opnMe by Boehringer Ingelheim

Capsid Assembly Inhibitor

BI-3257

Boehringer Ingelheim

Table of contents

Summary

BI-3257 inhibits replication of HIV-1 with nanomolar potency by inhibiting the assembly of the capsid core of the virus. A related negative control is available (BI-3545).

Chemical Structure

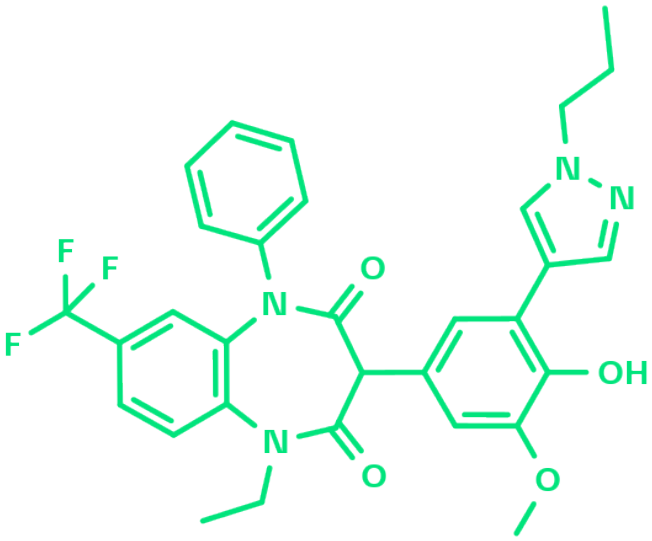

Figure 1: 2D structure of BI-3257

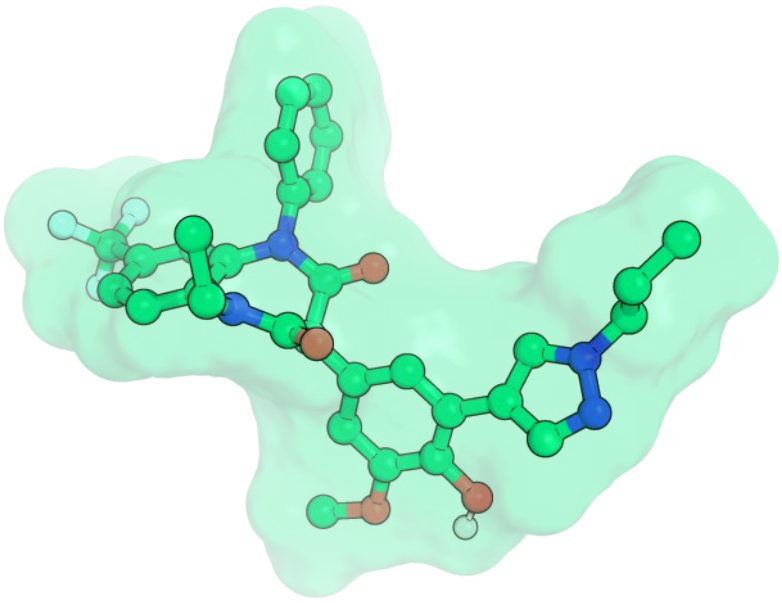

Figure 2: BI-3257, 3D conformation, derived from the conformation of a highly related compound observed in complex the capsid N-terminal domain

Highlights

BI-3257 is an inhibitor of HIV replication with nanomolar activity. It prevents the formation of the capsid core structure that encapsulates the viral genome and its associated enzymes, reverse transcriptase and integrase. BI-3257 binds to an induced pocket in the N-terminal domain of the capsid protein and induces significant structural changes (displacement of the a1-helix), likely affecting the protein-protein interactions that define the regular structures of the viral capsid core. The binding location of BI-3257-type compounds in the capsid monomer was elucidated by X-ray crystallography, and electron microscopy allowed the elucidation of its mode of action. Due to this innovative mechanism of viral replication, BI-3257 is equally potent against the wild-type as well as mutant viruses that are resistant to the four major classes of antiretroviral inhibitors[1].

Target information

BI-3257 targets the HIV-1 capsid (CA) protein. CA is initially synthesized as the central region of the 55-kDa Gag polyprotein, which is the protein that mediates the assembly and budding of the immature virion. In this context, CA provides key protein-protein interactions required for immature virion assembly [2,3]. During viral maturation, proteolytic cleavage of Gag releases CA, allowing the protein to assemble into a cone-shaped central capsid that surrounds the viral RNA genome and its associated enzymes, reverse transcriptase and integrase [4]. The capsid structure is stabilized by multiple weak protein-protein interactions.

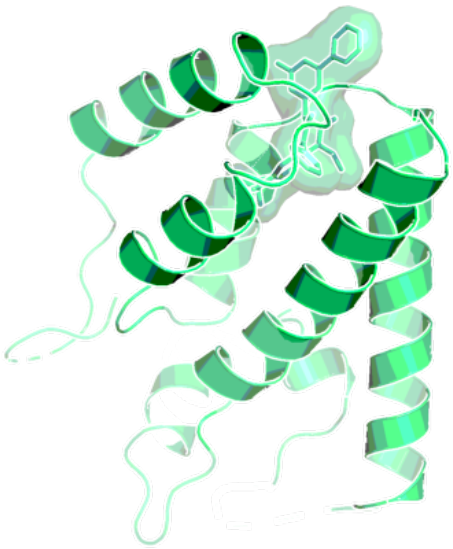

Figure 3: Complex of a compound highly related to BI-3257 with the N-terminal domain of the capsid monomer, as solved by X-ray crystallography (PDB code: 4E91) [1].

In vitro activity

BI-3257 displays an EC_{50} of 70 nM in a viral replication assay and induces disassembly of viral capsid structures with an IC_{50} of 160 nM. No cytotoxicity was found up to a concentration of 28 µM.

PROBE NAME / NEGATIVE CONTROL	BI-3257	BI-3545
MW [Da, free base][a]	578.6	468.4
Assay A (EC_{50}) [nM]	70 [1,6]	n.d.
Assay B (IC50) [nM]	>413	n.d.
Assay C (IC50) [nM]	160 [6]	>9,300
Assay D (CC_{50}) [nM]	>28,000	n.d.

[a] For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

Assay A: Standard antiviral replication assay [1]; C8166-LTR-luciferase cells were infected with HIV-1 NL4-3 for 1.5 h and were seeded in assay wells already containing inhibitors. The EC_{50} corresponds to a 50% reduction of viral replication as observed via spectroscopic measurement of the luciferase expression levels.

Assay B: Capsid assembly assay [7]; An assay that targets the assembly of the capsid protein (CA) *in vitro* into capsid-like oligomers. The assay makes use of a recombinant CA protein fused to nucleocapsid (NC) as found in the immature Gag polyprotein. *In vitro* assembly of the CA-NC fusion protein into capsid-like oligomers can be stimulated by single-stranded DNA oligonucleotides of TG repeat sequence at low protein concentrations. They stimulate assembly by binding to NC and remain bound in the assembled capsid-like oligomers. The assay will measure the assembly of CA-NC oligomers in the presence of a mixture of two TG repeat oligonucleotides: one labeled with biotin, the other labeled with fluorescein. CA-NC oligomers will be captured on strepavidin-coated 384 well plates and washed and detected by measuring the level of fluorescence they contain as only capsid-like oligomers will be able to bind both kinds of oligonucleotides simultaneously.

Assay C: Capsid disassembly assay [7]; This assay adds a step to the HIV Capsid Assembly assay (Assay B). The whole assembly process is performed, and then inhibitors are incubated with assembled CA complexes, that will lead to disassembly. The level of disassembly is measured by the fluorescence captured on neutravidin-coated 384 well plates.

Assay D: See reference 5

In vitro DMPK and CMC parameters

BI-3257 shows a good cell permeability and a half-life of 86 and 67 min in human and rat microsomes, respectively. BI-3257 shows rather low solubility.

PROBE NAME / NEGATIVE CONTROL	BI-3257	BI-3545
logD @ pH 2 / 11	4.5 / 4.4	4.1 / 0.9
Solubility @ pH 2.0 / 6.8 [µg/mL]	0.13 / <0.1	4.8 / 573
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	9.9 [6]	n.a
Microsomal stability (human/rat) [$t_{1/2}$, min]	86 / 67 [6]	n.a
CYP 3A4 (IC_{50}) [µM]	3.6 [6]	n.a
CYP 2D6 (IC_{50}) [µM]	>30 [6]	n.a

Negative control

BI-3545 is structurally highly related to BI-3257 but is much more soluble due to the presence of a carboxylate group. It was found inactive in the capsid disassembly assay at a concentration of 9.3 µM.

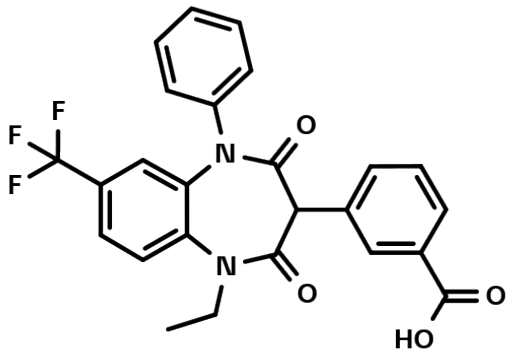

Figure 4: BI-3545 which serves as a negative control

Selectivity

BI-3257 displays an EC_{50} of 70 nM in a viral replication assay and induces disassembly of viral capsid structures with an IC_{50} of 160 nM. In selectivity panels it showed no relevant off-target effects. The negative control BI-3545 showed in 1 out of 44 targets (PDE4D2) inhibition with more than 50% @ 10µmol.

SELECTIVITY DATA AVAILABLE	BI-3257	BI-3545
SafetyScreen44™ with kind of support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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

The X-ray crystal structure of target in complex with BI-3257 was not determined. However, the structure with a highly related compound is available (PDB code: 4E91) [1].

Reference molecule(s)

We have disclosed a second chemotype binding to the same pocket of the CA N-terminal domain as BI-3257 (see compound BM1[1]). Compounds of this type have a distinct binding mode and induce a somewhat different structural change in the CA protein. Also, BM1-type compounds likely primarily inhibit the maturation of the conical capsid structure, while BI-3257 acts at an earlier stage of the replication cycle: it already inhibits the assembly of the CA-containing Gag polyprotein, which mediates the assembly and budding of the immature virion. Another compound described earlier and binding to a similar surface location on the capsid protein is CAP-1. However, this compound binds less deep in the protein and has a binding affinity of only ~800 µM[9]. Other molecules binding to at least two additional locations on CA have been described (see [1] and references therein).

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

1. Lemke C. T., Titolo S., Von Schwedler U., Goudreau N., Mercier J-F., Wardrop E., Faucher A-M., Coulombe R., Banik S. S R, Fader L., Gagnon A., Kawai S. H., Rancourt J., Tremblay M., Yoakim C., Simoneau B., Archambault J., Sundquist W. I., Mason S. W. Distinct Effects of Two HIV-1 Capsid Assembly Inhibitor Families That Bind the Same Site within the N-Terminal Domain of the Viral CA Protein *J Virol.* **2012,** 86:6643–6655. DOI:10.1128/jvi.00493-12 , PubMed.

2. Ganser-Pornillos B. K., Cheng A., Yeager M. Structure of Full-Length HIV-1 CA: A Model for the Mature Capsid Lattice *Cell* **2007**, 131:70–79. DOI:10.1016/j.cell.2007.08.018, PubMed.

3. Von Schwedler U. K., Stray K. M., Garrus J. E., Sundquist W. I. Functional Surfaces of the Human Immunodeficiency Virus Type 1 Capsid Protein *J Virol.* **2003**, 77:5439–5450. DOI:10.1128/jvi.77.9.5439-5450.2003, PubMed.

- Sundquist W. I., Hill C. P. How to Assemble a Capsid *Cell.* **2007**, 131:17–19. DOI:10.1016/j.cell.2007.09.028, PubMed.

4. Fader L. D., Bethell R., Bonneau P., Bös M., Bousquet Y., Cordingley M. G., Coulombe R., Deroy P., Faucher A.-M., Gagnon A., Goudreau N. Grand-Maître C., Guse I., Hucke O., Kawai S. H., Lacoste J.-E., Landry S., Lemke C. T., Malenfant E., Mason S., Morin S., O'Meara J., Simoneau B., Titolo S., Yoakim C. Discovery of a 1,5-dihydrobenzo[b][1,4]diazepine-2,4-dione series of inhibitors of HIV-1 capsid assembly *Bioorg Med Chem Lett.* **2011**, 21:398–404. DOI:10.1016/j.bmcl.2010.10.131, PubMed.

5. Fader L. D., Landry S., Goulet S., Morin S., Kawai S. H., Bousquet Y., Dion I., Hucke O., Goudreau N., Lemke C. T., Rancourt J., Bonneau P., Titolo S., Amad M., Garneau M., Duan J., Mason S., Simoneau B. Optimization of a 1,5-dihydrobenzo[b][1,4]diazepine-2,4-dione series of HIV capsid assembly inhibitors 2: Structure–activity relationships (SAR) of the C3-phenyl moiety *Bioorg Med Chem Lett.* **2013**, 23:3401–3405. DOI:10.1016/j.bmcl.2013.03.074, PubMed.

6. Simoneau B., Deroy P., Fader L., Faucher A. M., Gagnon A., Grand-Maitre C., Kawai S., Landry S., Mercier J. F., Rancourt J. 1-Phenyl-1,5-dihydrobenzo[b][1,4]diazepine-2,4-

dione derivatives as HIV replication inhibitors and their preparation and use for the treatment of HIV infection. WO 2011/100838, 2011

7. Fader L. D., Landry S., Morin S., Kawai S. H., Bousquet Y., Hucke O., Goudreau N., Lemke C. T., Bonneau P., Titolo S., Mason S., Simoneau B. Optimization of a 1,5-dihydrobenzo[b][1,4]diazepine-2,4-dione series of HIV capsid assembly inhibitors 1: Addressing configurational instability through scaffold modification *Bioorg Med Chem Lett*. **2013**, 23:3396–3400. DOI:10.1016/j.bmcl.2013.03.073, PubMed.

8. Kelly B. N., Kyere S., Kinde I., Tang C., Howard B. R., Robinson H., Sundquist W. I., Summers M. F., Hill C. P. Structure of the Antiviral Assembly Inhibitor CAP-1 Complex with the HIV-1 CA Protein *J Mol Biol*. **2007**, 373:355–366. DOI:10.1016/j.jmb.2007.07.070, PubMed.