FRAGMENT BASED APPROACH TOWARDS THE DESIGN OF MACROCYCLIC NATURAL PRODUCT ANALOGS

3\textsuperscript{RD} RSC BMCS MEDICINAL CHEMISTRY SYMPOSIUM ON MACROCYCLES, OCTOBER 8-9\textsuperscript{TH}, 2018
FBLD IN DRUG DISCOVERY

- 2 decades overview in Discovery
- FBLD successful on numerous difficult targets (Bcl-XL, BACE-1, MCL-1, ATAD2, ...)
- 30+ development candidates from FBLD in the clinics

WHAT IS A FRAGMENT?

- Astex Rule of 3: MW < 300, HBD ≤ 3, HBA ≤ 3, ClogP ≤ 3, NROT (≤ 3) and PSA (≤ 60), HAC ≤ 16

M. Congreve, H. Jhoti et al. DDT 2003, 8(19), 876-877.

» A fragment is just a small, weak hit »


Good aqueous solubility in PBS buffer and solubility in DMSO

Mostly flat heterocyclic structures

BEYOND RO3 TO ADDRESS PPI

“PPI fragments appear to be slightly larger (mean MWT of 278 vs. 221) and more lipophilic (mean logP of 2.48 vs. 1.77) and contain more rotatable bonds (mean of 4.01 vs. 2.50) than standard fragments“


“Using biophysical techniques commonly employed for screening, we could only detect binding of fragments that violate the Rule of Three”

FBLD TOWARDS THE DESIGN OF NEW MACROCYCLES

FK506/FKBP: PDB 4NNR

Towards more 3D-fragments?
NP FRAGMENTS BY NP CHEMICAL FRAGMENTATION

Construction of a 3D-shaped, natural product like fragment library by fragmentation and diversification of natural products
NP-DERIVED FRAGMENTS FOR FBLD

Fragment-like Filter

DNP 18.2 (Dictionary of Natural Products)
- 65 molecules with a MW > 2500 Da
Cheminformatic analysis

Fragments of all sizes and properties with attachment points and functional groups at natural positions

Fragment-filter & HTS-filter

Natural product-derived fragments

Representative NP-fragment library

Fragment structures / Phosphatasess

<table>
<thead>
<tr>
<th>Fragment</th>
<th>IC_{50} [nM]</th>
<th>LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 VEPTP</td>
<td>10.2 ± 3.3</td>
<td>0.49</td>
</tr>
<tr>
<td>4 VHR</td>
<td>68.2 ± 25.2</td>
<td>0.55</td>
</tr>
<tr>
<td>5 CDC65A</td>
<td>10.7 ± 0.8</td>
<td>0.66</td>
</tr>
<tr>
<td>6 MPTPE</td>
<td>80.8 ± 9.4</td>
<td>0.45</td>
</tr>
<tr>
<td>7 VEPTP</td>
<td>25.5 ± 5.2</td>
<td>0.44</td>
</tr>
<tr>
<td>8 PTP1B</td>
<td>20.9 ± 7.7</td>
<td>0.49</td>
</tr>
</tbody>
</table>

EDELIRS APPROACH TOWARDS 3D-FRAGMENTS

- Inspired by natural products
- Maximize exit vectors
- Diversify pharmacophores to mimic natural product diversity

2-ABN: 2-azabicyclo[3.3.1]nonane

morphine  daphniyumine A  tubifoline  FR901483  madangamine A
MIMICKING NATURAL PRODUCT DIVERSITY

2-ABN: 2-azabicyclo[3.3.1]nonane

Fragments elaborated from 2-ABN framework
RECENT ACHIEVEMENT: AZ MACROCYCLIC BCL6 INHIBITOR

- Virtual screening combined with fragment based screening strategies
- Low nanomolar inhibitors of PPI between BCL6 and its corepressor BCOR
- Antiproliferative effect on DLBCL cell lines

AZ MACROCYCLIC BCL6 INHIBITOR

On and off-rate matched molecular pairs analysis

Substantial decrease in off-rate combined with increase in on-rate constant due to macrocyclisation

CHALLENGES TACKLED BY EDELRRIS

Structural Biology

Innovative and validated chemical frameworks

SBDD (EDEN)

Drugs

Cyclosporin A
Cyclophilins are folding helper enzymes member of the Peptidyl Proline Isomerases (PPI) superfamily.

Cyclosporin A (CsA) is a potent inhibitor of CypD.

No SME disclosed as CyPD inhibitor when work was initiated.
INTEGRATED HIT GENERATION PROGRAM CONDUCTED AT EMD SERONO

- High Throughput Screening
- Knowledge Based Design
- Fragment Based Screening

- Full deck screening
- 575 primary hits
- 178 hits
- 165 hits
- 0
TOWARDS A 3D-FRAGMENTS ENRICHED COLLECTION

EMD Serono Fragment collection

~2100 Fragments

- Ro3
  - log P ≤ 3
  - MW ≤ 300
  - H bond donors ≤ 3
  - H bond acceptors ≤ 3
  - rotatable bonds ≤ 3
  - Solubility: logS > -3

~400 3D-Fragments from Edelris

- ~20 chemical series
- 10-20 fragments /series
- 2-3 exit vectors and good chemical tractability
EMD SERONO FRAGMENT SCREENING OUTCOME

- EMD-Serono Fragment Collection ~ 2500 Fragments
- 168 Primary Fragment hits
- 58 Confirmed hits: 2% Hit rate
- CypD SPR surface – Test at 2 mM
- Validation of Binding by SPR – $K_d$ determination
- 6 X-Ray
- X-Ray crystallography (Proteros)
CYCLOPHILIN D LIGANDABILITY ASSESSMENT

- 7 Ligandable pockets identified (Fpocket)
- Known inhibitor CsA binds mainly to pocket 4
FRAGMENT X-RAYS

- Edelris 1
  
  \[K_D = 7.1 \text{ mM (LE = 0.2)}\]

- Edelris 2
  
  \[K_D = 7.5 \text{ mM (LE = 0.16)}\]

- Tetrazole
  
  \[K_D = 3.9 \text{ mM (LE = 0.21)}\]

- Isoxazole
  
  \[K_D = 22 \text{ mM (LE = 0.15)}\]

- Oxalylamide
  
  \[K_D = 1.1 \text{ mM (LE = 0.22)}\]

- Succinimide
  
  \[K_D = 45 \text{ mM (LE = 0.11)}\]

Optimal space occupation for fragment growing and linking
EDELRIS FRAGMENTS VERSUS CYCLOSPORIN A

- Edelris 1
  - MW = 209
  - clogP = 0.6
  - HAC = 15
  - Fsp3 = 0.45
  - $K_D = 7.1$ mM
  - LE = 0.2

- Edelris 2
  - MW = 248
  - clogP = 1.4
  - HAC = 18
  - Fsp3 = 0.46
  - $K_D = 7.5$ mM
  - LE = 0.16

- Fragment highly 3D (Fsp3 $\uparrow$)
- Moderate Ligand efficiency (LE)
- Optimal occupancy of pocket 3 unexplored by CsA
TOWARDS A FRAGMENT MERGING APPROACH

Guichou urea (PDB 4J5B)

Edelris 1
$K_D = 7.1 \text{ mM}$
$LE = 0.2$

Edelris 2
$K_D = 7.5 \text{ mM}$
$LE = 0.16$

A MILLION FOLD IMPROVEMENT OF AFFINITY IN 3 MONTHS

10⁶ potency improvement in two optimization cycles through an optimized space occupancy of pocket 3 and the creation of interactions with two additional residues (Arg124 and Ser123)
MSC2530594: A HIGHLY POTENT LOW MW CYPD INHIBITOR

MSC2530594: MW 496
SPR: $K_D = 7 \text{ nM}$
Binding: $IC_{50} = 31 \text{ nM}$
PPI: $IC_{50} = 4 \text{ nM}$

CsA: MW 1202
SPR: $K_D = 30 \text{ nM}$
Binding $IC_{50} = 23 \text{ nM}$ (FP)
PPI: $IC_{50} = 40 \text{ nM}$

MSC2530594 X-Ray structure bound to cyclophilin D
CONCLUSION

- FBLD successful to address NP macrocycle modulated targets
- 3D-fragments allowed the identification of pockets that were not addressed by more conventional flat heterocyclic structures
- Merging strategy proved to be very successful and highly correlated to the superimposition of the aniline moiety in the pocket 3
ACKNOWLEDGMENTS

Fragment library generation

Gérald Coste
Amaury Patin
Doriane Bataillon
Anthony Willeume

Merck
Dirk Finsinger
Bertram Cezanne
Michel Calderini

Cyclophilin D project

Marine Gilardone
Hugues Lemoine
Patrice Fontaine
Frédéric Frébault

Merck
Catherine Jorand Lebrun
Ulrich Graedler
Xuliang Jian
Theresa Johnson
Daniel Schwarz
Andreas Marx
Birgitta Leuthner
Matthias Frech

Thank you