New approaches to traditional anti-mitotic chemotherapy: The structure based drug design of the Eg5 inhibitor NVP-BQS481, from computer to clinic

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Presentation outline

- Pyridone and Carboline hit-to-lead campaigns
  - use of crystal structure information

- Evolution of the BQS481 series
  - structure based drug design
  - SAR/medchem issues
  - efficacy, pre-clinical profiling
Why is Eg5 an attractive target?

- Eg5 is a motor protein required for chromatid separation
- Inhibition of Eg5 causes monopolar spindle formation
- Unlike other anti-mitotics (taxanes, epothilones & Vinca alkaloids), inhibition of Eg5 does not affect microtubule stability
- Evidence that Eg5 inhibition only targets dividing cells & thus should not cause neurotoxicities, unlike microtubule disruptors

Figures modified from Mayer et al: Science 1999, 286, 971
Cleveland et al: Cancer cell 2005, 8, 7-12
Eg5 offers the possibility for allosteric inhibition

“The concerted movement of switch-2 and of the necklinker region is the most significant conformational change of the KSP-ADP (Eg5-ADP) complex when monastrol binds”

“This comparison reveals that the KSP (Eg5) protein assumes the locked conformation when it is bound with ADP and monastrol”

Kuo et al; J. Mol. Biol. 2004, 335, 547-554
The Pyridones

Due to close structural similarities, pyridone series terminated for IP concerns and potential for similar liabilities as SB-715992
The Tetra-hydro-\( \beta \)-carbolines

**HTS Hit**

\[
\text{IC}_{50} = 2.5 \ \mu\text{M}
\]

\[
\text{IC}_{50} = 0.18 \ \mu\text{M}
\]

\[
\text{GI}_{50} = 0.34 \ \mu\text{M (Colo205)}
\]

\[
= 0.28 \ \mu\text{M (MDA435)}
\]

\[
= 0.92 \ \mu\text{M (HCT-15)}
\]

\[
= 0.64 \ \mu\text{M (KB3.1)}
\]

\[
= 1.0 \ \mu\text{M (KBV1)}
\]
Binding induced rearrangement of Eg5

$$IC_{50} = 0.183 \mu M$$
Superposition of THB-Carboline with Quinazolinone

Each class explores different interactions with protein

Pocket changes due to backbone movement of L214

Large pocket opens accommodating Benzyl when L214 Shifts
Each class explores different interactions with protein

Pocket changes due to backbone movement of L214
Tetra-hydro-β-carbolines: from hit to lead

IC$_{50}$ = 0.18 μM
GI$_{50}$ = 0.34 μM (Colo205)
= 0.28 μM (MDA435)
= 0.92 μM (HCT-15)
= 0.64 μM (KB3.1)
= 1.0 μM (KBV1)

IC$_{50}$ = 0.02 μM
GI$_{50}$ = 0.13 μM (Colo205)
= 0.08 μM (MDA435)
= >2 μM (HCT-15)
= 0.27 μM (KB3.1)
= >20 μM (KBV1)

IC$_{50}$ = 2.1 μM
GI$_{50}$ = 2.2 μM (Colo205)
= 2.2 μM (MDA435)
= 2.8 μM (HCT-15)
= 5.1 μM (KB3.1)
= 6.8 μM (KBV1)
Tetrahydro-β-carboline series terminated

The series was discontinued due to:
• Steep SAR
• Modeling based on crystal structure proved unpredictable
• High interdependence of phenolic OH/activity
• Major pGP issues
• Constrained IP

Open-chain Carboline analogs: opening the C-ring

IC$_{50}$ = 0.025 µM
(scored -35 kJ/mol)

IC$_{50}$ > 25 µM
(scored -39.9 kJ/mol)
Morphing the THB-carboline series into a new phenyl imidazole core

Open A ring

Open both A and C rings

Modeling score -30.2 kJ/mol
IC50 Eg5 23 μM

Pocket opens up when L214 shifts

IC50 Eg5 >25 μM

Transpose Ph

IC50 Eg5 0.106 μM
Phenylimidazoles: crystal structure

Resolution: 2.6 Å

- benzyl and toluamide share large hydrophobic pocket
- propyl amine extends into solvent channel to make the only H-bond, with Glu116

IC$_{50}$ = 0.225 μM
Modification of the toluamide region

Quite the “magic methyl”

Phenyl imidazole series not locked into toluamide
Unanticipated broad amide SAR observed

Eg5 IC<sub>50</sub> = 0.051 µM

Eg5 IC<sub>50</sub> = 0.082 µM

Eg5 IC<sub>50</sub> = 0.013 µM
Why do the t-butyl and chloride modifications increase potency?

\[
\text{IC}_{50} = 0.238 \ \mu\text{M}
\]

\[
\text{IC}_{50} = 0.063 \ \mu\text{M}
\]

\[
\text{IC}_{50} = 0.014 \ \mu\text{M}
\]

Why is such diversity now tolerated in the amide region?
Meta-chloride pushes amide moiety into a new binding pocket.

Green IC\textsubscript{50} = 0.238 \textmu M

Purple IC\textsubscript{50} = 0.072 \textmu M
t-Butyl has same effect as the meta-chloro phenyl

Green

IC$_{50}$ = 0.072 µM

Purple

IC$_{50}$ = 0.016 µM
Exploiting new binding mode

IC₅₀ = 0.003 µM
GI₅₀ = 0.026 µM (HCT-116)
0.028 µM (KB3.1)
0.423 µM (KB8.5)
RF = 15
Cyclic constraints based on modelling & crystallography addresses pGP

IC$_{50}$ = 0.042 μM
GI$_{50}$ = 0.250 μM (HCT-116)
   0.217 μM (KB3.1)
   0.406 μM (KB8.5)
RF = 1.9
New binding mode allows increased diversification

IC$_{50}$ = 0.02 $\mu$M

IC$_{50}$ = 0.007 $\mu$M

IC$_{50}$ = 0.007 $\mu$M
Addressing pGP through amine basicity

<table>
<thead>
<tr>
<th>R</th>
<th>KB3.1 GI\textsubscript{50}</th>
<th>KB8.5 GI\textsubscript{50}</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2</td>
<td>0.03</td>
<td>0.60</td>
<td>22</td>
</tr>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{OH} \textsubscript{NH}_2</td>
<td>0.50</td>
<td>6.6</td>
<td>13</td>
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<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2 \textsubscript{OH}</td>
<td>0.004</td>
<td>0.88</td>
<td>221</td>
</tr>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2 \textsubscript{OCH}_3</td>
<td>0.02</td>
<td>0.43</td>
<td>22</td>
</tr>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2</td>
<td>0.184</td>
<td>1.0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

IC\textsubscript{50} values in \textmu M

<table>
<thead>
<tr>
<th>R</th>
<th>KB3.1 GI\textsubscript{50}</th>
<th>KB8.5 GI\textsubscript{50}</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2</td>
<td>0.34</td>
<td>0.43</td>
<td>1.3</td>
</tr>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2 \textsubscript{F}</td>
<td>0.13</td>
<td>0.15</td>
<td>1.1</td>
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<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2</td>
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<td>0.09</td>
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<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2 \textsubscript{F}</td>
<td>0.23</td>
<td>0.35</td>
<td>1.5</td>
</tr>
<tr>
<td>\textsubscript{3}s\textsubscript{s} \textsubscript{NH}_2 \textsubscript{OH}</td>
<td>0.25</td>
<td>1.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Attenuation of pKa modulates hERG inhibition

attenuation of pKa modulates hERG inhibition

Di-F Methyl and oxetane successfully attenuates hERG channel activity
Potency / Cyp3A4 inhibition dichotomy

X-ray shows H-bond requires eclipsed methyl group stabilizes eclipsed conformation predicts dimethyl might be even better

ARG119

GI50 KB8.5 0.0005 µM

GI50 KB8.5 0.0002 µM

GI50 KB8.5 0.014 µM
Potency / Cyp3A4 inhibition dichotomy

X-ray shows H-bond requires eclipsed

methyl group stabilizes eclipsed conformation

predicts dimethyl might be even better

increased potency from bis-H bond to ARG119 also occurs with CYP3A4
Steric bulk around carbonyl and ether unable to resolve Cyp3A4 inhibition issue

IC50 Eg5: 0.7 nM
GI50 KB: 8.5 nM
Rf: 1.5

CYP3A4 (MDZ): 1 μM

IC50 Eg5: 0.7 nM
GI50 KB: 8.5 nM
Rf: 1.4

CYP3A4 (MDZ): 0.3 μM

all < 3 μM CYP3A4

5-6 μM CYP3A4
NVP-BQS481 selected as most balanced molecule to move forward

NVP-BQS481
HCT-116 = 0.1 nM
KB 8.5 = 0.6 nM
KB 3.1 = 0.6 nM
RF = 1

Rat PK (i.v, 1.5 mg/kg)
T½ = 4.9 h
Vss = 20 L/kg
Cl = 73 mL/min/kg
AUC = 0.7 μM*h

- Exceptionally potent in cellular assays including those that overexpress p-GP
- Well established PK/PD relationship; strong dose response (mitotic arrest and apoptosis)
- Monopolar spindles observed in tumors establishing in vivo MOA
- Consistent iv PK across species
- No red flags from Safety
  - >5 μM in pharmacology safety panel
  - hERG patch clamp; 27% @ 5μM; no findings during dog telemetry
  - CYP3A4 ~0.5μM
  - GLP rat and dog toxicology findings consistent with MOA (anti-mitotic)
Key Eg5 protein interactions for BQS481 series

Key Interactions:

5 Hydrogen Bonding Interactions:

• 5-fluoro with H-N of ALA218
• Carbonyl & methoxy to backbone H-N of ARG119
• Fluorine of amine arm with N of ARG221
• N of amine arm with O of GLU116

Other Interactions:

• Benzyl moiety extends into hydrophobic pocket formed by PRO137, TRP127, TYR211
• 2-Fluoro in hydrophobic pocket formed by LEU214, ILE136, PRO137
• t-Butyl, amide & amine moieties extend into a solvent accessible channel
NVP-BQS481 efficacy in KB8.5 (pGP positive) mouse xenograft model (q4dx3, i.v)

Mean KB8.5 Tumor Volumes

NVP-BQS481 differentiates itself from SB-715992 in p-GP over expressing model
Hematological malignancies are particularly sensitive to BQS481

- Cell lines derived from hematological malignancies are amongst the most sensitive to BQS481

* Log of GI$_{50}$ value relative to the log of the mean GI$_{50}$ value of the entire NCI-60 panel plus the GI$_{50}$ values of the hematological cell lines panel
Sustained tumor regression by NVP-BQS481 i.v. q4d x 3 in MV4;11 AML subcutaneous mouse xenograft model

**Tumor Volumes**

- Vehicle
- NVP-BQS481 0.25 mg/kg
- NVP-BQS481 0.5 mg/kg
- NVP-BQS481 1 mg/kg

**Body Weights**

- *p < 0.05 - ANOVA, Dunn’s post-hoc test

6 CR, 3 PR
NVP-BQS481 is efficacious against disseminated KMS-11-luc multiple myeloma in SCID-Bg mice
Available data supported clinical development of NVP-BQS481 in AML and Multiple Myeloma

**AML**
- **In vitro**
  - Leukemia cell lines (n=28) are the most sensitive; majority have GI50's below 350 pM
  - Circulating blasts from AML patients were tested in tissue culture with BQS481.
    - Survival /colony formation assays demonstrated cytotoxic effects of BQS481 with GI50's = 120pM to 400 pM
- **In vivo**
  - BQS481 is superior in efficacy compared to AraC or daunorubicin in 3 AML Xenograft Models (1 subcutaneous and 2 disseminated)

**MM**
- **In vitro**
  - MM cell lines are among the most sensitive cell lines to BQS481 with subnanomolar GI50 values as observed in AML
- **In vivo**
  - BQS481 is superior in efficacy compared to Velcade in 2/3 MM Xenograft Models (1 subcutaneous and 1 disseminated)
Human PK well predicted by Allometric Scaling

- Predicted human PK parameters
  - CL: 799 – 833 mL/min
  - Vss: 908 mL
  - T_{1/2}: 10 – 13 hours

Human Data:
- Half-life of BQS481 ~ 11-12h at the 1.2 mg/m² cohort
- Highest plasma levels of BQS481 always reached at end of the 30-min infusion
- No drug accumulation between Days 1, 8 and 15 were observed
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