From unique technologies to innovative drugs

Successful applications of novel constrained macrocycles in drug discovery
Agenda

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  - MacroFinder®
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    - Pin-1
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- Summary
Introduction

Innovation in drug discovery

- Founded in 1996, located in Allschwil near Basle
- Private, research driven BioTech company
- Ca. 100 employees (85% scientists)
- Focus on discovery and development of macrocycle drugs
- Rich and diverse product portfolio at discovery and clinical development stage (3 products)
- Portfolio of research collaborations
Macrocycle platform

Macrocycles are medium size, cyclic molecules complementing chemical space between small molecules and biopharmaceuticals

- Polyphor macrocycles
- Small molecule drugs
- MacroFinder®
- PEMfinder®

100 – 500 MW
Small Molecules

500 - 2’000 MW
Polyphor Macrocycles

10’000 - 200’000 MW
Biopharmaceuticals

(e.g. growth hormone)
Macrocycle platform

Special features of macrocycles

• A macrocycle provides diverse functionality and stereochemical complexity in a **conformationally pre-organized ring structure**;

• Macrocycles are **semi-rigid** compounds. They provide a compromise between structural pre-organization (entropy) and sufficient flexibility to mould to a target surface and maximize binding (**induced fit**);

• Macrocycles can demonstrate **drug-like** physicochemical and pharmacokinetic properties such as solubility, lipophilicity, metabolic stability, cell permeability and bioavailability **beyond the rule of 5**;

• Macrocycles delivered important **drugs** and **clinical stage compounds** (cyclosporin A; erythromycin; daptomycin; rapamycin; ramoplanin; eribulin etc.);


It is Polyphor’s strategy to generate macrocycles which have **natural product-like complexity** and **can be efficiently synthesized** by fully modular assembly of readily available building blocks using high-throughput parallel synthesis and purification.

Transfer natural product complexity to the world of drug discovery
Macrocycle platform

Translation of structural information

PEMfinder®

Lead structures / development candidates

MacroFinder®

PEMphage®

PEM like peptides expressed in phages
Protein Epitope Mimetics (PEM)

The structure of PEM

PEM mimic secondary structure motifs of proteins, such as the β-hairpin and the α-helix.

β-hairpin is the predominant structural motif involved in known protein-protein interactions.

Protein Epitope Mimetics - functional minimizations of proteins
Protein Epitope Mimetics (PEM)

Variables for PEM design

PEM Technology is very versatile - the PEM molecules offer many possibilities of variation.

1. Loop size and sequence: 6-20 AAs
2. Building blocks
   - Encoded amino acids
   - Post-translationally modified amino acids
   - Non-natural amino acids
   - Amino acid mimetics/isosteres
3. Secondary structure stabilizing templates

PEM are synthesized in a parallel format, purified in a high throughput mode and thus efficiently optimized in rapid iterative cycles.
Protein Epitope Mimetics (PEM)

Selected scaffold structures of PEM molecules

- **POL6326 scaffold**
  - (CXCR4 antagonist)

- **POL7080 scaffold**
  - (antibiotic)

- **POL6014 scaffold**
  - (hNE inhibitor)

- **PPI scaffold**
  - (PEM antagonist)

Key functionalities:
- Chemokine receptor modulator
- Protease inhibitor
- Bacterial transporter inhibitor
- Extracellular PPI inhibitor
MacroFinder®

Features of MacroFinder®

- Macrocycles of variable ring size (12-30, typically 12-18; MW: 400-800 Da);
- Modular design; several privileged structural motifs;
- Semi-rigid backbone conformations induced by built-in structural constraints;
- High degree of conformational fine tuning possible through variation of ring size and stereochemistry of modular building blocks;
- Low energy conformations of most scaffolds was determined by 2D NMR;
- Efficient production by automated parallel synthesis and purification;
- MacroFinder® molecules show natural product-like structural complexity but exhibit small-molecule-like ADMET properties, such as cell penetration and oral bioavailability;
- The MacroFinder® library consists currently of >13’000 single purified compounds based on diverse scaffolds.
MacroFinder® contains a broad structural diversity of macrocycles exhibiting biological activities on a variety of different target classes.
Integrated drug discovery process

Automated hit expansion and HtL optimization on solid support

**Parallel synthesis** on 576 cpds arrays.
Output: > 1.5 mg per macrocycle,
        production time: 4-5 weeks.

**Split-mix synthesis** on 400 - 600 cpds arrays.
Output: > 2 mg per macrocycle,
        production time: 5-6 weeks.

All compounds undergo high throughput prep.
HPLC purification on normal or reverse phase.
Integrated drug discovery process

Cheminformatics, molecular modeling

- Adapted macrocycles-specific molecular modeling and visualization tools and processes relying on biostructural data (X-ray crystallography and high field NMR).
- Proprietary algorithms and tools tailored for design, data and SAR analysis of macrocyclic compounds (PolyMiner, PEMdesigner).

Adapted cheminformatics tools
MacroFinder® conformational analysis

Structural adaptation of the macrocycle upon binding to the target

- Overlay of conformations of MF cpd in solution (NMR, brown) and bound to target (X-ray, blue) show significant differences;

- The conformation of the bound MF molecule is also different from the *in silico* calculated conformation (grey);

- Due to their semi-rigidity MacroFinder® molecules and the dynamic target protein surfaces can adapt their respective conformations in the binding event: *induced fit*.

- Understanding of 3D shape and the conformational dynamics of the macrocycles is key.

Polyphor has a good understanding of the conformational dynamics of MacroFinder® scaffolds - key information for design and optimization.
MacroFinder® example

Successful MF approach to a small molecule undruggable intracellular target

Aim to discover and develop Macrocycle target inhibitors for once daily oral treatment

• Hit family identified by screening of MacroFinder® collection with potency ~250nM
• Successful hit-to-lead chemistry led to several divers promising lead families with
  • Sub nM potency in cell free assay;
  • < 20 nM potency in various functional cellular assays;
  • Excellent selectivity towards all other members of the target family and a large panel of anti-targets;
  • Broad set of compounds with overall matched favorable properties (solubility, permeability, microsomal stability, Cyp-inhibition);
  • Good rodent PK profile with oral bioavailability of ~30%;
  • Successful modulation of key readouts in two relevant rodent models;
  • bRo5: MW >600; PSA 80-100 Å², other parameters compliant with Ro5. Number of HBD’s that can not form intramolecular H-bonds must be minimized!
  • MacroFinder® scaffolds (macrocycles) are generally chemically and metabolically stable. Metabolic liabilities were found in the side-chain groups appended to the macrocyclic scaffold and could be remedied by standard MedChem optimization.
PEM – MacroFinder® crosstalk

PEMfinder®

Translation of structural information

MacroFinder®

PEMphage®
PEM like peptides expressed in phages

Lead structures / clinical candidates
PEM – MacroFinder® crosstalk

PEM design input for MacroFinder® molecules

- Experimental determination of low energy conformations (NMR; X-ray) of PEM and MacroFinder® scaffolds.
- Computational methods capable of reproducing the experimental data.
Pin-1, cis-trans peptidyl prolyl isomerase, intracellular PPI

Pin-1 could become a promising anticancer target

Interacts with mitotic phosphoproteins
Plays an important role in mitotic regulation
Is overexpressed in many human cancers

Recognition site: $p$Ser-Pro or $p$Thr-Pro residues
Specific cis-trans isomerization at Pro
Conformational changes of substrate protein

Pin-1 induced conformational changes rather than the initial phosphorylation *per se* regulate protein function.

* Example 1 (Vernalis):
  $IC_{50} = 0.83 \mu M$
Blocked proliferation of PC3 prostate cancer cells;
$GI_{50} = 13 \mu M$

** Example 2 (Pfizer):
  $K_{i} = 6 nM$
inactive in whole cell assay

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PEM – MacroFinder® crosstalk

PEM design input for MacroFinder® molecules for Pin-1

Cyclic peptide (IC$_{50}$ ~ 0.5 µM) bound to Pin-1 (full-length, single mutant (R14A)) determined @ 1.7Å.

Initial MacroFinder® screening hit (IC$_{50}$ ~ 80 µM) bound to Pin-1 determined @ 1.7Å.

MacroFinder® lead designed with input from structure 1 with two optimization cycles (IC$_{50}$ ~ 100 nM) bound to Pin-1 @ 1.7Å

Lead family today: 2 nM – 10 nM in Pin-1 enzymatic assay, fully selective against Cyclophilin A and FKBP12 GI$_{50}$ 0.5 - 3 µM on several cancer cell lines

X-ray structures of PEMfinder® and MacroFinder® hits generate valuable input for the design of focused MacroFinder® libraries
PEM example: POL7080, from discovery to the clinic

The Infectious Disease Society of America (IDSA) released a “hit list” of the six top priority, most dangerous drug-resistant microbes:

**Gram-negative**
- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Klebsiella species*
- *Acinetobacter baumannii*

**Gram-positive**
- Methicillin-resistant *S. aureus* (MRSA)
- Vancomycin-resistant *Enterococcus* (VRE)

POL7080: from discovery to the clinic

POL7080, new mode of action antibiotic against Gram-negative bacteria

Currently in Phase II clinical development

POL7080: from discovery to the clinic

Conformational stability is essential for antibacterial activity

<table>
<thead>
<tr>
<th>LB-01 (MIC = 0.01 μg/mL; Pa ATCC27853)</th>
<th>LB-02 (MIC &gt; 32 μg/mL; Pa ATCC27853)</th>
</tr>
</thead>
<tbody>
<tr>
<td>well ordered β-hairpin conformation</td>
<td>highly disordered conformation</td>
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</table>


Only the stable conformers are active towards *Pseudomonas aeruginosa*
POL7080: from discovery to the clinic

A photoaffinity labeled analogue binds specifically to LptD

For identification, proteins extracted from the outer membrane were separated on 2D SDS-PAGE/IEF gels, and analyzed by in-gel protease digestion and MALDI-MS/MS.

No labeling observed with PA\textsuperscript{RES1}

N. Srinivas et al. Science \textbf{2010}, \textit{327}, 1010-1013
POL7080: from discovery to the clinic

LptD translocates LPS to the cell surface

![Diagram showing LptD translocating LPS]

LptD and other β-barrel transporters are conserved in most Gram-negative bacteria – the area has become a “hot” research topic

Broad spectrum Gram negative research program

POL7080, new mode of action antibiotic against Gram-negative bacteria

Protegrin I → POL0067 → POL6137 → POL7001 → POL7080

Pseudomonas specific!  PK/ADMET optimization

~ 300 analogues

POL0067 → POL6137 → POL7001 → POL7080

Plasma stability

POL7080

POL7001

~ 500 analogues

~ 700 analogues


Currently in Phase II clinical development
## Broad spectrum Gram negative research program

**MIC (µg/mL) against recent clinical isolates**

<table>
<thead>
<tr>
<th></th>
<th>P0194999</th>
<th>P0211386</th>
<th>P0239449</th>
<th>P0239743</th>
<th>Colistin</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Ciprofloxacin</th>
<th>Ceftazidime</th>
<th>Ceftriaxone</th>
<th>Imipenem</th>
<th>Meropenem</th>
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<td>0.125</td>
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<td>0.5</td>
<td>2</td>
<td>2</td>
<td>≤0.06</td>
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</table>

Green = Sensitive, Yellow = Intermediate, Red = Resistant  
EUCAST clinical breakpoints

Activity significantly improved to a level of standard antibiotics including MDR; PEM antibiotics show no cross-resistance with known antibiotics
Summary

Macrocycles complement the established drug classes biopharmaceuticals and small molecules

- The Polyphor macrocycle platform generates hits on a wide range of extra- and intracellular targets including those where other approaches have failed;

- The focus on in depth understanding of structure and conformations of macrocycles is key to enable transfer of pharmacophors from peptidic PEMfinder® to non-peptidic MacroFinder®;

- In all of our successful projects, the macrocyclic backbone structure forms part of the pharmacophore and not only a scaffold providing correct 3D-vector display;

- Selectivity towards related targets or in safety panels so far has never been an issue. On the contrary, in 2 projects pronounced species selectivity despite high sequence homology for the target was hampering rapid project progress.

- For MacroFinder®, oral bioavailability can be readily (typically 2-3 optimization rounds) achieved with bR5 macrocycles (4 projects to date);

- Phenotypical, whole cell or pathway screening of macrocycles can be very rewarding: Discovery of antimicrobials against Gram negative pathogens with a new mode of action
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