HIV Integrase inhibitor I BI 224436

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Summary

BI 224436 was the first non-catalytic-site integrase inhibitor (NCINI) in the clinic. Its excellent potency and ADME profile make it a valuable *in vivo* tool compound.

Chemical Structure

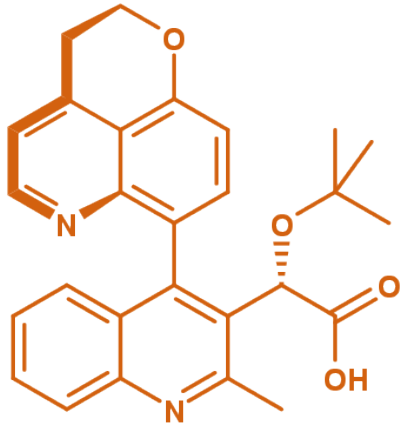

Figure 1: 2-D structure of BI 224436, a non-catalytic-site Integrase inhibitor (NCINI) of HIV-1

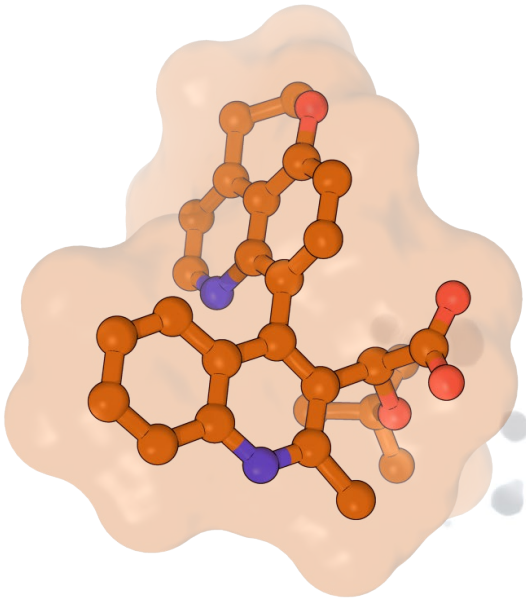

Figure 2: BI 224436, 3D conformation.

Highlights

BI 224436 is the first non-catalytic-site integrase inhibitor (NCINI) reaching a clinical trial. It combines high solubility at all relevant physiological pH values with good cell permeability and good metabolic stability. BI 224436's optimized *in vitro* ADME profile also translates into a good *in vivo* PK profile in preclinical animal species, with oral bioavailability ranging from 54-100%.

Worth noting is the excellent antiviral potency of BI 224436 in the presence of 50% human serum ($ssEC_{95}$ 22-75 nM). In addition, two-drug combination studies with other HIV-1 antiviral agents produced additive to synergistic effects. Thus BI 224436 could be a key component of new first-line cART regimen.

Wikipedia

Target information

Following the reverse transcription of viral RNA into cDNA, HIV-Integrase (IN) is responsible to integrate newly synthesized viral cDNA into the host cell genome. IN fulfils this function via a two-steps process: a 3'-dinucleotide processing reaction and a strand transfer reaction. In the first step, IN binds to viral DNA as part of the pre-integration complex (PIC) in the cytoplasm and excises a dinucleotide from each 3'-end. Thereafter, the PIC is transported into the nucleus where the 3'-ends of the viral DNA are covalently linked to the 5'-ends of the host cell DNA in a process known as strand transfer.[(1)]

Currently approved IN inhibitors (raltegravir, elvitegravir, dolutegravir) are IN strand transfer inhibitors (INSTIs) binding to the IN active site. In contrast, BI 224436 binds to a conserved allosteric pocket at the dimer interface of the catalytic core domain (CCD) of IN and acts through a distinct mechanism. The term non-catalytic-site integrase inhibitors (NCINIs) is used to differentiate both series of compounds having different MOA.

BI 224436 is the first NCINI validated in a phase-1a clinical trial and differs from INSTIs in many regards: 1) BI 224436 binds to an allosteric pocket, which functional effect is to inhibit the 3'-processing step of IN. Additionally, binding to this allosteric pocket also prevents protein-protein interaction with the host cell Lens Epithelial Derived Growth Factor (LEDGF) required for HIV-1 viral replication; 2) BI 224436 shows a distinct resistancy profile against virus mutations and maintains its antiviral activity against a panel of mutants that emerge during treatment failure with other inhibitor classes (INSTIs and NNRTIs).[(2)]

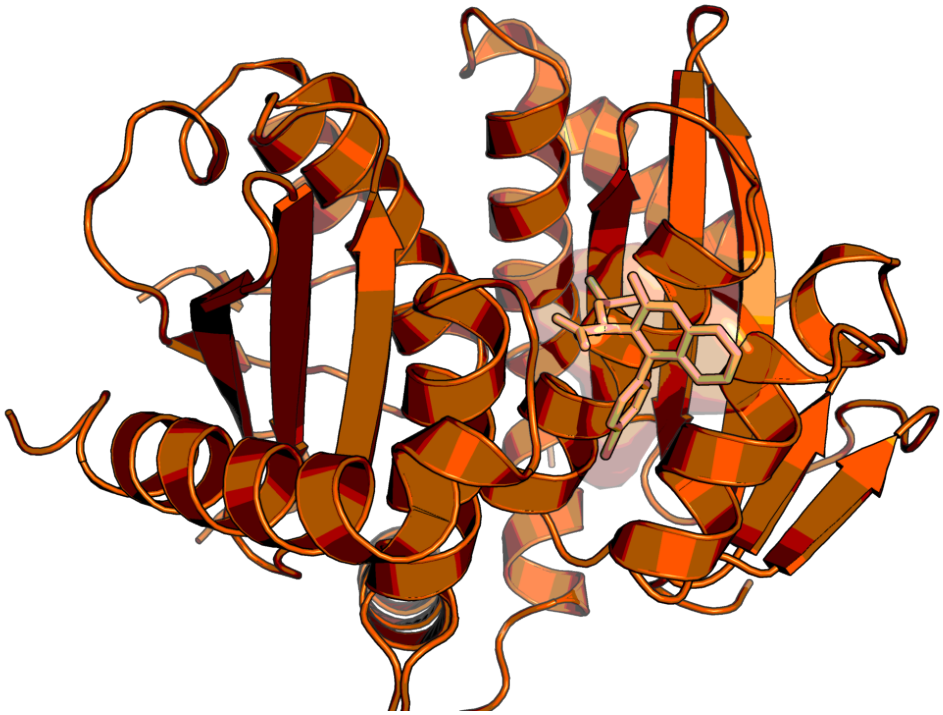

Figure 3: HIV integrase in complex with an analog of BI 224436 (PDB code: 4NYF)

In vitro activity

BI 224436 displays an IC_{50} = 15 nM in a LTR-cleavage assay measuring the 3'-processing hydrolysis reaction of a dinucleotide from the DNA 3'-end of each viral long terminal repeat. Moreover, BI 224436 exhibits excellent antiviral potency (EC_{50} 11-27 nM) in a panel of wild-type and recombinant viruses with different aa124/aa125 variants of IN.

A hallmark of BI 224436 is the low influence of human serum on antiviral potency ($ssEC_{50}$ 2.1-fold) which is the assay used for human dose predictions for HIV clinical candidates.

PROBE NAME / NEGATIVE CONTROL	BI 224436	BI-0449
MW [Da]	442.5	311.8
LTR-cleavage assay (IC_{50}) [nM][a]	15	6,840
Luc-RGA (EC_{50}) [nM][b]	11-27	>40,000

ssEC$_{50}$ (50% HS), fold-change[c]	2.1	-
MTT-C8166 (CC$_{50}$) [nM][d]	110,000	-

[a] Long Terminal Repeat (LTR) DNA 3'-processing assay measures the enzymatic activity of HIV-1 integrase to perform the essential 3'-processing reaction. Integrase binds to the viral DNA LTR ends at the CAGT-3' sequence and catalyzes the removal of the two terminal nucleotides. In this homogeneous assay, the HIV-1 LTR DNA substrate consists of two annealed oligonucleotides, a 31-mer modified at the 3' end with a black hole quencher (BHQ) and a 31-mer modified at the 5' end with rhodamine red-X *N*-hydroxysuccinimide (NHS) ester (5RhoR-XN). Enzymatic cleavage by integrase releases the terminal dinucleotides and black hole quencher, which allows the rhodamine fluorescence to be detected.

[b] Luciferase reporter gene assay (Luc-RGA): C8166 LTR-Luc cells infected with different HIV-1 viral strains was incubated at 37°C in presence of various concentration of inhibitors for 3 days. Viral strains used: HxB2 virus (A124/T125 IN variant); NL4.3 virus (T124/T125 IN variant); or recombinant NL4.3 virus (A124/T125, A124/A125, N124/T125, or N125/A125 IN variants)

[c] Determined by measurement of EC$_{50}$ +50% human serum. C8166 LTR-luciferase reporter cells were infected with HIV-1 NL4.3 virus in presence of 50% human serum. After 3-days of cell incubation at 37°C, Steady Glo was added and luminescence was monitored as a measurement of the HIV replication in the presence of various concentrations of inhibitor.

[d] Cytotoxicity for C-8166 LTR-Luc cells was determined using the tetrazolium salt MTT metabolic assay after 3 days of incubation.

In vitro DMPK and CMC parameters

BI 224436 combines excellent solubility at all physiologically relevant pH values (Sol. pH 2.0-6.8 >0.84 mg/mL) with very good cell permeability as measured in the caco-2 assay (A-B: 14 x10^{-6} cm/s) and minimal cytochrome inhibition (CYP2C9 IC$_{50}$: 20 µM). BI 224436's optimized quinoline C4-aryl substituent improved metabolic stability in human hepatocytes (13% QH).

PROBE NAME / NEGATIVE CONTROL	BI 224436	BI-0449
TPSA / cLogP / LogD$_{7.4}$	82 / 4.7 / 0.44	50 / 4.1 / ---
Solubility @ pH 2.0 / 6.8 [µg/ml]	840 / >1,000	-

CACO permeability @ pH 7.4 [*10^{-6} cm/s]	14	-
CACO efflux ratio	0.3	-
Microsomal stability [% Q_H] (human/mouse/rat/monkey/dog)	14 / 6.4 / 7 / 11 / 16	-
Hepatocyte stability [% Q_H] (human/mouse/rat/monkey/dog)	13 / 12 / 9 / 13 / 32	-
Plasma protein binding [%] (human/mouse/rat/monkey/dog)	84.3 / 97.3 / 98.2 / 78.0 / 75.5	-
hERG [inh. % @ 100 µM]	5.8 %	-
CYP 3A4 (IC_{50}) [µM]	23	>50
CYP 2C9 (IC_{50}) [µM]	20	19
CYP 1A2 (IC_{50}) [µM]	>30	-
CYP 2C19 (IC_{50}) [µM]	>30	>50
CYP 2D6 (IC_{50}) [µM]	>30	>50

In vivo DMPK parameters

BI 224436 combines excellent aqueous solubility across pH values (2.0 – 6.8) and good cellular permeability. In addition, BI 224436 displays a good PK profile in all investigated animal species with oral bioavailability typically ranging from 54-100%. For rodents and dogs, *in vivo* clearance (CL, % Q_H) is lower than values predicted by *in vitro* hepatocyte stability whereas monkey species is showing an opposite trend (i.e. higher *in vivo* clearance than anticipated). For rats, low *in vivo* clearance might be attributed to biliary enterohepatic recirculation of the parent compound/acyl glucuronide.[6, 7]

Given the overall favourable potency, *in vitro* ADME-CMC properties, *in vivo* PK profile, and clean animal toxicology studies, BI 224436 was advanced into phase-1a clinical development.

PK profile of BI 224436[a]

BI 224436	MOUSE[b]	RAT[b]	MONKEY[c]	DOG[c]
Clearance [% Q_H]	0.8	0.7	23	8
V_{ss} [l/kg]	0.20	0.45	0.54	0.88
$t_{1/2}$ [h]	2.6	8.8	1.4	5.9
C_{max} [µM]	15 [b]	13 [b]	4.8	12
AUC [µM*h]	99 [b]	75 [b]	6.3	24
F [%]	100	54	82	81

[a] The oral formulation contained 1% MP, 0.3% Tween 80, 0.5% MC; the i.v. formulation contained 70% PEG, 30% water.

[b] Mouse and rat doses: 0.2 mg/kg i.v.; 0.4 mg/kg oral. The oral exposure was dose normalized to 2 mg/kg to allow for an appropriate comparison to monkey and dog PK studies.

[c] Monkey and dog doses: 1.0 mg/kg i.v.; 2.0 mg/kg oral

In vivo pharmacology

No HIV-1 animal model routinely available for PD testing.

Negative control

BI-0449 is a representative compound of the 3-acetic acid-4-aryl quinoline hit series discovered during HTS campaign. Compared to optimized BI 224436, it is ~450-fold less active in the biochemical LTR-cleavage assay. However BI-0449 is inactive in any HIV-1 replicon cellular assay up to the highest concentration tested (>40 µM).

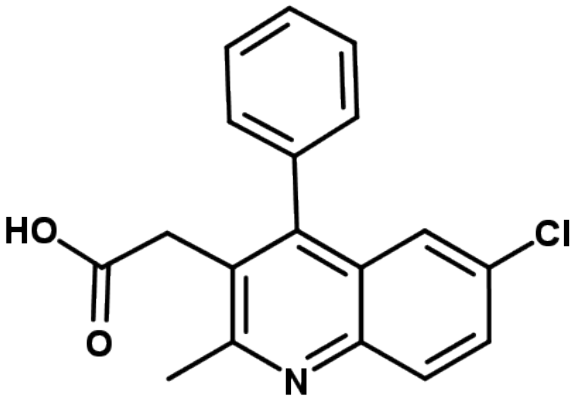

Figure 4: BI-0449, which serves as a negative control.

Selectivity

No panel data available.

BI 224436	SELECTIVITY DATA AVAILABLE
Cerep®	No
Panlabs®	No
Invitrogen®	No
DiscoverX®	No
Dundee	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

The Xray co-crystal structure of target in complex with BI 224436 is not available. However a closely related analog binding to a conserved allosteric pocket of the catalytic core domain of integrase has been reported (Figure 3, PDB code: 4NYF)[1].

Reference molecule(s)

For a review on investigational HIV integrase inhibitors see reference 8.

Summary

BI 224436 is the first non-catalytic-site integrase inhibitor (NCINI) reaching a clinical trial. It combines high solubility at all relevant physiological pH values with good cell permeability and good metabolic stability. BI 224436's optimized *in vitro* ADME profile also translates into a good *in vivo* PK profile in preclinical animal species, with oral bioavailability ranging from 54-100%.

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The chemical structure of BI 224436 is also worth highlighting, since unsymmetrical C4-aryl substituent of the quinoline scaffold produced a stable chiral atropisomer that raised development complexity.[5]

Overall, as the first representative of a new class of HIV-1 integrase inhibitors tested in clinical trials, BI 224436's attractiveness stems from its potent antiviral activity in addition to its good *in vivo* ADME-PK profile following oral (or *i.v.*) dosing.

Supplementary data

2-D structure files can be downloaded free of charge from opnMe.

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