IND-Directed Development of Dual-Acting Pyrimidinediones IQP-0410 and IQP-0528: Enhancement of Solubility and Metabolic Stability

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Abstract

The pyrimidinediones are highly potent nonnucleoside inhibitors of both HIV-1 and HIV-2, inhibiting HIV-1 RT at subnanomolar concentrations and the entry of both HIV-1 and HIV-2 at nanomolar concentrations, including all HIV subtypes (except subtype O) and MDR strains. The primary development hurdles associated with IQP-0410 (solubility and metabolic stability with rapid metabolism in both human liver microsomes and human hepatocytes) have been chemically addressed and a new lead (IQP-0528) has been identified and evaluated. IQP-0410 and IQP-0528 were well tolerated in animals with no test article-related findings noted from in-life data, clinical pathology, necropsy or histology. Pharmacokinetic studies demonstrated oral bioavailability with effective concentrations (EC90) of the compounds exceeded by 30-50-fold at 24 hours. Safety pharmacology studies showed no signs of pharmacologic or toxicologic activity and all genotoxicology testing was negative. We have defined two different major pathways of metabolism and new compounds were synthesized to block metabolic degradation. The N1-cyclopentenyl group in IQP-0410 undergoes multiple oxidations modification of this substituent to N1-cyclopropyl (IQP-0528) yielded significantly greater stability without loss of antiviral efficacy or enhanced toxicity. With IQP-0528 the major metabolic pathway is oxidation of one of the methyls of the C6-linked C6H4Me2 group, as has been reported for the metabolism of other uracil-based NNRTIs, such as Emivirine (MK-442). Replacement of the 3,5-dimethylphenyl with 3,5-dichloro, resulted in molecules which were less active than the parent. IQP-0528 is now being developed as a lead product for both HIV therapy and prevention. Our chemical modification efforts suggest a strategy to develop analogs with improved solubility and reduced metabolism via additional modifications to the 'right hand side' of the molecule, including (1) changes at C5 which will improve solubility and oral absorption, (2) decreasing the ClogP of the compounds to decrease metabolism, and (3) decrease metabolism in the C6 chain by introducing electron withdrawing groups to the aromatic ring.

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
JDJ01	iPr	F	Me	C=O	Cyclopropyl	4	ND	ND
JDJ02	iPr	F_3C	Me	C=O	Cyclopropyl	41	ND	ND
JDJ03	iPr	Me	Me	C=O	Cyclopropyl	47	ND	ND

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

Antiviral Activity Versus Drug Resistant Viruses

Compound	CEMSS EC_{50} (μM)		PBMC EC_{50} (μM)	
	HIV-1_{IIIB}	A17 (Y181C/K103N)	MDR769	AD MDR01
AZT	0.003	0.003	>1.0	0.029
EFZ	0.002	>10.0	0.0036	0.0025
NVP	0.015	0.04	ND	ND
DLV (μg/mL)	0.01	6.9	0.34	1.54
IQP-0405	0.004	1.6	**0.12**	**0.18**
IQP-0406	0.003	**0.5**	0.61	1.51
IQP-0407	0.002	**0.3**	**0.22**	**0.06**
IQP-0528	0.01	**0.7**	3.14	**0.79**
IQP-0529	0.004	11.0	2.4	1.36
IQP-0531	0.08	50.0	**1.94**	**1.85**
IQP-0532	0.01	15.5	**5.34**	**6.07**
IQP-0533	0.008	4.8	**0.56**	**0.36**
IQP-0546	0.02	4.4	14.9	10.5
IQP-0548	0.0005	6.3	0.99	1.45
IQP-0549	0.02	4.5	**0.37**	**0.58**
IQP-0550	0.002	12.9	**0.12**	0.91
IQP-0551	0.002	3.0	**0.05**	**0.15**
IQP-0558	0.01	50.0	20.0	65.9
IQP-0565	0.02	16.0	ND	ND
IQP-0410	0.004	**0.7**	2.09	1.51

IQP0410 Safety Pharmacology

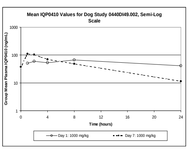
hERG potassium channel effects	8.8% inhibition
Neuropharmacological profile in mice	No effect
Pulmonary assessment in mice	No abnormalities
Genotoxicology	All negative

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

Oral IQP-0410 PK Study in Dogs



PK Parameters	Day 1 (mean)	Day 7 (mean)
AUC (0 to 24) ng/mL*hrs	1254	979
AUC (0 to infinity) ng/mL*hrs	981	1098
T_{max} (0 to 24) hrs	4.0	1.5
C_{max} (0 to 24) ng/mL	76	119
Ke (1/hr)	0.029	0.094
MTT (0 to 24) (hrs)	10.84	8.16
T1/2 (hrs)	23.88	7.46
CL/F (0 to 24) (L/kg/hr)	1125	1451
CL/F (0 to infinity) (L/kg/hr)	1020	1268
Vz/F (0 to 24) (L/kg)	68.11	16.14
Vz/F (0 to infinity) (L/kg)	35.13	14.08

14 Day Oral IQP-0410 Mouse Toxicity Study

• 72 mice (half male/half female) were assigned to toxicokinetics groups dosed with either 60, 200, or 600 mg/kg of IQP-0410

• 30 mice (half male/half female) were assigned to toxicology groups dosed with 0, 60, 200, or 600 mg/kg

• Daily administration via oral gavage for 14 days followed by a 14 day recovery period

• In-life data showed no signs of IQP-0410 related effects

•Males dosed at 600 mg/kg/day had significantly significant changes in organ weights (decreases in adrenals, kidney, liver; increases in brain) on Day 31. Females at same concentration had increase in absolute thymus weight.

•Histopathology revealed no microscopic lesions

•NOAEL in mice is 200 mg/kg/day

14 Day Oral IQP-0528 Rat Toxicity Study

• 6 Sprague Dawley rats (half male/half female) were dosed with either 100, 250, 500 or 1000 mg/kg of IQP-0528 in a Dose Range Finding Study

• Single administration via oral gavage. 100 mg/kg starting dose then increased every 24 hours up to 1000 mg/kg. Necropsy performed on day 3. 1000 mg/kg was well tolerated.

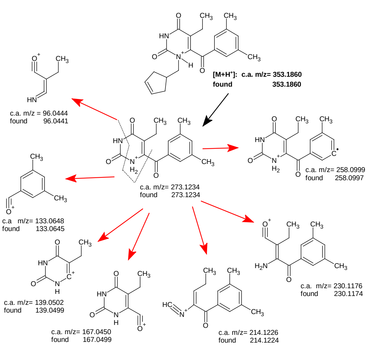
•10 rats (half male/half female) were assigned to toxicology groups dosed with 0 or 1000 mg/kg for 3 or 15 days.

•In-life data showed no signs of IQP-0528 related effects

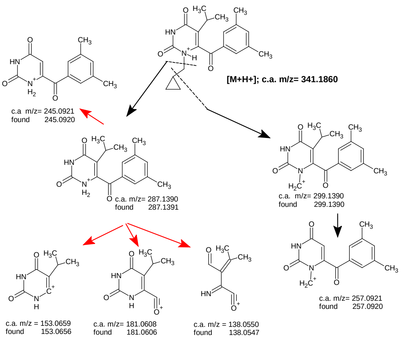
•No definitive IQP-0528 related changes in body weight

•Histopathology revealed no microscopic lesions

Elaborated Fragmentation Pathway of IQP-0410



Elaborated Fragmentation Pathway of IQP-0528



Metabolic Stability in Human Liver Microsomes and Pooled Human Hepatocytes

Compound	R Substituent	X Substituent	Human Liver Microsomes		Pooled Human Hepatocytes	
			Half-life (min)	Intrinsic Clearance (mL/mim/mg protein)	Half-life (min)	Intrinsic Clearance (mL/mim/mg protein)
IQP-0406	Cyclopropyl	O	17	0.083	292	0.0016
IQP-0528	Cyclopropyl	C=O	32	0.043	193	0.0024
IQP-0532	Cyclobutyl	O	<10	0.140	122	0.0032
IQP-0549	Phenyl	O	15	0.092	259	0.0018
IQP-0410	3-cyclopenten-1-yl	C=O	17	0.081	206	0.0022
JDJ01	Cyclopropyl	C=O	>60	ND	>120	ND
JDJ02	Cyclopropyl	C=O	55	0.025	>120	ND
JDJ03	Cyclopropyl	C=O	30	0.047	>120	ND

Compounds were evaluated at 1 μM for metabolic stability measured by LC/MS.

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). Dual mechanism of action could provide benefit of three drug when combined in HAART regimen.

• Congeners of IQP-0410 with cyclopropyl substutions at the N-1 of pyrimidinedione represent better clinical candidates. IQP-0528 shares its high therapeutic index and dual mechanism of action with a significantly higher stability and longer half-life in metabolic assays.

• IQP-0528 demonstrates enhanced genetic barrier to resistance for a significant potential in salvage therapy regimens.

• In combination with FDA approved inhibitors of HIV entry, RT, integrase and protease additive to highly synergistic interactions were observed for IQP-0528.

• Structure elucidation indicates IQP-0410 generated a pool of M1-M3 metabolites resulting from mono-oxygenation into a methyl group (M2) and into a cyclopentenyl fragment (M1/M3). The cyclopentenyl fragment underwent rapid metabolism.

• Structure elucidation indicates IQP-0528 generated a single major putative metabolite (M1) as a result of mono-oxygenation into a methyl group.

• Medicinal chemistry was employed to development analogs by eliminating sites of rapid metabolism from IQP-0528. JDJ01 was more metabolically stable in the human liver microsome assay and in pooled human hepatocytes than IQP-0528.

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