CMV polymerase inhibitor | BI-9553

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Summary

BI-9553 is a selective, potent and well characterized non-nucleoside CMV polymerase inhibitor. BI-0309 is available as its negative control.

Chemical Structure

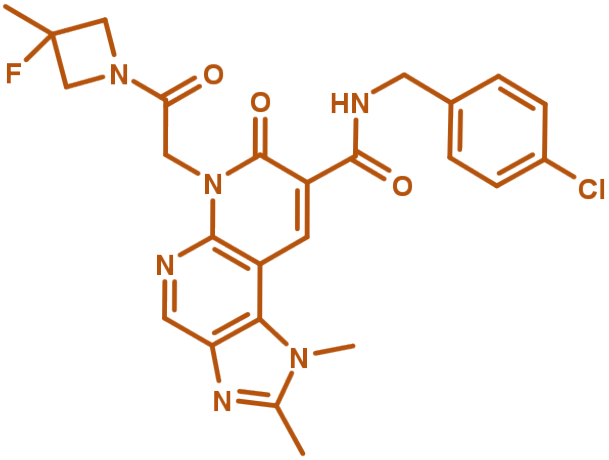

Figure 1: 2-D structure of BI-9553, a potent and selective CMV polymerase inhibitor

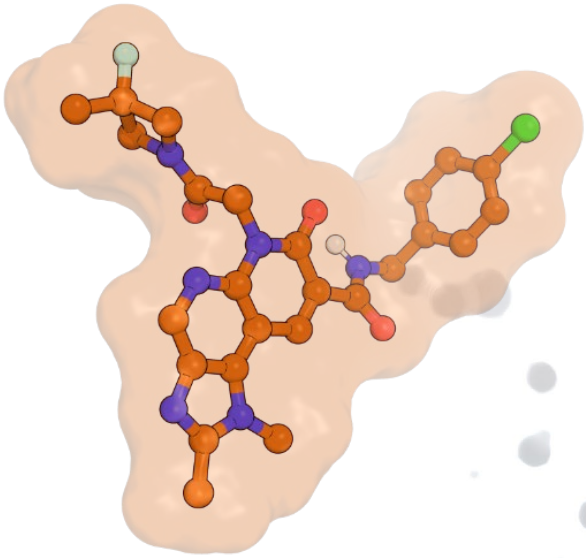

Figure 2: BI-9553, 3D low energy conformation

Highlights

BI-9553 is a potent and selective non-nucleoside CMV polymerase inhibitor (EC_{50} < 30 nM). This compound has been extensively characterized. It shows good cell permeability, reasonable hepatocyte stability across species, and good bioavailability in rat and mouse. Thus, BI-9553 is suitable for both *in vitro* and *in vivo* experiments.

Target information

Human cytomegalovirus (HCMV) belongs to the beta-herpes virus family and is among the largest of the DNA viruses.[1] HCMV can cause severe life-threatening infections especially in immunocompromised and immunonaïve patients. Congenital CMV infection is also a leading cause of birth defects, such as hearing loss. One essential enzyme for viral replication is the CMV DNA polymerase encoded by the UL54 gene.[2] Inhibition of CMV polymerase enzymatic activity has been clinically validated, however current gold standard therapies face considerable challenges such as drug resistance and poor tolerability.[3]

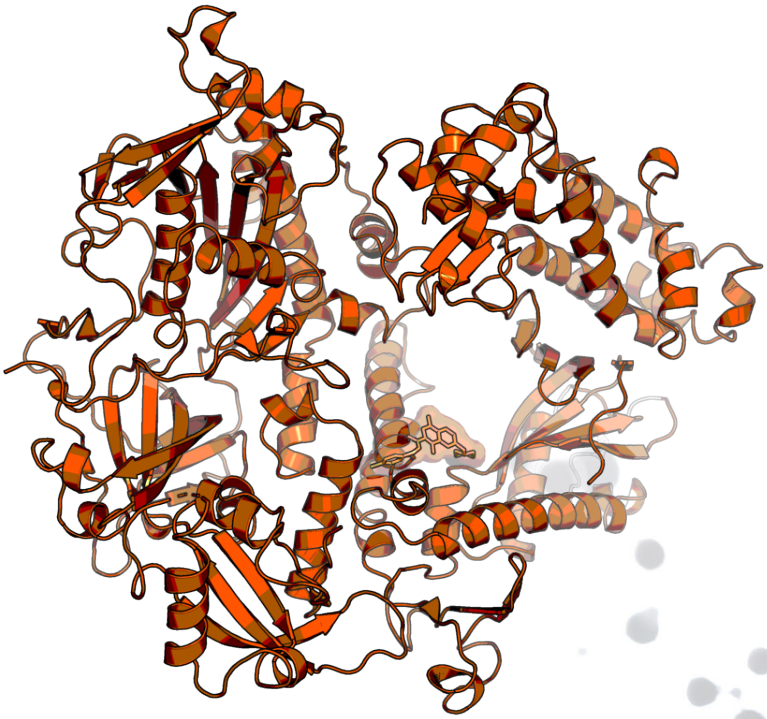

Figure 3: Homology model of HCMV polymerase showing the binding mode of an inhibitor structurally related to BI-9553, bound to the active site. (Homology model based on the X-ray structure of Herpes simplex virus polymerase, PDB code: 2gv9

In vitro activity

BI-9553 shows a good potency with an $EC_{50} < 30$ nM in a qPCR cell-based assay. Cell activity could be dissociated from cytotoxicity depending on different cell lines.

PROBE NAME / NEGATIVE CONTROL	BI-9553	BI-0309
MW [Da]	510.9	492.5
HCMV Pol. LAN (IC_{50}) [nM][a]	46	>5,000
HCMV qPCR_AD169 (EC_{50}) [nM][b]	28	>5,800
Syber Green II (IC_{50}) [nM][c]	>100,000	n.a.
Cytotoxicity (CC_{50}) [µM][d]	1-30	n.a.

[a] HCMV Polymerase LANCE TR-FRET Assay: purified recombinant HCMV polymerase (UL54) using a Digoxigenin-labeled oligonucleotide priming a heteropolymeric template. The enzymatic activity is measured by incorporating biotin-dUTP in the nascent complementary strand. The signal is generated by FRET from the donor (anti-Digoxigenin-Europium chelate binding with the primer) to the acceptor (Streptavidin-APC) binding to the biotin of the labelled nucleotides incorporated in proximity.

[b] qPCR cell-based assay: this assay evaluates the propensity of a compound to inhibit the replication of HCMV viral DNA during the first 72h. MRC-5 cells (5% FBS), HCMV virus strain is AD169, MOI= 0.05; in MRC-5 cells a SI with >1000 could be measured.

[c] DNA intercalation biochemical assay.

[d] Variable cytotoxicity observed in different cell lines.

In vitro DMPK and CMC parameters

BI-9553 has a good cell permeability and reasonable hepatocyte stability across species.

PROBE NAME / NEGATIVE CONTROL	BI-9553	BI-0309
cLogP / LogD pH 7.4 / LogD pH11	-/ 2.8 / 2.9	-/-/ 1.09
Solubility @ pH 7 [µg/ml]	0.8	< 1
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	20.4	0.4
CACO efflux ratio	n.a.	37
Microsomal stability [% Q_H] (human/mouse/rat)	27 / <24 / <23	27 / <23 / <22
Hepatocyte stability [% Q_H] (human/mouse/rat)	38 / 24 / 8	n.a.
Plasma protein binding [%] (human/mouse/rat)	89.9 / 94.2 / 93.0	n.a.
hERG [µM]	n.a.	n.a.
CYP 3A4 (IC_{50}) [µM]	12.4	>50
CYP 2C8 (IC_{50}) [µM]	18	>50
CYP 2C9 (IC_{50}) [µM]	13.5	>50
CYP 2C19 (IC_{50}) [µM]	>30	>50
CYP 2D6 (IC_{50}) [µM]	21.3	>50

In vivo DMPK parameters

BI-9553 shows a good clearance and MRT in rat and moderate ones in mouse. Bioavailability is good in both species.

BI-9553	MOUSE [A]	RAT [B]
Clearance [% Q_H]	30.2	10.3
Mean residence time (MRT) after iv dose [h]	0.8	2.5
t_{max} [h]	0.5	3.2
C_{max}_DN [nM]	1,509	1,158
F [%]	32	82
V_{ss} [l/kg]	1.5	1.5

[a] Dose for mouse i.v. and oral: 2.0 mg/kg and 10 mg/kg
[b] Dose for rat i.v. and oral: 2.0 mg/kg and 5.0 mg/kg

Negative control

Despite having high structural similarity to BI-9553, the negative control BI-0309 is inactive in biochemical and cellular assays, due to a substitution of a non-polar para-chlorine by a polar para-phenol group.

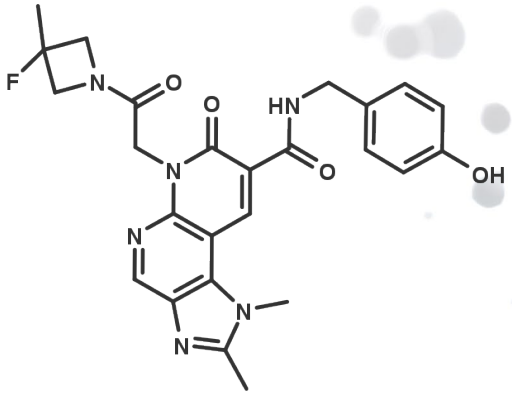

Figure 4: BI-0309 which serves as a negative control

Selectivity

BI-9553 is not showing any effect on kinase activity (82 kinase panels tested @10 µM, all <36% inhibition) and did not show any activity in a panel of 44 receptors at 10µM (all <39% inhibition @10 µM). Negative control BI-0309 did not hit any receptor out of 44 targets @10µM.

SELECTIVITY DATA AVAILABLE	BI-9553	BI-0309
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

Other CMV polymerase inhibitors are commercially available e.g. ganciclovir or valganciclovir.

Supplementary data

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

References

1. Ye L., Qian Y., Yu W., Guo G., Wang H., Yue X. Functional Profile of Human Cytomegalovirus Genes and Their Associated Diseases: A Review *Front Microbiol.* **2020**, 11, 2104. DOI: 10.3389/fmicb.2020.02104, PubMed.

2. Dunn W., Chou C., Li H., Liu F. Functional profiling of a human cytomegalovirus genome *PNAS* **2003**, 100(24), 14223-14228. DOI: 10.1073/pnas.2334032100, PubMed.

3. Bogner E., Egoroa A., Makarov V. Small Molecules – Prospective Novel HCMV Inhibitors, *Viruses* **2021**, 13, 474. DOI: 10.3390/v13030474, PubMed.

4. Beaulieu P.L., Bailey M., Bilodiau F., Carson R., Giroux A., Godbout C., Hucke O., Joly M-A., Leblanc M., Lepage O., Moreau B., Naud J., Poirier M., Villemure E. Patent WO 2013152065, **2013**.