HIV NNRTI | BI-2540

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Summary

BI-2540 is a potent inhibitor of HIV non-nucleoside reverse transcriptase (NNRT) and cross-reactive against clinically relevant mutants of reverse transcriptase.

Chemical Structure

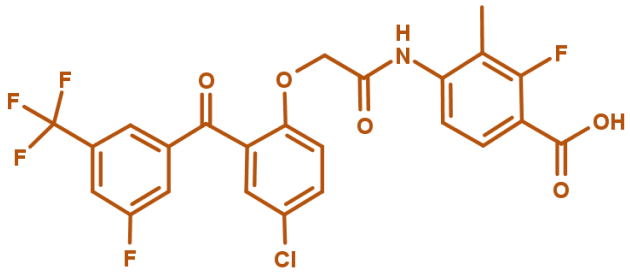

Figure 1: 2-D structure of BI-2540, a non-nucleoside reverse transcriptase inhibitor (NNRTI)

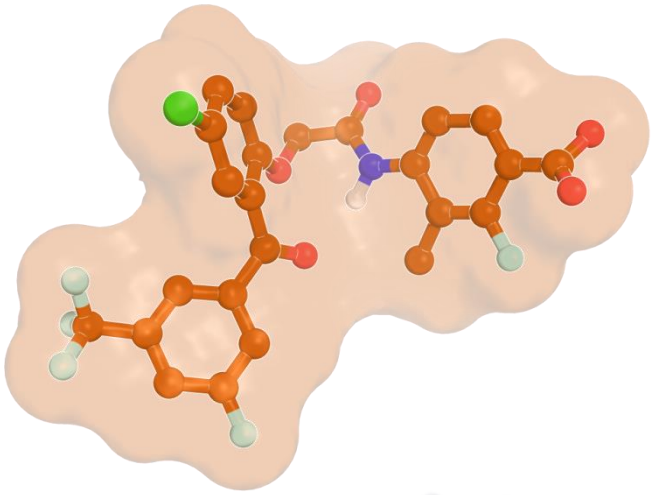

Figure 2: BI-2540, 3D conformation.

Highlights

BI-2540 is a potent inhibitor of HIV non-nucleoside reverse transcriptase (NNRT) and cross-reactive against clinically relevant mutants of reverse transcriptase. BI-2540 shows low clearance and good

bioavailability upon *p.o.* dosing in rats. With BI-2439, a relatively close analog of BI-2540 with significantly lower activity (420-fold) is available as negative control for in vitro experiments.

Target information

Description of the protein: The human immunodeficiency virus (HIV-1) reverse transcriptase (RT) enzyme performs the retrotranscription of the viral single-stranded RNA into double-stranded linear DNA. The viral genomic retrotranscription arises from the cooperative effect of two enzymatic functions of RT: a DNA polymerase activity (copy of the RNA/DNA template) and ribonuclease H activity (cleavage of RNA when part of a RNA/DNA duplex).

Protein domain structure: RT of HIV-1 is a heterodimer composed of subunit p66 (560 AA) and p51 (440 AA). The larger subunit p66 contains both active sites responsible for RT enzymatic functions (DNA polymerase and RNase H activities).[1]

Molecular mechanism: there are two different classes for RT inhibitors: nucleoside RT inhibitors (NRTI) and non-nucleoside RT inhibitors (NNRTI). NNRTI bind to an allosteric site adjacent to the polymerase active site (~10 Å). Binding of NNRTI interferes with the chemical step of DNA synthesis by affecting the alignment of the primer terminus with the polymerase active site. Thus, NNRTIs efficacy stems from the structural rearrangement in the p66 subunit which precludes viral DNA synthesis.

Biology: HIV-1 largely infects CD4-positive T lymphocytes and macrophage cells, thus destroying the immune system. Lymphoid organs are a major reservoir of ongoing HIV-1 replication. HIV life cycle consists of seven steps: 1) binding, 2) fusion, 3) reverse transcription, 4) integration, 5) replication, 6) assembly, and 7) budding.

Disease link: HIV-1 infects >30 million people worldwide. Infected patients cannot be cured; therefore a “triple drug cocktail” of antiretroviral therapy (ART) must be continuously administered. Combination drug therapies suppress viral load by blocking viral replication and improving efficacy to overcome resistant variants.[1,2]

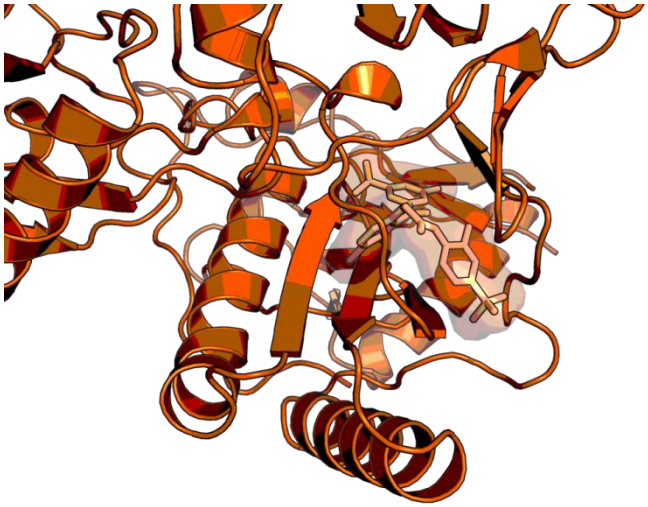

Figure 3: HIV NNRTI in complex with related structure GW564511 (PDB code: 3DLG)

In vitro activity

BI-2540 is a potent HIV non-nucleoside reverse transcriptase (NNRT) inhibitor. For cellular efficacy against RT mutants please refer to the selectivity section.

PROBE NAME / NEGATIVE CONTROL	BI-2540	BI-2439
MW [Da]	528	476
HIV1-RT Pico, WT (IC_{50}) * [nM]	12	5053
HIV replicon ELISA, WT (EC_{50}) § [nM]	0.76	n.d.
HIV replicon LUC, WT (EC_{50}) ς [nM]	2.6	n.d.

* Enzymatic assay: HIV-1 reverse transcriptase (Picogreen Fluorescence Assay); 15 min pre-incubation before adding substrate solution, 50 min incubation at 37°C, PicoGreen as a fluorescent intercalator.

§ HIV viral replication assay: C8166 cells, 72 h incubation at 37°C, readout: ELISA quantification of p24

ς HIV-1 luciferase assay: C8166 cells, 72 h incubation at 37°C, readout: RLU of luciferase, Steady Glo.

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-2540	BI-2439
cLogP	5.7	4.2
Solubility @ pH 7.0 [µg/ml]	>52	n.d.
CACO permeability @ pH 4 / 7.4 [*10^{-6} cm/s]	0.2 / 23	n.d. / 12
CACO efflux ratio	0.1	1.4
Microsomal stability [% Q_H] (human)	<24	n.d.
Plasma protein binding [%] (human)	99.5	n.d.
CYP 3A4 (IC_{50}) [µM]	18	>50
CYP 2C9 (IC_{50}) [µM]	>30	8.5
CYP 1A2 (IC_{50}) [µM]	>30	>50
CYP 2C19 (IC_{50}) [µM]	>30	>50
CYP 2D6 (IC_{50}) [µM]	>30	>50

In vivo DMPK parameters

BI-2540 shows a low clearance and good bioavailability upon *p.o.* dosing in rats.

BI-2540	RAT
Clearance [% Q_H][a]	2.9
V_{ss} [l/kg][a]	2.26
MRT [h][a]	12.8
t_{max} [h][b]	1.0
C_{max} [µM][b]	11860
AUC [µM*h][b]	125620
F [%][b]	53

[a] *i.v.* (2 mg/kg)

[b] *p.o.* (10 mg/kg)

In vivo pharmacology

No data available.

Negative control

BI-2439 is a relatively close analog of BI-2540 with significantly lower activity (420-fold) in the HIV-RT Picogreen Fluorescence assay (IC_{50} = 5 µM) and is therefore offered as an *in vitro* negative control.

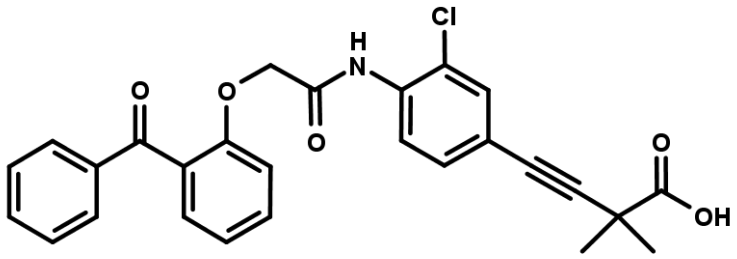

Figure 4: BI-2439 which serves as a negative control

Selectivity

Cellular efficacy against RT mutants: HIV-1 luciferase assays: C8166 cells, 72 h incubation at 37°C; readout: RLU of luciferase, Steady Glo

MUTATIONS	BI-2549	X-FOLD VS. WT
Wild type (WT) EC_{50} [nM]	2.58	--
A98G EC_{50} [nM]	3.94	1.5
K103N EC_{50} [nM]	2.36	0.9
V106A EC_{50} [nM]	12.8	5.0
V106I EC_{50} [nM]	4.19	1.6
E138K EC_{50} [nM]	15.0	5.8
Y181C EC_{50} [nM]	2.44	0.9
Y188C EC_{50} [nM]	0.38	0.1

Y188L EC_{50} [nM]	39.1	15
G190A EC_{50} [nM]	3.10	1.2
P236L EC_{50} [nM]	7.53	2.9
L100I/K103N EC_{50} [nM]	3.18	1.2
K103N/G190A EC_{50} [nM]	8.16	3.2
K103N/V108I EC_{50} [nM]	4.82	1.9
K103N/Y181C EC_{50} [nM]	6.54	2.5
K103N/P225H EC_{50} [nM]	4.77	1.8
V106A/E138K EC_{50} [nM]	119	46
V106A/P236L EC_{50} [nM]	199	77
V106I/E138K EC_{50} [nM]	38.1	15
V106I/P236L EC_{50} [nM]	36.1	14
E138K/P236L EC_{50} [nM]	60.3	23

No data panel available

BI-2540	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.

No X-ray available.

Reference molecule(s)

Commercially available NNRTIs: Nevirapine, Delavirdine, Efavirenz, Dapivirine, Etravirine, Rilpivirine.[2]

Summary

BI-2540 is a potent inhibitor of HIV non-nucleoside reverse transcriptase (NNRT) and cross-reactive against clinically relevant mutants of reverse transcriptase. BI-2540 shows low clearance and good bioavailability upon *p.o.* dosing in rats. With BI-2439, a relatively close analog of BI-2540 with significantly lower activity (420-fold) is available as negative control for in vitro experiments.

A practical synthesis for BI-2540 was reported.[3]

Supplementary data

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

References

1. Stefan G. Sarafianos, Bruno Marchand, Kalyan Das, Daniel M. Himmel, Michael A. Parniak, Stephen H. Hughes and Eddy Arnold Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition *J. Mol Biol.* **2009**, *385*, 693-713. DOI: 10.1016/j.jmb.2008.10.071, PubMed.

2. Iris Usach, Virginia Melis and Jose-Esteban Peris Non-nucleoside reverse transcriptase inhibitors: a review on pharmacokinetics, pharmacodynamics, safety and tolerability *J. Int AIDS Soc.* **2013**, *16*, 1-14. DOI: 10.7448/IAS.16.1.18567, PubMed.

3. Xiao-jun Wang, Li Zhang, Xiufeng Sun, Heewon Lee, Dhileepkumar Krishnamurthy, Jeff A. O'Meara, Serge Landry, Christiane Yoakim, Bruno Simoneau, Nathan K. Yee, and Chris H. Senanayake Practical Synthesis of A Benzophenone-Based NNRT Inhibitor of HIV-1 *Org. Process Res. Dev.* **2012**, *16*, 561-566. DOI: 10.1021/op200301h.