Telomerase Inhibitor | BIBR1532

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Summary

BIBR1532 is a highly potent and selective inhibitor of the human telomerase. With excellent permeability and sufficient metabolic stability, it induces telomere shortening and is suitable for both *in vitro* and *in vivo* experiments.

Chemical Structure

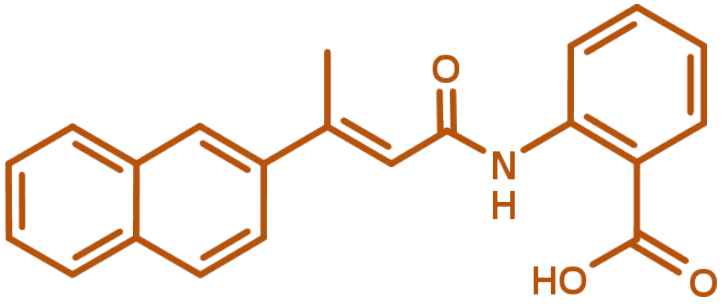

Figure 1: 2-D structure of BIBR1532, a human telomerase inhibitor

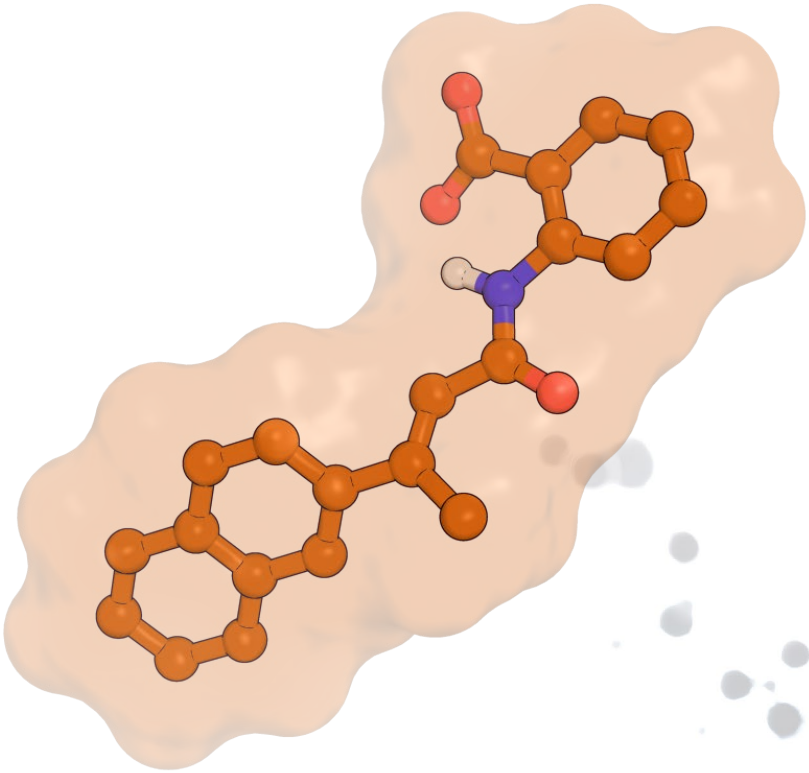

Figure 2: 3-D structure of BIBR1532, a human telomerase inhibitor

Highlights

BIBR1532 is a highly potent and selective inhibitor of the human telomerase. With excellent permeability and sufficient metabolic stability, it induces telomere shortening by interfering with the processivity of the enzyme, whilst also lacking acute cytotoxicity. Its *in vivo* PK profile is good, with high bioavailability and plasma exposure shown in mice. Thus, BIB1532 is suitable for both *in vitro* and *in vivo* experiments.

Target information

Telomerase is a ribonucleoprotein responsible for the maintenance of the telomere. The cellular RNA-dependent DNA polymerase is an enzyme comprised of a template-containing RNA subunit (TR) and a protein component including the catalytic subunit telomerase reverse transcriptase (TERT). The tetrameric enzyme complex of human telomerase consisting of two hTR and two hTERT molecules is capable of elongating a short single-stranded DNA by adding multiple TTAGGG repeats to the 3'-end of a suitable DNA primer. Telomerase activity is detected in the majority of tumors, but is absent in most somatic tissues and there is scientific evidence that it could be used as a prognostic marker for certain cancer types. It was shown that inhibition of telomerase disrupts telomere maintenance leading to telomere erosion which in turn results in proliferation arrest and cell death as part of *in vitro* and *in vivo* experiments.

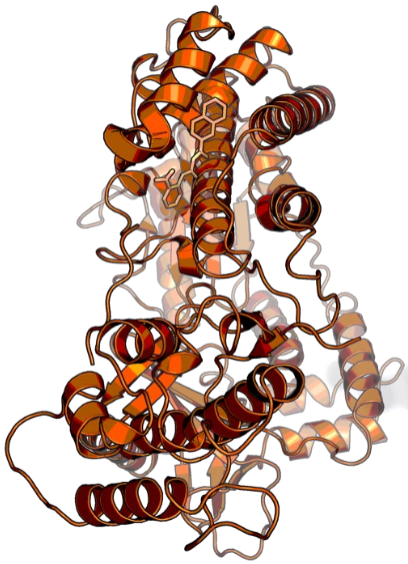

Figure 3: 3-D structure of telomerase

In vitro activity

BIBR1532 shows excellent selectivity for hTERT over RNA polymerases (IC_{50} (hTERT) = 93 nM; IC_{50} (human RNA polymerase I) >100 µM, IC_{50} (human RNA polymerase II + III) >100 µM).

BIBR1532 induces a senescence phenotype and anti-proliferative effects demonstrated by growth arrest in various cancer cell lines, including NCI-H460 lung carcinoma, HT1080 fibrosarcoma, MDS-MB231 breast carcinoma and DU145 prostate carcinoma cells.

PROBE NAME / NEGATIVE CONTROL	BIBR1532	BIBR1654
MW [Da]	353.35 (sodium salt)	418.50
hTERT (IC_{50}) [nM][a]	93	5,000
mTERT (IC_{50}) [µM][a]	>50	-
TRAP (IC_{50}) [nM][b]	200	-

[a] conventional enzyme activity assay, assay conditions see reference 1.
[b] PCR-based TRAP assay, assay conditions see reference 2.

On a cellular level, BIBR1532 efficiently induces telomere shortening and limits cell proliferation in a number of cancer cell lines (Figure 4).[1]

A **B** **C**

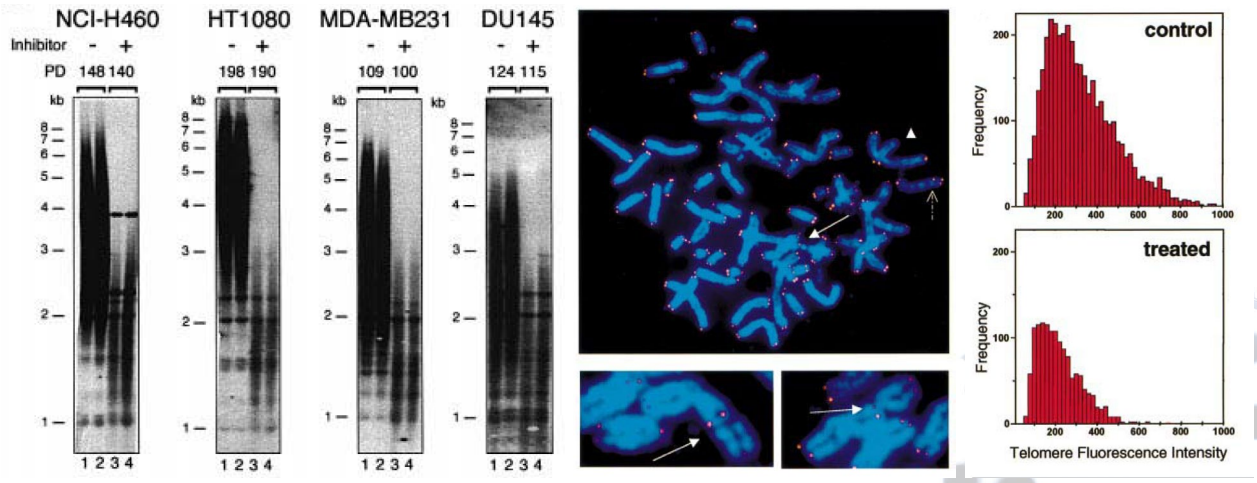

Figure 4: A Total genomic DNA from untreated (lane 1), solvent-treated (lane 2) or inhibitor-treated (lanes 3 and 4) cancer cell lines, including NCI-H460 lung carcinoma, HT1080 fibrosarcoma, MDS-MB231 breast carcinoma and DU145 prostate carcinoma cells, was assessed for telomere restriction by Southern blot analysis. **B** Telomere analysis of inhibitor-treated NCI-H460 cells; Q-FISH analysis of metaphase chromosomes (Arrowhead denotes missing telomeres; arrow denotes fused chromosomes; dashed arrow denotes interchromosomal telomere signal). **C** Telomere analysis of inhibitor-treated NCI-H460 cells; histograms expressing fluorescence intensity and frequency of all individual telomere spots from NCI-H460 derived metaphases.

In vitro DMPK and CMC parameters

BIBR1532 displays excellent cell permeability and minimal cytochrome inhibition. A rather high logP causes low solubility and high plasma protein binding.

PROBE NAME / NEGATIVE CONTROL	BIBR1532	BIBR1654
logP	4.10	3.71
Solubility @ pH 6.8 [µg/ml]	66.0	66.0
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	5.9	13.0
CACO efflux ratio	1.0	0.8
Microsomal stability (human/mouse) [% Q_H]	87/43	60/74
Hepatocyte stability (human/mouse) [% Q_H]	<25 / <25	-
Plasma protein binding (human/mouse) [%]	>99 / >99	-
CYP 3A4 (IC_{50}) [µM]	50	-
CYP 1A2 (IC_{50}) [µM]	10	-
CYP 2C9 (IC_{50}) [µM]	1.5	-
CYP 2C19 (IC_{50}) [µM]	42	-

In vivo DMPK parameters

BIBR1532 displays a good PK profile in mice[a] with a high bioavailability and plasma exposure while exhibiting a low acute toxicity (LD_{50} of 2000 mg/kg). Due to its good bioavailability and high plasma exposure levels, the compound can be used as a tool for *in vitro* as well as *in vivo* studies.

PROBE NAME	BIBR1532
$t_{1/2}$ [h]	12.7
AUC [ng*h/mL]	140761
F [%]	80
V_{ss} [l/kg]	0.1

[a] Mouse doses: 1 mg/kg *i.v.*; 5 mg/kg oral. The *i.v.* formulation contained 25% HP-β-CP in water; the oral formulation was a natrosol suspension.

In vivo pharmacology

Due to favourable PK and toxicity (see section *in vivo* DMPK parameters), BIBR1532 can be used as a tool in rodent *in vivo* models. *In vivo* efficacy of BIBR1532 was previously demonstrated in an immunodeficient mice model, where effects on tumor growth were observed.[1] NMRI mice carrying subcutaneous implants of telomere-shortened HT1080 cells, were treated with BIBR1532 at a dose of 100 mg/kg/day orally. Over the 60 days of treatment, the mice developed no or only small tumors.

A B C

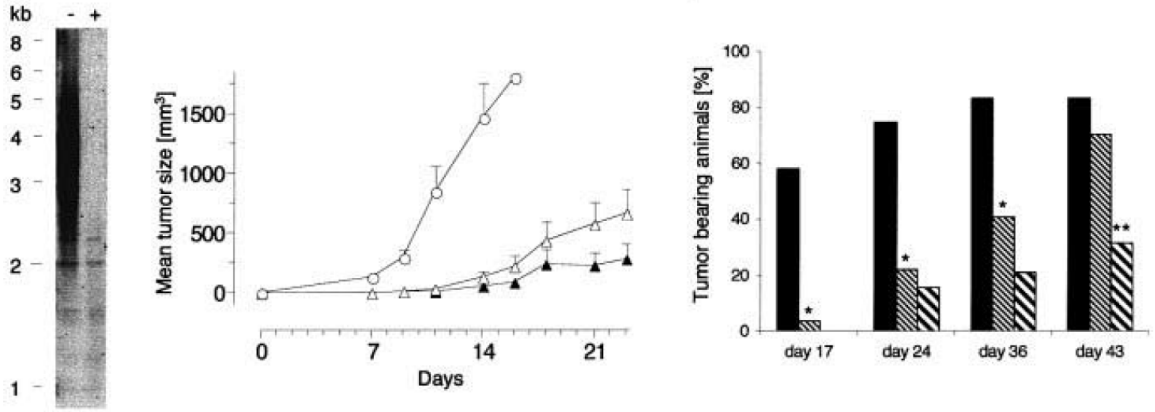

Figure 5: Tumorgenicity assay. **A** Telomere length of untreated control (-) and inhibitor-treated (+) HT1080 cells used to inject mice. **B** Mean tumor size of animals bearing control (open circles) and pre-senescent cells in the absence (open triangles) or presence (closed triangles) of BIBR1532. **C** Number of animals (percentage) with a tumour > 1000 mm^3 for untreated control (black) and pre-treated cells in the absence (thin stripes) or presence (bold stripes) of BIBR1532 at the indicated days after injection.

Negative control

The structurally related molecule BIBR1654 serves as a negative control with IC_{50} (hTERT) = 5 µM.

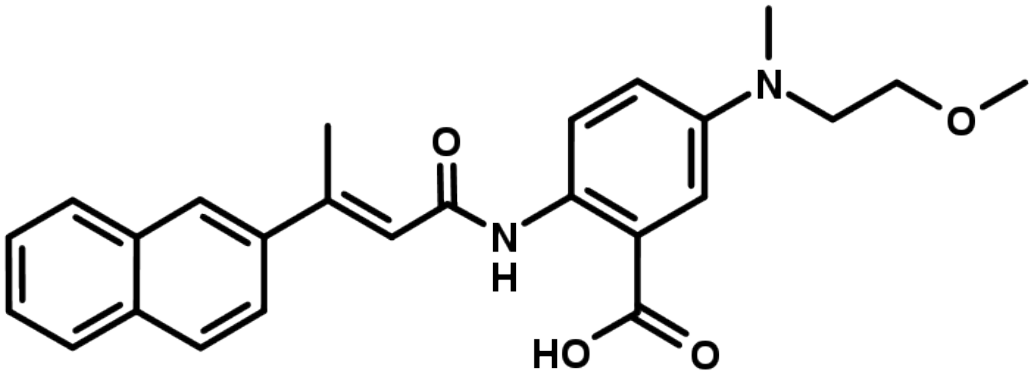

Figure 6: BIBR1654, negative control

Selectivity

The selectivity profile for BIBR1532 was assessed in a panel of DNA and RNA polymerases. Enzymatic activity was assayed in the presence of 0-50 µM BIBR1532. No effects were observed on all tested targets with up to 50 µM BIBR1532.[1]

ENYZME / ASSAY	IC_{50} [µM]
Human telomerase	0.093
Taq DNA polymerase	-
Human DNA polymerase α, β, γ	-
Calf thymus DNA polymerase α	-

Human RNA polymerase I	>100
Human RNA polymerase II + III	>100
In vitro translation	-
Bacterial DNA helicase	-
HIV-1 reverse transcriptase	-

SELECTIVITY DATA AVAILABLE	BIBR1532	BIBR1654
SafetyScreen44™ with kind 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 Xray crystal structure of Tribolium castaneum catalytic subunit of telomerase (*tc*TERT) in complex with BIBR1532 is available (PDB code: 5CQG).[3]

Reference molecule

With BRACO19 trihydrochloride a similarly potent telomerase inhibitor is available as reference molecule. The mechanism of this inhibitor is however different from BIBR1532 as it inhibits the telomere-end-binding protein 1.[4]

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

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3. Bryan C., Rice C., Hoffman H., Harkisheimer M., Sweeney M., Skordalakes E. Structural basis of telomerase inhibition by the highly specific BIBR1532. *Cell Structure* **2015**, *23*, 1934-1942. DOI:10.1016/j.str.2015.08.006, PubMed.
4. Mengual Gomez D. L., Armando R. G., Cerrudo C. S., Ghiringhelli P. D., Gomez G. E. Telomerase as a cancer target. Development of new molecules. *Curr. Top. Med. Chem.* **2016**, *16*, 2432-2440. DOI: 10.2174/1568026616666160212122425, PubMed.