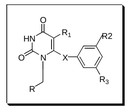
Development of Next Generation Pyrimidinedione Inhibitors of HIV

Tracy Hartman and Robert Buckheit, Jr. ImQuest Biosciences, Frederick, MD, USA

Abstract

Although the currently approved NNRTIs are highly potent, significant improvements in therapeutic utility are still required. The pyrimidinediones are small molecule HIV inhibitors with two distinct mechanisms of action, inhibiting HIV-1 RT at subnanomolar concentrations through interaction at the hydrophobic NNRTI binding pocket, and the entry of both HIV-1 and HIV-2 at low nanomolar concentrations by interaction with a novel conformational structure formed upon co-culture of virus and CD4+ target cells. From a SAR series of 68 congeners, twelve lead therapeutic compounds have been identified with greater inhibitory potential and biochemical and metabolic stability for further evaluation and development. These pyrimidinediones remain highly active against multi-drug resistant (MDR) viruses with mutations in RT and/or protease, suggesting that the compounds may be highly useful as a potential salvage therapy in patients failing HAART, or as a primary therapy in those patients infected with drug-resistant strains. Drug-resistant virus selections have resulted in virus isolates which are completely resistant to the selecting pyrimidinedione. Genotypic and phenotypic evaluations of the strains which emerge during the selection process demonstrates the initial appearance of mutations in the RT, resulting in approximately 100-fold loss in sensitivity, followed by the accumulation of mutations in gp120, gp41 and gag proteins which allow the virus to escape entry or maturation inhibition, yielding viruses with 1000-10,000-fold resistance. Finally, multiple additional changes occur in the NNRTI binding pocket, resulting in complete resistance. The mutational profile and associated mechanism of action studies suggest that the compounds act as typical NNRTIs but also recognize a unique conformational structure formed upon co-culture of virus and target cells which requires the interaction of viral envelope and gag proteins and host CD4 and chemokine receptor molecules. The appearance of mutations in gp120 and gp41 is consistent with regions of envelope associated with chemokine receptor engagement and fusion. Each of the resistant viruses have been evaluated for their sensitivity to other nonnucleoside, nucleoside and nucleotide RT inhibitors, entry and fusion inhibitors, and protease inhibitors. Cross-resistance is only detected with other NNRTIs and enhanced sensitivity was observed with entry inhibitors such as T20. In combination with FDA approved inhibitors of HIV entry, RT, integrase and protease additive to highly synergistic interactions were observed. Comparative evaluation of compound metabolism and protein binding is also being utilized to help prioritize development efforts, since our current clinical candidate IQP-0410 is metabolized quickly by human liver microsomes. The pyrimidinediones represent an excellent therapeutic candidate based the dual mechanism of action, favorable interactions in combination with FDA-approved anti-HIV compounds, and a higher genetic barrier to resistance.

Chemical Structure of Pyrimidinediones



Compound	Substituents					CEMSS/HIV-1_{IIIB} EC_{50} (nM)	PBMC/Clade B EC_{50} (nM)	Entry Inhibition EC_{50} (nM)
	R1	R2	R3	X	R			
IQP-0405	Et	Me	Me	O	Cyclopropyl	4	0.2	475
IQP-0406	iPr	Me	Me	O	Cyclopropyl	3	0.2	257
IQP-0407	Et	Me	Me	C=O	Cyclopropyl	2	0.3	238
IQP-0528	iPr	Me	Me	C=O	Cyclopropyl	10	0.4	560
IQP-0529	Et	Me	Me	S	Cyclobutyl	4	2	648
IQP-0531	Et	Me	Me	O	Cyclobutyl	80	>10	861
IQP-0532	iPr	Me	Me	O	Cyclobutyl	10	25	594
IQP-0533	Et	Me	Me	C=O	Cyclobutyl	8	0.02	>1000
IQP-0546	iPr	Me	Me	C=O	Cyclohexyl	20	9	670
IQP-0548	iPr	Me	Me	S	Phenyl	0.5	0.2	200
IQP-0549	Et	Me	Me	O	Phenyl	20	2	>1000
IQP-0550	iPr	Me	Me	O	Phenyl	2	0.01	2540
IQP-0551	Et	Me	Me	C=O	Phenyl	2	0.06	220
IQP-0558	iPr	Me	Me	C=O	1-Cyclopenten-1-yl	10	0.5	>1000
IQP-0565	Et	Me	Me	O	3-Cyclopenten-1-yl	20	3	435
IQP-0410	Et	Me	Me	C=O	3-Cyclopenten-1-yl	4	0.1	173
IQP-1187	iPr	Me	Me	C=O	3-Cyclopenten-1-yl	4	0.5	580

Dose Escalation Drug Resistant HIV-1_{IIIB} Selections

Compound	R Group	Passage/Drug Concentration (μM)	Fold-Resistance	Envelope Mutations	RT Mutations
IQP-0405	Cyclopropyl	p9/0.8	150,000	Y136N/T138X/N143X/F423I/V701I /V704I	V106A/F227L
IQP-0406	Cyclopropyl	p9/1.6	12,200	In progress	V106A/F227L/F346S
IQP-0407	Cyclopropyl	p6/0.13	250,000	In progress	V106A/F227L
IQP-0528	Cyclopropyl	p12/20.8	10,000	F175L/F353Y/H638Y/K660N/T671 S/N672S/V696I	L214F/Y181C/K101E/V108I/F227L
IQP-0529	Cyclobutyl	p12/16.4	2,500 (ongoing)	In progress	V108I/E169X/R277S
IQP-0531	Cyclobutyl	p9/5.2	184,000	K306R/V701I	E169K/Y181X/Y188L
IQP-0532	Cyclobutyl	p9/5.1	2,680 (due to toxicity)	In progress	V106A
IQP-0533	Cyclobutyl	p12/2.1	625 (ongoing)	In progress	V108I/E169K/Y181C
IQP-0546	Cyclohexyl	p9/32.0	150 (ongoing)	In progress	S162X/Y181C
IQP-0549	Phenyl	p11/10.4	4,200 (due to toxicity)	K306R	V179D/Y181X/Y318F/S322L
IQP-0558	1-cyclopenten-1-yl	p10/25.4	1,750 (due to toxicity)	In progress	K101E/P236X
IQP-0565	3-cyclopenten-1-yl	p9/5.2	4,160 (due to toxicity)	K306R/V701X/V704I	Y188L/Y318F
IQP-0410	3-cyclopenten-1-yl	p12/16.8	125,000	T138I/V696I/R737K	L214F/E169K/L234I/K103N

Antiviral Activity Versus Drug Resistant Viruses

Compound	CEMSS EC_{50} (μM)											PBMC EC_{50} (μM)	
	HIV-1_{IIIB}	IQP0405-R_{IIIB} (V106A/ F227L)	IQP0406-R_{IIIB} (V106A/ F227L/ F346S)	IQP0407-$R_{III\ B}$ (V106A/ F227L)	IQP528-R_{IIIB} (L214F/Y181C/ K101E/V108I/ F227L)	IQP0532-R_{IIIB} (E169K/ Y188L)	IQP0533-R_{IIIB} (V106A)	IQP0549-R_{IIIB} (V179D/Y181C/ Y318F/S322L)	IQP0410-R_{IIIB} (L214F/E169K/ L234I/K103N)	NL4-$3_{M41L/L74V/V106A/T215Y}$	A17 (Y181C/ K103N)	MDR769	AD MDR01
AZT	0.003	0.004	0.0006	0.0006	0.01	0.006	0.001	0.002	0.001	0.02	0.003	>1.0	0.029
EFZ	0.002	>0.1	0.07	0.07	>0.1	>0.1	>0.1	0.1	0.06	>10.0	>10.0	0.0036	0.0025
NVP	0.015	>10.0	>10	>10.0	>10.0	>10.0	>10	9.9	>10.0	0.003	0.04	ND	ND
DLV (μg/mL)	0.01	0.02	1.34	0.06	>10.0	0.05	8.71	0.01	**0.03**	0.9	6.9	0.34	1.54
IQP-0405	0.004	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	0.4	1.6	**0.12**	**0.18**
IQP-0406	0.003	>100.0	>50	>50.0	>50.0	**17.7**	>50	**2.2**	>100.0	0.7	**0.5**	0.61	1.51
IQP-0407	0.002	>100.0	>50	>50.0	**0.89**	>100.0	**20**	>100.0	>100.0	5.4	**0.3**	**0.22**	**0.06**
IQP-0528	0.01	>100.0	**10.5**	**7.09**	>50.0	**73.0**	**1.24**	**6.1**	>100.0	**0.11**	**0.7**	3.14	**0.79**
IQP-0529	0.004	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	1.6	11.0	2.4	1.36
IQP-0531	0.08	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	1.0	50.0	**1.94**	**1.85**
IQP-0532	0.01	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	0.19	15.5	**5.34**	**6.07**
IQP-0533	0.008	>100.0	>50	>50.0	>50.0	>100.0	>50	**83.1**	>100.0	**0.09**	4.8	**0.56**	**0.36**
IQP-0546	0.02	>100.0	>50	**1.23**	>50.0	>100.0	>50	>100.0	>100.0	**0.15**	4.4	14.9	10.5
IQP-0548	0.0005	>100.0	>50	ND	ND	>100.0	ND	>100.0	**58.1**	0.02	6.3	0.99	1.45
IQP-0549	0.02	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	**0.12**	4.5	**0.37**	**0.58**
IQP-0550	0.002	>100.0	>50	>50.0	>50.0	**19.2**	>50	>100.0	**25.3**	0.16	12.9	**0.12**	0.91
IQP-0551	0.002	>100.0	>50	ND	ND	>100.0	ND	>100.0	>100.0	0.7	3.0	**0.05**	**0.15**
IQP-0558	0.01	>100.0	>50	**1.0**	>50.0	>100.0	>50	>100.0	>100.0	0.3	50.0	20.0	65.9
IQP-0565	0.02	>100.0	>50	>50.0	>50.0	>100.0	>50	>100.0	>100.0	**0.07**	16.0	ND	ND
IQP-0410	0.004	>100.0	>50	**21.7**	>50.0	>100.0	>50	**40.8**	>100.0	0.08	**0.7**	2.09	1.51

Combination Therapy Evaluations

Compound Tested in Combination with:	IQP-0405			IQP-0406			IQP-0407			IQP-0528			IQP-0558			IQP-0410			IQP-1187		
	Defined Interaction of Combined Therapy																				
	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C	CEMSS/RF	PBMC/ Clade B	PBMC/ Clade C
Chicago Sky Blue (EI)	HS	A	A	A	S	A	S	A	A	S	S	A	S	A	A	A	A	A	A	S	A
Cyanovirin (EI)	S	HS	HS	A	A	A	HS	A	HS	S	S	HS	S	A	A	A	HS	S	S	S	A
Efavirenz (NNRTI)	A	S	A	S	S	A	S	S	A	S	S	A	S	A	A	A	HS	S	S	HS	A
ISIS 5320 (EI)	HS	A	A	S	HS	A	HS	S	A	S	HS	A	HS	A	A	S	S	A	S	HS	A
UC781 (NNRTI)	S	HS	A	A	A	A	A	S	S	S	A	A	A	A	A	S	S	A	A	S	S
T20 (FI)	S	HS	S	S	HS	S	A	A	A	S	S	S	A	A	A	S	S	A	A	S	HS
AZT (NRTI)	S	S	A	S	HS	S	S	S	A	S	HS	A	A	S	S	HS	HS	A	S	S	A
Tenofovir (NRTI)	A	HS	S	A	A	HS	A	HS	HS	A	A	HS	A	A	HS	A	A	HS	A	A	S
Ritonavir (PI)	S	S	S	A	S	HS	S	S	HS	A	S	HS	S	S	A	A	S	A	A	S	S
Raltegravir (II)	S	IP		A	IP		S	IP		S	IP		S	IP		S	IP		HS	IP	

HS: Highly Synergistic S: Synergistic A: Additive IP: In Progress

Rapid Drug Resistant HIV-1_{IIIB} Selections at Fixed Concentrations

Compound	Sterilizing Concentration (μM)	Concentration Selecting Mutant (μM)	Reverse Transcriptase Mutations
IQP-0405	1.25	0.05	Y181C
IQP-0406	0.25	0.01	V108I/Q152P/M357T
IQP-0407	6.25	0.01	Y181C
IQP-0528	0.5	0.01	Y181C/G142R
IQP-0529	62.5	2.5	Y181C
IQP-0531	62.5	2.5	Y181C
IQP-0549	25	0.2	Y181C/P97T/T351A
IQP-0554	62.5	2.5	Y181C/E40K
IQP-0558	15.6	0.025	Y181C/E40K
IQP-0565	62.5	2.5	Y181C
IQP-0410	12.5	0.1	Y181C
IQP-1187	0.02	0.004	Y181C

Metabolic Stability in Human Liver Microsomes

Compound	R Substituent	X Substituent	Half-life (min)	Intrinsic Clearance (mL/mim/mg protein)
IQP-0403	Cyclopropyl	S	22	0.062
IQP-0404	Cyclopropyl	S	21	0.067
IQP-0405	Cyclopropyl	O	39	0.036
IQP-0406	Cyclopropyl	O	17	0.083
IQP-0407	Cyclopropyl	C=O	31	0.045
IQP-0528	Cyclopropyl	C=O	32	0.043
IQP-0532	Cyclobutyl	O	<10	0.140
IQP-0549	Phenyl	O	15	0.092
IQP-0410	3-cyclopenten-1-yl	C=O	17	0.081
IQP-1187	3-cyclopenten-1-yl	C=O	17	0.083

Summary

- Lead compounds were chosen based on their ability to reduce virus replication by inhibiting both virus entry (79 to 2000 nM range) and RT (3 to 158 nM range).
- Drug resistant HIV-1 isolates were selected and compared for the relative rate of resistance and defined mutations: IQP-0528, IQP-0532 and IQP-0549 demonstrate enhanced genetic barrier to resistance.
- Cross resistance evaluations determined IQP-0407 and IQP-0528 in the cyclopropyl series demonstrated a higher genetic barrier to resistance: active versus 5-8 drug resistant viruses.
- IQP-0405, IQP-0407 and IQP-0528 in the cyclopropyl series were the most metabolically stable in the liver microsome assay. IQP-0532 of the cyclobutyl series was the least stable.
- Additional studies are in progress to synthesize analogs with increased bioavailability by increasing the thermodynamic solubility of the drug solid and improving the plasma half life.

Acknowledgements

The authors would like to acknowledge Samjin Pharmaceutical Co. LTD for providing the pyrimidinedione compounds. This work was funded by SBIR grants 1R43A1084676-01 and 2R44A1078858-02A1.