weight: 406.39

Pale Yellow Crystalline Compound, Mp. 196-198 °C, UV (CH₂OH): λmax 338 nm (ε: 17,300).

HIV Integrase Inhibition

Strong inhibitor of both the 3'-Processing and Strand Transfer Steps of HIV-1 Integrase

(Nair et al., *J. Med. Chem.* 2006, 49, 445-447; *Antiviral Res.* 2006, 70, A26; *Rev. Med. Virol.* 2007, 17, 277-295)

Molecular Level Representation of the Interaction of Inhibitor, INH-I001, with Integrase-Viral DNA Complex



Viral DNA (green), integrase (purple), Mg²⁺ ions (magenta spheres), catalytic triad, Asp64, Asp116 and Glu152 (yellow). The De Luca's model of the integrase-LTR complex was used for docking. The compound binds to integrase by coordinating the metal ions present in the catalytic core. The docked position is also stabilized by hydrogen bond between Asp64 and the enolate hydroxyl (Cox and Nair, *Antiviral Chem. & Chemother.* 2006, 17, 343-355).

Results

Antiviral Data of INH-I001 and the Positive Control, AZT, in Inhibition of HIV-1 Replication in PBMC

Compound	High Conc.	HIV-1 Isolate	EC ₅₀	CC ₅₀	Therapeutic Index (TI)
INH-I001	200 µM	TEKI	50 nM	>200 µM	>4,000
INH-I001	200 µM	NL4-3	<20 nM	>200 µM	>10,000
AZT	1 µM	TEKI	0.14 nM	>1 µM	>7,143
AZT	1 µM	NL4-3	0.18 nM	>1 µM	>5,556

EC₅₀ Conc. of drug required to inhibit virus-induced cytopathicity by 50%.

CC₅₀ Conc. of drug required to reduce cell viability by 50% (cytotoxicity).

Stability of INH-I001 in Pooled Human Liver Fractions*

Time (min)	% Remaining ^a	
	in Cytosol ^b	in Microsome ^c
	40 µM ^b	40 µM ^c
60	82.0	61.5
120	69.6	45.1
180	61.8	33.3
240	53.3	27.2

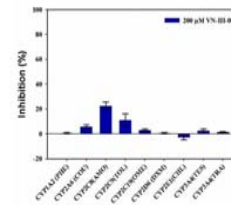
*One cleavage metabolite A was produced slowly in human liver microsome. In cytosol, little reduction to metabolite B occurred without the addition of high (mM) concentrations of NADH or NADPH.

^a % Remaining = (substrate conc. at time t / initial substrate conc.) × 100

^b Initial compound concentration.

Human pooled liver microsome half-life at 100 µM ~ 3 h

Cytochrome P450 Studies on INH-I001



Oxidation of INH-I001 by CYP450 enzymes was not detected.

Cytochrome P450 Inhibition Data

CYP450	Inhibition (%)
CYP1A2 (Phenacetin O-deethylation)	0.80 (SD=0.80)
CYP2A6 (Coumestrol 7-hydroxylation)	2.70 (SD=0.80)
CYP2C8 (Amisulaprine N-deethylation)	22.80 (SD=1.14)
CYP2C9 (Tubalansin 4-methylhydroxylation)	11.87 (SD=0.30)
CYP2C19 (Omeprazole 5-hydroxylation)	2.10 (SD=0.30)
CYP2D6 (Dextromethorphan O-demethylation)	0.80 (SD=0.80)
CYP2E1 (Chlorzoxazone 6-hydroxylation)	2.70 (SD=0.30)
CYP3A4 (Testosterone 6β-hydroxylation)	2.47 (SD=0.30)
CYP3A4 (Triazolam 6-hydroxylation)	1.40 (SD=0.30)

For CYP 2C8 and 2C9, IC₅₀ values were >400 µM.

Caco-2 Cell Permeability Data of INH-I001 Compared to Clinically Known Compounds

Compd.	MW	P _{app} (cm ² /s, ×10 ³)	%F ₂ ¹
INH-I001 (VN-III-083)	406	16.821.7*	
Ketoprofen	254	47.922.7*	100
Carbamazepine	236	36.823.1*	100
Metoprolol	267	19.621.7 ²	95
Furosemide	331	0.850.2*	61
Atenolol	266	2.650.8 ¹	50
Sulfasalazine	398	0.750.2*	12-13

¹ % F₂ is the percent absorbed in humans; and the data are from the literature.

² % F₂ of ketoprofen is from *J. Pharm. Sci.* 2001, 90(6), 749-754

* P_{app} for other compounds are from *J. Med. Chem.* 2001, 44, 923-930

¹ P_{app} = 0.8374 × 10³ cm²/s; ² P_{app} = 1.97 × 10³ cm²/s

Summary of Oral Dosing Data for INH-I001 from Rat PK Studies*

Pharmacokinetic Parameters	Oral Dose 1 mg/kg	Oral Dose 10 mg/kg	Oral Dose 12.5 mg/kg
F (Bioavailability)	84%	58%	80%
V _d (F) (L/kg)	1.84 (SD=0.24)	1.54 (SD=0.14)	1.48 (SD=0.38)
CL/F (ml/min-kg)	4.83 (SD=0.56)	4.13 (SD=0.21)	4.38 (SD=0.30)
T _{1/2α} (min)	90	90	90
C _{max} (mg/l)	2.73 (SD=0.32)	6.17 (SD=0.32)	7.83 (SD=1.25)
T _{1/2β} (min)	2.83 (SD=0.37)	4.27 (SD=0.17)	3.79 (SD=0.68)

*Values indicated are the mean ± SD. F = bioavailability; V_d(F) = adjusted volume of distribution at steady state; CL = clearance rate; T_{1/2α} = half-life.

Conclusions

The potent *in vitro* anti-HIV activity profile of INH-I001/VN-III-083, together with its low toxicity, microsome stability, P450 data, and favorable pharmacokinetics, suggest that it has potential for further preclinical studies and development as an anti-HIV agent. (Research Support: NIH, Georgia Research Alliance, Terry Endowment, Inhibitex, Inc.).