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Discovery of GS-9350: A Novel Pharmacoenhancer without Anti-HIV Activity

L Xu,[1] H Liu,[1] BP Murray,[2] C Callebaut,[3] A Hon,[1] R Vivian,[1] MS Lee,[2] L Tsai,[3] K Stray,[3] G Eisenberg,[2] J Chau,[2] R Strickley,[4] J Koziara,[4] Y Choi,[1] C Cannizzaro,[5] S Swaminathan,[5] GR Rhodes,[2] and MC Desai[1]

Department of [1]Medicinal Chemistry; [2]Drug Metabolism; [3]Biology; [4]Formulation and [5]Structural Chemistry, Gilead Sciences, Inc., Foster City, CA, USA

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: (650) 522-5828
Fax: (650) 522-5899

Introduction

- Ritonavir (RTV), an HIV protease inhibitor (PI), is also a potent mechanism-based inhibitor of human CYP3A. It is now mainly used as a pharmacoenhancer to improve pharmacokinetics of coadministered HIV PIs, which are primarily metabolized by CYP3A
- Coadministeration of low dose ritonavir with HIV PIs has reduced pill burden and simplified regimens, and has served as a cornerstone of PI-based regimens
  - Ritonavir has been used for over 10 years in HIV-infected patients
- Elvitegravir (EVG, GS-9137), an integrase inhibitor, can be dosed once-daily when boosted with ritonavir
- Ritonavir has limitations when used as a CYP3A inhibitor
  - Potent anti-HIV activity, may cause emergence of resistance when used at a low/subtherapeutic dose
  - Poor aqueous solubility results in inconvenient dose form
  - Requirement for refrigeration and challenging for co-formulation
  - Associated with lipid disorders and GI-side effects
  - Induction liability for off-target drug interactions (CYP, Pgp, UGT)
- CYP inhibitors that can overcome these limitations were designed
- The discovery, structure-activity relationships (SAR), pharmacokinetic profile, and synthesis of a novel series of CYP3A inhibitors are presented in this poster

Methods

HIV protease enzyme inhibition and cell inhibition assay

- Inhibition of HIV protease was evaluated using synthetic fluorescent substrates previously described. Standard viability assays were used to determine antiretroviral activity in MT-2 cells infected for 5 days with HIV-1 IIIB

CYP inhibition Assays

- IC_{50} values for inhibition of human hepatic microsomal CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A activities were generated using industry and FDA recommended methods. CYP3A inhibition data described were generated using midazolam as the probe substrate
- CYP3A inactivation kinetic parameters were generated using a two-step protocol with pooled human hepatic microsomal fractions and midazolam as the probe substrate

PXR Activation Assay

- Human PXR activation was determined by transactivation analysis in cell lines containing a construct with the CYP3A4 promoter fused to a firefly luciferase reporter gene

Pharmacokinetics

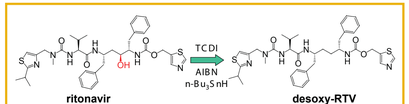
- GS-9350 or RTV were dosed orally as solutions to Sprague Dawley rats and intact or portal vein cannulated beagle dogs. Plasma samples were analyzed using specific LC-MS/MS methods

Adipocytes

- Effect on lipid accumulation and insulin-stimulated glucose uptake was assessed in human adipocytes (Cambrex) and mouse OP9 adipocytes (ATCC), respectively

Results and Discussion

Desoxy-Ritonavir



ritonavir — desoxy-RTV

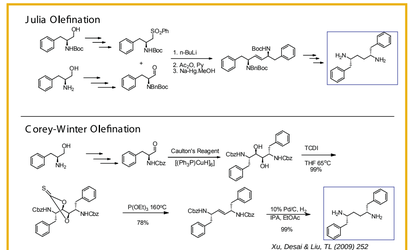
Compound	HIV EC_{50}(nM)	CYP3A IC_{50}(μM)	CYP2C9 IC_{50}(μM)	CYP2D6 IC_{50}(μM)	PXR activation%
1 Ritonavir	15	0.11	4.9	2.3	51
2 Desoxy-RTV	290	0.11	3.9	3.9	70

- Desoxy-RTV has reduced anti-HIV activity
- Comparable CYP3A activity and selectivity
- Desoxy-RTV served as the lead compound for SAR studies

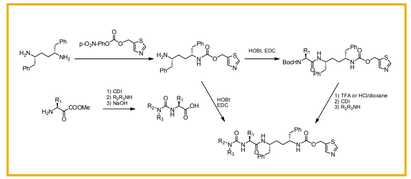
Results and Discussion (cont'd)

Synthetic Methods

Synthesis of Core Diamine

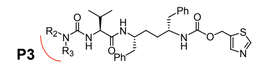


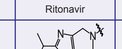
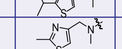
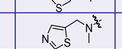
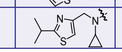
Synthesis of Analogs



SAR Results

SAR of P3 Region

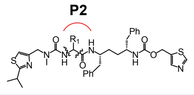


Compound	R_2-N(R_3)	HIV replication IC_{95} (μM)		CYP 3A4 IC_{50} (μM)
		Protease	Replication	
1	Ritonavir	0.0006	0.015	0.11
2		0.005	0.29	0.11
3		0.035	0.40	0.10
4		0.386	0.7	0.08
5		0.550	0.75	0.21

- Modifications to P3 region still allow potent CYP3A inhibition
- Removal of isopropyl moiety from thiazole or incorporation of bulky substitutents at urea N did not significantly reduce anti-HIV activity

SAR Results (cont'd)

SAR of P2 Region



Compound	R_1	HIV IC_{95} (μM)		CYP3A4 IC_{50} (μM)
		Protease	Replication	
1	Ritonavir	0.0006	0.015	0.11
2		0.005	0.29	0.11
6		0.05	1.5	0.15
7		0.005	10	0.50
8		0.14	1.0	0.13
9		3.0	>10	0.12
10		>30	>30	0.11
11		0.03	0.3	0.10
12		>18	>30	0.21
13		>23	20	0.14
GS-9350		>30	>30	0.15
14		>30	>30	0.09

- P2 groups modulate anti-HIV activity
- Compound 10, 12, 14 and GS-9350 are potent CYP3A inhibitors, while having no activity against HIV

CYP450 Enzyme Inhibition Specificity

Compound	CYP450 Enzyme IC_{50} (μM)					PXR activation %
	3A	1A2	2C19	2C9	2D6	
Ritonavir	0.11	>25	12.7	4.9	2.3	51
10	0.11	>25	2.2	4.7	0.6	15
12	0.21	>25	>25	>25	0.8	5
GS-9350	0.15	>25	>25	>25	9.2	10
14	0.09	>25	1.27	8.1	3.1	32

- GS-9350 is a more specific CYP3A inhibitor, has minimal effects on PXR and was selected for further evaluation

Key Profiles of GS-9350

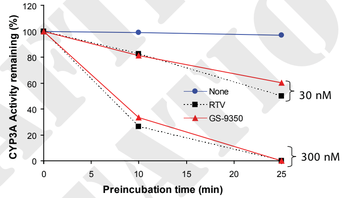
- **GS-9350 is a Mechanism-based Inhibitor of CYP3A**

Kinetics of Inactivation of Human CYP3A

	GS-9350	RTV
k_{inact} (min^{-1})	0.44	0.23
K_I (μM)	0.94	0.26

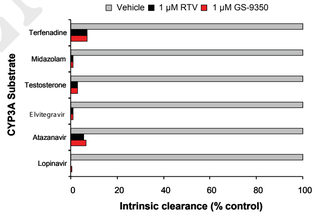
mean of 5 experiments

Inhibition of Microsome CYP3A Activity



- Inhibition of CYP3A by GS-9350 is both time- and concentration-dependent

- **GS-9350 inhibits the metabolism of a broad range of CYP3A substrates**

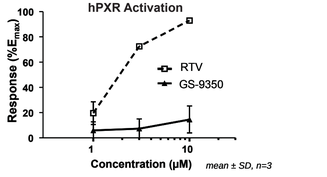


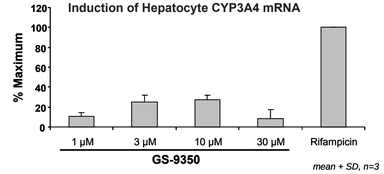
- **GS-9350 shows reduced potential for lipid abnormalities**

Compound	Adipocyte Function Assays	
	Inhibition of Normal Lipid Accumulation (EC_{50} μM)	% Inhibition of Glucose Uptake @ 10μM
Ritonavir	16	55
GS-9350	> 30	9.5
Atazanavir	> 30	1.1

mean of 5 experiments

- **GS-9350 exhibits minimal induction of drug metabolizing enzymes and transporters**

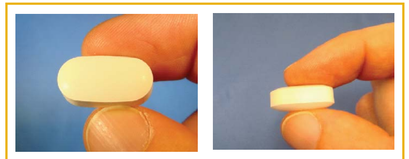




- **GS-9350 shows good *in vitro* DMPK profile, improved aqueous solubility**
- **GS-9350 has comparable DMPK profile to ritonavir with high absorption potential after oral dosing**

Compound	Aqueous solubility (ug/mL)		Log D	Caco-2 Papp (x10^{-6} cm/s)		Portal vein absorption in dog
	pH 7.4	pH 2.2		A to B	B to A	
Ritonavir	~2.0	3.1	3.3	6.3 (5 μM)	27.1 (5 μM)	>50%
GS-9350	75	>6500	3.1	2.4 (1 μM) 7.6 (10 μM)	22.7 (1 μM) 8.5 (10 μM)	>50%

Integrase Fixed-Dose Combination Tablet (QUAD)



Integrase Fixed-dose Regimen
(Elvitegravir/ Emtricitabine/ Tenofovir DF/ GS-9350)

- GS-9350 was successfully co-formulated with elvitegravir/ emtricitabine/tenofovir as a single tablet (QUAD)
- QUAD is smaller in size than ATRIPLA™

Clinical Studies

- In phase I, at 100 and 200 mg, once daily GS-9350 reduced the clearance of midazolam (a CYP3A substrate) by 90% and 95%, respectively
- In phase I, GS-9350 (150 mg, once daily), when used as a component of integrase fixed-dose regimen QUAD, enhanced the PK of elvitegravir to provide comparable C_{trough} to that boosted with 100 mg once daily ritonavir
- In phase I, GS-9350 (150 mg, once daily) enhanced the PK of atazanavir bioequivalent to that obtained when coadministered with 100 mg once daily ritonavir (see Poster A1-1301)
- GS-9350 is being evaluated in Phase II studies in HIV-infected patients

Conclusions

- **GS-9350 is a potent, selective, mechanism-based CYP3A inhibitor that lacks anti-HIV activity and has limited effects on adipocyte function *in vitro***
- **GS-9350 has reduced potentials than ritonavir for off-target drug-interactions due to enzyme inhibition or induction**
- **GS-9350 shows much improved physicochemical properties over ritonavir, allowing tablet co-formulations with other agents**
- **GS-9350 boosts CYP3A substrates comparable to ritonavir in humans**
- **Single pill, once daily integrase fixed-dose regimen (Elvitegravir/Emtricitabine/Tenofovir DF/GS-9350) is in Phase II study in HIV-infected patients**

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