

Antiviral Characterization and Human Experience with BILR 355 BS, a Novel Next-Generation Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) with a Broad Anti-HIV-1 Profile

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ABSTRACT

BACKGROUND

NNRTIs are potent components of combination antiretroviral regimens. Currently available NNRTI regimens are limited by the emergence of broad cross-resistance to NNRTIs and by toxicities. BILR 355 BS is a next-generation NNRTI with potent antiviral activity.

METHODS

The *in vitro* inhibitory activity (IC_{50} value) and anti-HIV-1 activity (EC_{50} value) of BILR 355 BS were determined against wild-type (WT) and recombinant viruses containing one or more mutations that confer phenotypic resistance to NNRTIs. This panel of viruses was selected based on the clinical prevalence of NNRTI-resistant mutants, and included K103N, Y181C, G190A, K103N/Y181C, K103N/V108I, K103N/P225H, and L100I/K103N. BILR 355 BS was also tested against a broad panel of HIV-1 isolates from various clades (A, B, C, D, G, O, A/E, A/G) originating from treatment-naïve patients. Single (SD) and multiple-dose (MD) healthy male volunteer studies have been conducted with BILR 355 BS + RTV 100 mg (7 to 11 days) to determine its pharmacokinetics, safety, and tolerability.

RESULTS

The EC_{50} of BILR 355 BS against WT HIV-1 is 0.26 ng/mL and against common NNRTI-resistant viruses ranges from 1.5 to 13 ng/mL for the more clinically common single and double NNRTI mutations. BILR 355 BS maintains potent antiviral activity against the panel of HIV-1 clades tested (median EC_{50} = 1.3 ng/mL) but is inactive against HIV-2. A serum shift of 2-fold in 50% human serum will limit the impact of protein binding. Orally administered SD BILR 355 BS resulted in modest plasma levels and $t_{1/2}$ of 2 hours. Exposure is increased when RTV 100 mg is added: C_{max} by approximately 2.3- to 5-fold, and $t_{1/2}$ by 3.5- to 5.5-fold. MD exposure at 150 mg QD + RTV 100 mg QD results in C_{max} 870 ng/mL (SD: 130), C_{24h} 279 ng/mL (SD: 54) and $t_{1/2}$ of 16 to 17 hours. Time to steady state is 5–6 days. With the exception of 1 subject with a transient asymptomatic Grade 3 ALT elevation in the 25-mg dose cohort, there have been no significant clinical or laboratory safety abnormalities observed to date.

CONCLUSION

BILR 355 BS displays an excellent antiretroviral profile against HIV-1 WT and NNRTI-resistant mutants and promising biopharmaceutical properties. When co-administered with RTV, BILR 355 BS has favorable human PK and safety profiles for up to 11 days of exposure. These data support the continued development of BILR 355 BS as a novel NNRTI with activity against viruses resistant to first-generation NNRTIs.

INTRODUCTION

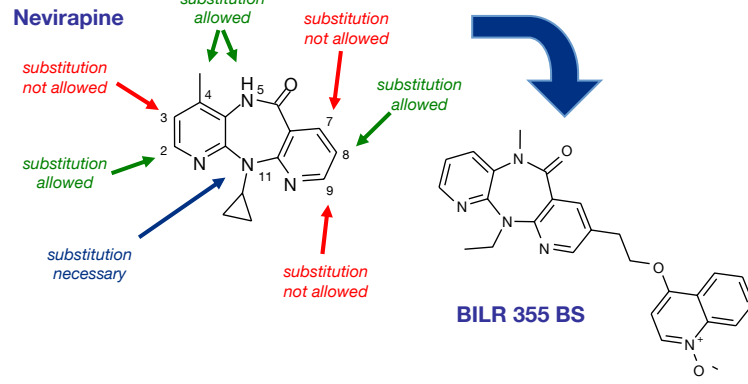
NNRTIs are potent components of combination antiretroviral regimens. However, patients failing on an NNRTI-containing regimen typically select for viruses that are cross-resistant to all members of the class, leaving them with no further NNRTI options. Thus there is a need for new NNRTIs with potent and durable antiviral activity vs both wild-type and clinically relevant NNRTI-resistant strains in order to complement and further improve existing combination therapies.

We have performed extensive SAR studies on the nevirapine dipyrrodozepinone core and discovered a new series of molecules with broad antiviral activity and desirable biopharmaceutical properties.¹ BILR 355 BS has been identified as a promising member of this novel series and is currently advancing in development.

The antiviral characterization of BILR 355 BS against a panel of recombinant NNRTI-resistant viruses, as well as against various wild-type HIV-1 clades, is presented. The evaluation of key biopharmaceutical parameters along with Phase I safety and pharmacokinetics data in healthy male volunteers (following single- and repeat-dose co-administration with ritonavir) are also presented.

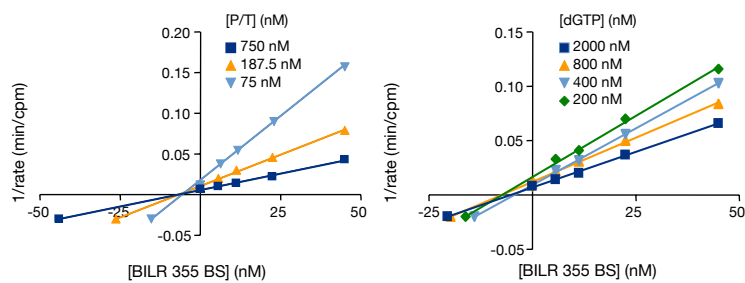
STRUCTURE OF DIPYRIDODIAZEPINONE CORE

Figure 1. Schematic of Structure-Activity Studies



RESULTS: MODE OF INHIBITION

Figure 2. *In Vitro* Reverse Transcriptase Scintillation Proximity Assay



- Dixon plots vs both substrates (primer/template & dGTP) intercept on the x-axis
- Cornish-Bowden plots (not shown) also intercept on the x-axis²
- This is consistent with BILR 355 BS being a non-competitive inhibitor of HIV-1 RT wild type with $K_i \sim 6$ nM (same mode of inhibition as nevirapine and efavirenz)

RESULTS: IN VITRO ACTIVITY OF BILR 355 BS

Table 1. RT Inhibition & Antiviral Profile vs HIV-1-Recombinant Viruses

BILR 355 BS	IC ₅₀ /EC ₅₀ (nM)						
	WT	K103N	Y181C	G190A	V106A	Y188L	P236L
nevirapine	208	13180	34680	>10000	16210	>10000	430
efavirenz	/0.3	/5.5	/4.1	5.1	2.1	52	2.2
BILR 355 BS	95/4.5	121/5.1	40/2.9	167/5.9	nd/4.1	nd/30	nd/235
nevirapine	>10000	>10000	>10000	/400	nd	nd	nd
efavirenz	30	83	91	2820	nd	nd	nd

¹IC₅₀: RT inhibition (SPA assay)
²EC₅₀: antiviral activity (p24 readout)
10 nM = 4 ng/mL

RESULTS: BILR 355 BS ANTIVIRAL ACTIVITY AGAINST CLADE B VIRUSES

Table 2. Antiviral Activity vs a Panel of HIV-1 Clade B Clinical Isolates*

Virus lab #	Treatment history	RT mutations	PI mutations	BILR 355 BS EC ₅₀ (nM)	NVP	EFV
5054	No treatment	None	None	1.2	50	0.7
3350	NVP, AZT	K103N/Y181C, T215Y	None	3.6	50000	nd
5403	AZT, 3TC, EFV, ddI, ddV, RTV, SDV, NFV	M41L, D67N, T69D, K70R, L74V, L100L, K103N, M184V, T215F, K219Q	L10I, K20I, E4V, L63P, A71V, G73S, L90M	2.1	nd	832
5477	AZT, 3TC, EFV, ddI, ddV, RTV, SDV, NFV	A62V, K103N	L10I, M46L, L63P, I84V, L90M	8.7	nd	1190
5730	NVP, AZT	V106A	None	21	1504	nd
5707	AZT, ABC, NVP, SDV	M41L, M184V, Y181C, L210W, T215Y	L10I, M46L, E4V, A71V, V82T, I84V, L90M	1.7	nd	nd
5555	AZT, 3TC, ddI, ddV, RTV, SDV	M41L, D67N, M184V, T215F, K219Q	L10I, E4V, L63P, G73S, V82A, L90M	4.1	nd	nd
5729	AZT, 3TC, SDV	None	L10R, M46L, L63P, V82T, I84V	0.5	nd	nd
3722	AZT, 3TC	D67N, T69D, K70R, A62S, M184V, T215F, K219Q	L63P	1.8	nd	nd
3788	AZT, 3TC	D67N, T69D, K70R, M184V, T215F, K219Q	L63P	3.5	nd	nd
5685	AZT, 3TC, EFV, NFV	M41L, Y181C, M184V, L210W, T215D	L10I, K20I, M36L, L63P, A71V, L90M	15.4	nd	nd
5652	AZT, 3TC, ddI, ddV, RTV, SDV	M41L, D67N, T69D, K70R, L74V, K103N, Y181C, M184V, T215F	L10I, M36L, M46L, L63P, A71V, G73S, L90M	87	nd	nd

*The small number of isolates in this study is not yet fully representative of the spectrum of resistance in clinical isolates.

RESULTS: BILR 355 BS ANTIVIRAL ACTIVITY AGAINST VIRUS FROM DIFFERENT CLADES

Table 3. Antiviral Activity vs Different HIV-1 Clades & CRF (from treatment-naïve patients) EC₅₀ (nM)

Clade	A	A	B	B	C	C	D	G	G	O	O	CRF01_AE	CRF01_AG	CRF02_AG	CRF02_AG
BILR 355 BS	4	14.7	4.6	1.4	4.9	3	12	1.2	0.5	352	10	3.1	0.8	2.6	1.1
nevirapine	130	>400	55	35	42	16	>80	32	30	10000	823	41	1.5	5.7	6.3
efavirenz	1.4	2.4	1	0.1	1	0.2	1.4	0.2	0.5	412	68	1.1	0.5	nd	0.3

- BILR 355 BS maintains potent antiviral activity against this panel of non-clade B isolates (except one group O isolate containing the Y181C polymorphism)
- BILR 355 BS, nevirapine and efavirenz are not active vs HIV-2 isolates (EC_{50} > 1000 nM)

RESULTS: BIOPHARMACEUTICAL PARAMETERS OF BILR 355 BS

Table 4A. Protein Binding & Serum Shift

	Cell culture medium	Human plasma	Human serum albumin	α-acid glycoprotein	β-lipo protein	Serum shift* in 50% Human serum
BILR 355 BS	45.8	95.8	83.4	97.4	41.0	2X
nevirapine	16.1	55.3	57.1	29.5	0	1X
efavirenz [†]	80.9	99.4	98.9	92.8	99.2	13X

*Serum shift = EC_{50} with 50% human serum/ EC_{50} with 0% human serum.
[†]Values for efavirenz are those determined at Boehringer Ingelheim.

4B. Metabolic Stability & CYP450 Inhibition

	1A2	2C9	2C19	2D6	3A4-BFC*	3A4-BQ*	t _{1/2} (min)
BILR 355 BS	>30	8	4	>30	0.8	3	223

*7-benzoyl-4-(4-fluoromethyl)-coumarin (BFC) & 7-benzoylquinoline (BQ) are fluorogenic substrates for CYP3A4.

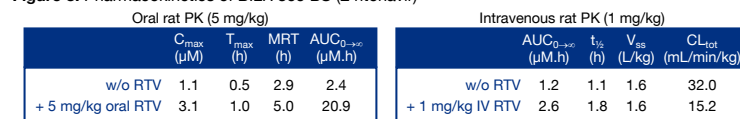
4C. Solubility & Cell Permeability

	Solubility* μg/ml	Log D* Oct/buffer	Caco-2 permeability [†] (10 ⁻⁶ cm/s)		
			A→B	B→A	ratio
BILR 355 BS	7	2.1	35.2	63.1	1.8

*Buffer = 100 mM phosphate, pH 7.2, I = 165 mM. Solubility measured after 24h equilibrium in buffer.
[†]A = apical, B = basal.

RESULTS: PHARMACOKINETIC CHARACTERIZATION (RAT MODEL)

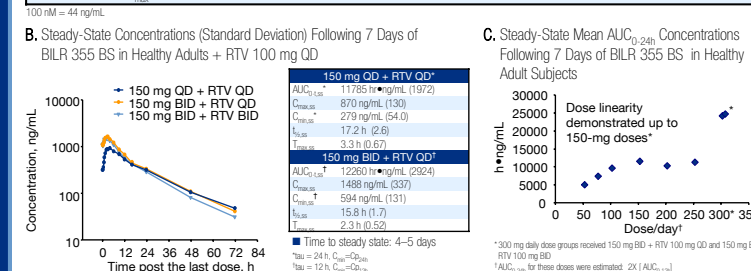
Figure 3. Pharmacokinetics of BILR 355 BS (± ritonavir)



RESULTS: HUMAN PHARMACOKINETICS OF BILR 355 BS

Figure 4. Results of (A) Dose-Escalation Studies and (B) Steady-State Concentrations

	BILR 355 BS regimen	50 mg single dose, no RTV	50 mg single dose + RTV 100 mg
AUC _{0-∞}		419 ng/mL	6654 ng/mL
C _{max}		164 ng/mL	431 ng/mL
t _{1/2}		2.5 h	11.2 h
T _{max}		0.67 h	0.96 h



RESULTS: SAFETY PROFILE OF BILR 355 BS

Table 5. Adverse Events Reported in Healthy Adult Subjects Receiving BILR 355 BS + RTV 100 mg for 7 Days*

	Not Related/ Related*	Mild/mod†
Headache	6/0	6/0
Diarrhea	2/2	4/0
Cold symptoms	2/0	2/0
Sore throat	1/0	1/0
Food poisoning	1/0	0/1
Stomach ache	0/1	1/0
Nausea/ermsis	1/1	1/1
Shingles	1/0	1/0
Angioedema (lip/pharynx)	0/1	1/0

*Addressed to BILR 355 BS or RTV, as indicated by investigator. †Doses of 150 mg QD, 150 mg BID, 200 mg QD, and 250 mg QD.
■ No serious adverse events reported.
■ There was no apparent dose–event relationship across all dose groups.
■ One subject in lower-dose group (25-mg dose) discontinued BILR 355 BS for Grade 4 ALT elevation.

CONCLUSIONS

- BILR 355 BS displays potent antiviral activity against isolates of HIV-1 resistant to currently available NNRTIs as well as against a broad panel of HIV-1 clades. A larger evaluation of clinical isolates is ongoing
- A low serum shift of 2-fold in 50% human serum will limit the impact of protein binding on its antiviral activity
- Rat PK parameters predicted the boosting effect in humans of BILR 355 BS by the CYP3A4 inhibitor ritonavir
- BILR 355 BS + RTV was generally well tolerated clinically
- Concentrations are achieved that are expected to be effective vs WT HIV-1 and many viruses from NNRTI-experienced patients
- PK and safety profile that justify evaluation of safety and efficacy in HIV-1-infected patients

REFERENCES

1. Bonneau et al. Characterization of a novel series of NNRTIs with broad antiviral potency against NNRTI-resistant HIV. Poster 530. Presented at: 11th CROI, February 8–11, 2004, San Francisco, Calif. Poster 530.
2. Cornish-Bowden. A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. *Biochem J.* 1974;137:143–144.