Table of contents

Summary 2
Chemical Structure 2
Highlights 3
Target information 4
In vitro activity 4
In vitro DMPK and CMC parameters 5
In vivo DMPK parameters 5
In vivo pharmacology 5
Selectivity 6
Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein. Reference molecule(s) - Inhibitors 6
Summary 6
Supplementary data 7
References 7
Summary

BI-2536 was the first potent and selective PLK1 inhibitor which entered clinical trials. It is a suitable *in vitro* and *in vivo* tool to study PLK function.

Chemical Structure

![Figure 1: 2-D structure of BI-2536, an inhibitor of PLK1](image-url)
Figure 2: 3-D structure of BI-2536, as observed in complex with PLK1 by X-ray crystallography

**Highlights**

BI-2536 inhibits the PLK1 enzyme with an IC$_{50}$ of 0.8 nM and is active in a large variety of human tumor cell lines in the range of EC$_{50}$ = 2-25 nM. In vivo, BI-2536 is efficacious in mouse xenograft models in the range of 30-60 mg/kg (once or twice weekly i.v. administration).
Target information

Polo-like kinase 1 (PLK1) is a key regulator of cell division in eukaryotic cells. PLK1 contributes to the activation of the cyclin B1/CDK1 complex and is involved in centrosome maturation and bipolar spindle formation at the onset of mitosis. Moreover, PLK1 controls mitotic exit by regulating the anaphase-promoting complex, and it is also involved in the temporal and spatial coordination of cytokinesis.

Figure 3: BI-2536 bound to PLK1 (Xray structure solved at Boehringer-Ingelheim)

In vitro activity

BI-2536 shows EC50 values in a large panel of human tumor cell lines (carcinomas, sarcomas, melanomas and tumors derived from hematological malignancies) in the range of 2 to 25 nM:

<table>
<thead>
<tr>
<th>PROBE NAME / NEGATIVE CONTROL</th>
<th>BI-2536</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLK1 (IC50) [nM]</td>
<td>0.83</td>
</tr>
<tr>
<td>NCI-H460 (EC50) [nM]</td>
<td>12</td>
</tr>
</tbody>
</table>
**In vitro DMPK and CMC parameters**

<table>
<thead>
<tr>
<th>PROBE NAME / NEGATIVE CONTROL</th>
<th>BI-2536</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW [Da]</td>
<td>521.7</td>
</tr>
<tr>
<td>Solubility @ pH 5, McIlvaine buffer [µg/ml]</td>
<td>320</td>
</tr>
<tr>
<td>CACO permeability @ pH 7.4 [*10^-6 cm/s]</td>
<td>16</td>
</tr>
<tr>
<td>CACO efflux ratio</td>
<td>3</td>
</tr>
<tr>
<td>Plasma protein binding human / mouse / rat [%]</td>
<td>91 95 95</td>
</tr>
</tbody>
</table>

**In vivo DMPK parameters**

<table>
<thead>
<tr>
<th>BI-2536</th>
<th>MOUSE</th>
<th>RAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL [% Q_{e}}</td>
<td>116</td>
<td>56-200</td>
</tr>
<tr>
<td>MRT [h]</td>
<td>0.8</td>
<td>0-9-2.5</td>
</tr>
<tr>
<td>V_{ss} [l/kg]</td>
<td>5.6</td>
<td>4.8-55</td>
</tr>
<tr>
<td>F [%]</td>
<td>n.d.</td>
<td>14</td>
</tr>
</tbody>
</table>

**In vivo pharmacology**

BI-2536 is efficacious in mouse xenograft models in the range of 30-60 mg/kg (once or twice weekly i.v. administration).¹
**Selectivity**

Low selectivity considering closest family members: PLK2: IC$_{50}$ = 3.5 nM

PLK3: IC$_{50}$ = 9 nM

High overall kinase selectivity:

$>$1000-fold (panel of 63 protein kinases, supplemental data)$^1$

<table>
<thead>
<tr>
<th>PROBE NAME</th>
<th>BI-2536</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerep$^*$</td>
<td>No</td>
</tr>
<tr>
<td>Eurofins-Panlabs$^*$</td>
<td>No</td>
</tr>
<tr>
<td>Invitrogen$^*$</td>
<td>No</td>
</tr>
<tr>
<td>DiscoverX$^*$</td>
<td>Yes$^4$</td>
</tr>
<tr>
<td>Dundee</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

X-ray co-crystal structure available: PDB-code: 2RKU

**Reference molecule(s) - Inhibitors**

- Volasertib (BI 6727) shows similar in-vitro profile, but in-vivo longer half-life$^7$
- GSK-461364A is reported with PLK family selectivity$^6$

**Summary**

BI-2536 is a very well-studied tool for PLK1 function *in vitro* and *in vivo*. In house experiments do not provide evidence for BRD4 involvement at doses relevant for *in vivo* efficacy.
Supplementary data

Selectivity data can be downloaded free of charge from this site.

References

1. Steegmaier et al., BI-2536, a Potent and Selective Inhibitor of Polo-like Kinase 1, Inhibits Tumor Growth In Vivo, Current Biology 2007, pp 316-322

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5. Petronczki M et al., Polo on the Rise—from Mitotic Entry to Cytokinesis with PLK1, Dev Cell, 2009 May;14(5):646–59

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