NMR-based screening of combinatorial libraries to target protein-protein interactions with reversible or covalent agents

North Jersey ACS NMR Topical Group
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School of Medicine, University of California Riverside

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands

3. Introduction of an anchoring moiety in the library (focused HTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates

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4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates.
Protein-protein interactions (PPIs) represent a large class of potentially viable therapeutic targets that are deemed “undruggable”

- Targeting PPIs has been notoriously difficult using conventional biochemical HTS approaches (identification of artefacts or PAINS compounds)
- Contact surface area is typically too large for a “small-molecule” (MW 500 -1000)
- Binding pockets tend to be flat with induced fit grooves
- Lack natural small molecule binders onto which design mimetics
- Some success using fragment- and structure based approaches (i.e. SAR by NMR)
- Short peptides or peptide-mimetics targeting PPIs (HTS by NMR) as chemical probes or even therapeutics
The design of potent (low nM) and selective PPIs antagonists is not a trivial task.
*SAR by ILOEs

**INPHARMA


**Fragment based drug discovery (FBDD)**

SAR by NMR (structure guided fragment linking)

- **Fragments**
  - NMR screen of fragments

- **Bi-dentate**

**Structure guided**

- **Fragment growing or merging**

**Optimized lead**

- **Vemurafenib**
  - First FBDD designed kinase inhibitor.
  - Targets V600E-RAF protein in melanoma.

**Venetoclax**
- First rationally designed PPI inhibitor


Maurizio Pellecchia¹, Ivano Bertini, David Cowburn, Claudio Dalvit, Ernest Giralt, Wolfgang Jahnke, Thomas L James, Steve W Homans, Horst Kessler, Claudio Luchinat, Bernd Meyer, Hartmut Oschkinat, Jeff Peng, Harald Schwalbe, Gregg Siegal
When targeting PPIs, short peptide mimetics can be effective pharmacological agents and even therapeutics.

(a) Loop
EphA4/ephrin

(b) β-strand
XIAP/Smac

(c) α-helix
Bcl-2/Bim

Inhibitors

UCR
Development stage (150E8)

Genentech
Chen, K. F. et al., *Biochem Pharm.* 2012
Phase II

Abbott
Souers et al., *Nature Medicine* 2013
FDA approved 2016
For a library of all possible tri-peptides or tetra-peptides composed by let’s say 50 natural or non natural aminoacids:

- Synthesize a library of $50 \times 50 \times 50 = 125,000$ molecules
- Test them in assays that are sensitive enough to detect weak binders against PPIs (NMR)
- For tetra-peptides and using let’s say 50 amino-acids $50 \times 50 \times 50 \times 50 \Rightarrow 6$ Million

However we can reduce the complexity by synthesizing pools using the position scanning method and by testing the mixtures by protein NMR:

$$\begin{align*}
\text{P1} & \quad \text{P2} & \quad \text{P3} \\
A X X & \quad X A X & \quad X X A \\
G X X & \quad X G X & \quad X X G \\
. & \quad . & \quad . \\
. & \quad . & \quad . \\
W X X & \quad X W X & \quad X X W
\end{align*}$$

$A X X = A A A, AAG, AGA, \ldots, AWW$

$50 \times 50 = 2500$

If $AGW$ is a positive then we expect that the mixtures $AXX, XGX, \text{and } XXW$ will be positive by NMR

$$50 + 50 + 50 = 150 \text{ mixtures}$$

Ligand binding by solution NMR spectroscopy

Overlay of NMR spectra measured in absence and presence of test ligands

Protein concentrations 5-20 μM
Mixtures 1-2 mM (individual agents ~ 1-2 μM)

Barile and Pellecchia, Chem. Rev. 2014, 114, 9, 4749-4763
Ligand binding by solution NMR spectroscopy

Overlay of NMR spectra measured in absence and presence of test ligands

Barile and Pellecchia, Chem. Rev. 2014, 114, 9, 4749-4763
Dihydrodipicolinate reductase (DHPR), a homo-tetramer of 120 kDa, which is involved in the biosynthesis of lysine and bacterial cell wall components.

Assignment of active site $^{13}\text{C}/^{1}\text{H}$ Met via mutagenesis or differential chemical shift

2D [$^{13}\text{C}$, $^{1}\text{H}$] HMQC spectra recorded with a 150 µl of a 50 µM sample of U-$^{2}\text{H}$, $^{13}\text{C}/^{1}\text{H}$ Met, $^{13}\text{C}/^{1}\text{H}$ Thr, $^{13}\text{C}/^{1}\text{H}$ δ Ile labeled DHPR ([MIT]-DHPR). (C) and (D): Met $^{13}\text{C}/^{1}\text{H}$ sub-spectra. (C) Black, unbound [MIT]-DHPR; blue, [MIT]-DHPR bound to PDC. (D) Red, [MIT]-DHPR bound to 4-Cl PDC; blue, [MIT]-DHPR bound to PDC. Pellecchia et al. J Biol NMR 22: 165–173, 2002.
HTS by NMR as a way to design/discover potent and selective peptide mimetics: basic principles

General POS libraries of

- 3-mers or 4-mers using natural and non-natural aa.
- Biophysical detection/selection
- Optimization strategies

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands.

3. Introduction of an anchoring moiety in the library (focused HTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins.

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates.
EphA4/ephrin interactions modulate MN cell death and viability: ephrin mimetics (EphA4 agonists) may revert EphA4 induced MN cell death.

Xua, et al., Nikolov. PNAS (2013) 110, 14634–14639

EphA4 subtype is involved in preventing nerve regeneration, and is associated with spinal cord injury and ALS.

However:
Targeting the EphA4 receptor with small molecules is challenging because its ephrin binding site is large.

Phage display identified a weak 12-mer linear peptide (KYL or KYLPYWPVLSL)\(^1\). Subsequent HTS campaigns (300,000 compounds) using biochemical assays resulted in in 1 hit molecule (compound 1)\(^1\)

**However:**

- When we tested compound 1 by NMR or by ITC we could not detect any appreciable binding to the EphA4 !

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HTS by NMR targeting EphA4-LBD

Tested by NMR a combinatorial library of 48 x 48 x 48 tripeptoids
(144 mixtures covering >110,000 peptoids)

Initial hit

Optimized compound

Final compound

\[ K_d \approx 233 \text{ } \mu\text{M} \]

\[ K_d \approx 12 \text{ } \mu\text{M} \]

\[ K_d \approx 1.2 \text{ } \mu\text{M} \]
HTS by NMR targeting EphA4-LBD

Potent and Selective EphA4 Agonists for the Treatment of ALS. Wu et al., Pellecchia Cell Chem Biol 2017

<table>
<thead>
<tr>
<th>ID</th>
<th>R1</th>
<th>R2</th>
<th>Kᵢ (µM) by FPA</th>
<th>Kᵢ (µM) by ITC</th>
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<td>120H10</td>
<td>H₂N</td>
<td>-</td>
<td>0.96</td>
<td>ND</td>
</tr>
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<td>-</td>
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<tr>
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<td>13.96</td>
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<tr>
<td>123C2</td>
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<td>-</td>
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<tr>
<td>123C4</td>
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</tr>
<tr>
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<td>-</td>
<td>2.57</td>
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<td>123C6</td>
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<tr>
<td>123C7</td>
<td>H₂N</td>
<td>-</td>
<td>1.59</td>
<td>0.54</td>
</tr>
<tr>
<td>123C8</td>
<td>H₂N</td>
<td>-</td>
<td>0.64</td>
<td>0.42</td>
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</table>
Molecular basis for the selectivity of 123C4

<table>
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<tr>
<th>Protein</th>
<th>$K_d$ (µM)</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$T\Delta S$ (kcal/mol)</th>
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<tbody>
<tr>
<td>WT-EphA4 LBD</td>
<td>0.42 ± 0.02</td>
<td>-11.06 ± 0.06</td>
<td>-2.42</td>
</tr>
<tr>
<td>WT-EphA3 LBD</td>
<td>4.55 ± 1.09</td>
<td>-1.54 ± 0.30</td>
<td>5.75</td>
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<td>WT-EphA2 LBD</td>
<td>N.B.</td>
<td>-</td>
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<tr>
<td>I59G-EphA4 LBD</td>
<td>3.66 ± 0.71</td>
<td>-5.69 ± 0.74</td>
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<td>I59A-EphA4 LBD</td>
<td>9.09 ± 1.49</td>
<td>-3.43 ± 0.41</td>
<td>3.43</td>
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<tr>
<td>I159A-EphA4 LBD</td>
<td>0.29 ± 0.02</td>
<td>-8.53 ± 0.06</td>
<td>0.34</td>
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<tr>
<td>M60A-EphA4 LBD</td>
<td>0.35 ± 0.03</td>
<td>-9.98 ± 0.21</td>
<td>-1.24</td>
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<tr>
<td>M164A-EphA4 LBD</td>
<td>0.062 ± 0.01</td>
<td>-11.14 ± 0.13</td>
<td>-1.38</td>
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Targeting the LBD of EphA4
## HTS by NMR versus phage display

### HTS by NMR derived*

**123C4**

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
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<tbody>
<tr>
<td>123C4</td>
<td>MW ~ 800 Kd ~ 400 nM</td>
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<tr>
<td><img src="123C4.png" alt="Chemical Structure" /></td>
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</table>

### Phage display derived and optimized**

**APY-d3**

<table>
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<th>ID</th>
<th>Structure</th>
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<tbody>
<tr>
<td>146C8</td>
<td>MW ~ 1300 Kd ~ 67 nM βA-PYCVYR-βA-SWSC-CONH₂</td>
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<tr>
<td><img src="146C8.png" alt="Chemical Structure" /></td>
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<table>
<thead>
<tr>
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<td>150B8</td>
<td>MW ~ 800 Kd ~ 45 nM</td>
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<tr>
<td>146C8 (APY-d3)</td>
<td>MW ~ 1300 Kd ~ 67 nM</td>
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<tr>
<td>βA-PYCVYR-βA-SWSC-CONH₂</td>
<td></td>
</tr>
</tbody>
</table>

** HTS by NMR derived and optimized**

**Phage display derived and optimized**

Wu et al., Pellecchia, Cell Chem. Biol 2017;
*Baggio et al., Pellecchia,2020 in preparation

** Lamberto et al. Pasquale ACS Chem Bio, 2014;
123C4 is an effective EphA4 agonist
EphA4 is a disease modifier of amyotrophic lateral sclerosis in animal and in humans.


Potent and Selective EphA4 Agonists for the Treatment of ALS. Wu et al., Pellecchia
Cell Chem Biol 2017

123C4 crosses the BBB (%F Brain /IV ~64% after 30 min).

Compound 123C4 in saline was injected daily (i.p. 30 mg/kg) in SOD1(G93A) mice.
HTS by NMR derived potent EphA4 agonistic agents for the treatment of ALS

Wu et al., Ethell and Pellecchia, Cell Chem Biol 2017
*Baggio et al., Pellecchia, 2020 in preparation

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands

3. Introduction of an anchoring moiety in the library \((\text{focused HTS by NMR or } f\text{HTS by NMR})\) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates
Focused POS using an anchoring moiety: fHTS by NMR

(a) Fragment hits

(b) SAR and/or structural studies to define linker position on selected fragment hit

(c) Design, synthesis, and test by HTS by NMR of a fragment-inspired POS library

(d) Synthesis and testing of combinations of fragment-hit components

(e) Biophysical studies, SAR, cell based assays

**Curr Top Med Chem. 2015; 15(20): 2032–2042.**

**High-throughput screening by Nuclear Magnetic Resonance (HTS by NMR) for the identification of PPIs antagonists**

Bainan Wu, Elisa Barile, Surya K. De, Jun Wei, Angela Purves, and Maurizio Pellecchia*

Bottini, Wu, Barile, Leone, Pellecchia
HTS by NMR Guided Identification of Novel Agents Targeting the Protein Docking Domain of YopH
ChemMedChem. 2016 Apr 19;11(8):919-27
fHTS by NMR using -CONHOH as anchoring moiety for metallo-proteins

Baggio, Cerofolini, Luchinat, Fragai, Pellecchia

HTS by NMR for the Identification of Potent and Selective Inhibitors of Metalloenzymes

fHTS by NMR using -CONHOH as anchoring moiety for metallo-proteins

HTS by NMR using -CONHOH as anchoring moiety for metallo-proteins

fHTS by NMR using -CONHOH as anchoring moiety for metallo-proteins

FDA Approves New Kyprolis (Carfilzomib) Combination Therapy for the Treatment of Patients with Relapsed or Refractory Multiple Myeloma

Inhibition of apoptosis and cancer resistance

Adapted from: Biology, Medicine Experimental oncology. The inhibitor of apoptosis (IAP) proteins are critical regulators of signaling pathways and targets for anti-cancer therapy by De Almagro and Vučić
AVPI (or AVPF) mimetics as potent Smac mimetics targeting IAPs

<table>
<thead>
<tr>
<th>A φ P φ motifs</th>
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<tbody>
<tr>
<td>GDC-0152 (Genentech)</td>
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<tr>
<td><img src="image1" alt="GDC-0152" /></td>
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<tr>
<td>LCL161 (Novartis)</td>
</tr>
<tr>
<td><img src="image2" alt="LCL161" /></td>
</tr>
</tbody>
</table>


fHTS by NMR using Ala as anchoring moiety for XIAP targeting agents
A tri-peptoid library of 46 natural and non-natural amino acids (23 L/D aa + 23 modified aa) has been synthesized.

- **46x3 = 138 mixtures**
- each containing **1x46x46 = 2116 compounds**
- **46x46x46 = 97,336** compounds have been tested
- Average MW: 450 Da.

**H-AXXX-NH$_2$ LIBRARY:**

- **46x3 = 138 mixtures**
- each containing **1x46x46 = 2116 compounds**
- **46x46x46 = 97,336** compounds have been tested
- Average MW: 450 Da.
fHTS by NMR using Ala as anchoring moiety reveals the known binding motif, AVPI/F, for the Bir3 domain of XIAP.

2D [³¹H,³¹⁵N] HTS reveals the known consensus AVXX.

The known consensus is identified.
fHTS by NMR using Ala as anchoring moiety reveals novel binding motifs for the Bir3 domain of XIAP.

The fHTS by NMR also identifies new binding elements.
Synthesis of few agents lead to initial compound that seems as potent but more selective than GDC0152 towards XIAP

HTS by NMR using Ala as anchoring moiety reveals novel binding motifs for the Bir3 domain of XIAP

Can we likewise identify suitable electrophiles for the design of Lys covalent PPIs targeting ligands?

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands.

3. Introduction of an anchoring moiety in the library (focused HTS by NMR or fHTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins.

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates.
Can sulfonlfuorides or fluoro-sulfates be used for covalent PPIs targeting ligands? 

Can the agents react efficiently and selectively with any of these amino acids in PPIs?

Are the resulting agents suitable as chemical probes for Lys, Tyr, Ser, Thr, or His?

Stable in buffer and media, cell permeable, engage the target in cell

Are the resulting agents suitable as possible therapeutics

In vivo stability and bioavailability


XIAP BIR3 Lys-covalent inhibitors

Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His

Sulfonyl fluorides agents rapidly react with Lys 311

<table>
<thead>
<tr>
<th>Agent</th>
<th>BIR3wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>I D</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>FO_{2}SO</td>
</tr>
<tr>
<td>3</td>
<td>FO_{2}SO</td>
</tr>
<tr>
<td>4</td>
<td>FO_{2}SO</td>
</tr>
</tbody>
</table>
Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His


<table>
<thead>
<tr>
<th>Agent</th>
<th>BIR3wt</th>
<th>Lys311Ala</th>
<th>Lys311Tyr</th>
<th>Lys311His</th>
<th>Lys311Thr</th>
<th>Lys311Ser</th>
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<tbody>
<tr>
<td><strong>IC₅₀ [nM]</strong></td>
<td><strong>ΔTm [°C]</strong></td>
<td><strong>IC₅₀ [nM]</strong></td>
<td><strong>ΔTm [°C]</strong></td>
<td><strong>IC₅₀ [nM]</strong></td>
<td><strong>ΔTm [°C]</strong></td>
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<tr>
<td><strong>I D</strong></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11 ± 1</td>
<td>31</td>
<td>86 ± 2</td>
<td>1.5</td>
<td>12 ± 2</td>
<td>37.5</td>
</tr>
<tr>
<td>3</td>
<td>17 ± 0.1</td>
<td>35.5</td>
<td>61 ± 4</td>
<td>5</td>
<td>6.4 ±0.1</td>
<td>36</td>
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<tr>
<td>4</td>
<td>71 ± 7</td>
<td>24 ± 1</td>
<td>8.5</td>
<td>65 ± 1</td>
<td>11</td>
<td>37 ± 5</td>
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<tr>
<td>5</td>
<td>190 ± 20</td>
<td>11.5</td>
<td>86 ± 9</td>
<td>16</td>
<td>105 ± 10</td>
<td>8.5</td>
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<td>6</td>
<td>140 ± 1</td>
<td>11.5</td>
<td>89 ± 0.2</td>
<td>15</td>
<td>100 ± 5</td>
<td>11.5</td>
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<td>7</td>
<td>590 ± 65</td>
<td>4.5</td>
<td>218 ±8</td>
<td>31</td>
<td>100 ± 7</td>
<td>8.5</td>
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<td>8</td>
<td>2000 ± 20</td>
<td>11.5</td>
<td>70 ± 2</td>
<td>11</td>
<td>90 ± 1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Two values are for 2 hr or 6 hr incubation time, respectively.

Thermal shift (ΔTm) assay

Sulfonyl fluorides and fluorosulfates with Lys311Tyr and Lys311His mutants

Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His

- Can sulfonyl-fluorides or fluoro-sulfates be used for covalent PPIs targeting ligands?
  Can the agents react efficiently and selectively with any of these amino acids in PPIs

- Are the resulting agents suitable as chemical probes for Lys, Tyr, Ser, Thr, or His?
  Stable in buffer and media, cell permeable, engage the target in cell

- Are the resulting agents suitable as possible therapeutics
  In vivo stability and bioavailability

### Sulfonyl fluorides (-SO$_2$F) versus aryl-fluorosulfates (-OSO$_2$F)

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>IC$_{50}$ XIAP-BIR3 [nM]$^a$</th>
<th>Chemical stability</th>
<th>Plasma Stability$^c$</th>
<th>$\Delta T_m$ [°C]$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCL161</td>
<td><img src="image" alt="LCL161 Structure" /></td>
<td>48 ± 5 53 ± 3</td>
<td>&gt; 5 h</td>
<td>&gt; 2 h</td>
<td>14 18.5</td>
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<tr>
<td>10</td>
<td><img src="image" alt="10 Structure" /></td>
<td>63 ± 6 28 ± 6</td>
<td>&gt; 5 h</td>
<td>&gt; 2 h</td>
<td>9.5 33.5</td>
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<tr>
<td>11</td>
<td><img src="image" alt="11 Structure" /></td>
<td>24 ± 1 3.5 ± 0.1</td>
<td>~ 2.5 h</td>
<td>~15 min</td>
<td>35 34.5</td>
</tr>
</tbody>
</table>

- **Rapid reaction with target**
- **Very stable in water and plasma**

$>=$

- **Rapid reaction with water and plasma**
- **Slower reaction with target**

$^a$Chemical stability
$^c$Plasma Stability
$^d$DT$_m$ [°C]
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
**Sulfonyl fluorides (-SO₂F) versus aryl-fluorosulfates (-OSO₂F)**

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>IC₅₀ X1AP-BIR3 [nM]³</th>
<th>Chemical stability</th>
<th>Plasma Stability⁵</th>
<th>ΔTₘ [°C]⁴</th>
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<tbody>
<tr>
<td>LCL161</td>
<td></td>
<td>48 ± 5 53 ± 3</td>
<td>&gt; 5 h</td>
<td>&gt; 2h</td>
<td>14 18.5</td>
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<tr>
<td>10</td>
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<td>35 34.5</td>
</tr>
</tbody>
</table>

Rapid reaction with target

Very stable in water and plasma

=>

Rapid reaction with water and plasma

Slower reaction with target
### Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M


<table>
<thead>
<tr>
<th>ID</th>
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<th>XIAP-BIR3</th>
<th>cIAP1-BIR3</th>
<th>cIAP2-BIR3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ [nM]ᵃ</td>
<td>ΔTm [°C]ᵇ</td>
<td>IC₅₀ [nM]</td>
<td>IC₅₀ [nM]</td>
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<tr>
<td>LCL161</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>50 ± 5</td>
<td>14.0</td>
<td>18.6 ± 0.1</td>
<td>11 ± 4</td>
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<tr>
<td></td>
<td>40 ± 14</td>
<td>18.5</td>
<td>21 ± 3</td>
<td>19 ± 2</td>
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<tr>
<td>1</td>
<td>63 ± 6</td>
<td>9.5</td>
<td>24 ± 1</td>
<td>40 ± 3</td>
</tr>
<tr>
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<td>28 ± 6</td>
<td>33.5</td>
<td>20 ± 5</td>
<td>47 ± 23</td>
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<tr>
<td>2</td>
<td>38 ± 1</td>
<td>37.5</td>
<td>25 ± 1</td>
<td>28 ± 1</td>
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<tr>
<td></td>
<td>12 ± 2</td>
<td>38.0</td>
<td>14 ± 2</td>
<td>9 ± 3</td>
</tr>
</tbody>
</table>

*Distal* -OSO₂F

*Proximal* -OSO₂F

Plasma stable

Slow reaction with target

Fast reaction with target
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

Compound 1

Compound 2

Compound 3
Cell permeability assay

Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M

Aryl-fluorosulfate-based pan-IAP antagonist is bioavailable

\[ F = \frac{[AUC]}{Dose} \]

\[ F_{PO} = 36\% \]

\[ F_{IP} = 38\% \]

PK data from the UCSD In vivo pharmacology core facility (ms in preparation)
Taming the sulfonyl fluorides

<table>
<thead>
<tr>
<th>ID</th>
<th>X group</th>
<th>aqueous stability</th>
<th>DELFIA IC50 [nM]</th>
<th>$\Delta T_m$ [°C]</th>
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<tbody>
<tr>
<td></td>
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<td>wt-BIR3 (Lys311)</td>
<td>mut-BIR3 (Lys311Tyr)</td>
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<tr>
<td>1</td>
<td></td>
<td>&gt;99% &gt;99%</td>
<td>348 ± 8</td>
<td>4.7 ± 0.1 5.4 ± 0.1 3.0 ± 0.2 2.7 ± 0.5</td>
</tr>
<tr>
<td>2</td>
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<td>54% 0%</td>
<td>15 ± 2</td>
<td>30.1 ± 0.3 29.0 ± 0.4 26.8 ± 0.2 24.7 ± 0.1</td>
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<tr>
<td>3</td>
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<td>57% 0%</td>
<td>47 ± 3</td>
<td>5.1 ± 0.1 5.2 ± 0.3 24.8 ± 0.1 23.4 ± 0.2</td>
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<tr>
<td>4</td>
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<td>39% 0%</td>
<td>173 ± 6</td>
<td>6.7 ± 0.2 6.1 ± 0.1 26.6 ± 0.2 25.2 ± 0.2</td>
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<tr>
<td>5</td>
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<td>58% 0%</td>
<td>12 ± 1</td>
<td>28.5 ± 0.4 28.4 ± 0.4 25.9 ± 0.1 24.7 ± 0.1</td>
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<tr>
<td>6</td>
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<td>80% 15%</td>
<td>21 ± 3</td>
<td>30.4 ± 0.1 30.0 ± 0.2 26.4 ± 0.1 25.1 ± 0.1</td>
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<tr>
<td>7</td>
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<td>94% 73%</td>
<td>122 ± 1</td>
<td>25.8 ± 0.4 25.5 ± 0.2 26.3 ± 0.2 25.0 ± 0.1</td>
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<tr>
<td>8</td>
<td></td>
<td>96% 46%</td>
<td>28 ± 3</td>
<td>26.8 ± 0.7 27.4 ± 0.3 26.0 ± 0.2 24.6 ± 0.1</td>
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<tr>
<td>9</td>
<td></td>
<td>90% 71%</td>
<td>261 ± 16</td>
<td>5.3 ± 0.4 26.0 ± 0.4 24.6 ± 0.1 23.5 ± 0.2</td>
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<tr>
<td>10</td>
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<td>92% 47%</td>
<td>47 ± 2</td>
<td>25.8 ± 0.2 26.0 ± 0.1 25.5 ± 0.2 24.3 ± 0.1</td>
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<tr>
<td>11</td>
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<td>86% 39%</td>
<td>268 ± 5</td>
<td>5.5 ± 0.2 25.6 ± 0.5 25.2 ± 0.1 24.1 ± 0.1</td>
</tr>
</tbody>
</table>

Taming the sulfonyl fluorides
Taming the sulfonyl fluorides

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>Thermal shift data ΔTm [°C] (30min/2hr incubation)</th>
<th>DELFIA IC₅₀ values [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>XIAP-BIR3</td>
<td>cIAP1-BIR3</td>
</tr>
<tr>
<td>LCL161</td>
<td><img src="image" alt="Structure" /></td>
<td>9.8 ± 0.3</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Structure" /></td>
<td>26.7 ± 0.2</td>
<td>24.0 ± 0.7</td>
</tr>
<tr>
<td>13</td>
<td><img src="image" alt="Structure" /></td>
<td>27.0 ± 0.2</td>
<td>15.6 ± 0.8</td>
</tr>
</tbody>
</table>

Design of potent covalent inhibitors of PPIs

Identify initial peptide mimetic (Kd << 10 μM)

HTS by NMR (general library)
fHTS by NMR (target specific library)

Systematic introduction of –SO₂F or –OSO₂F (library)

Design of covalent PPIs antagonists

Structural studies and design of –SO₂F or –OSO₂F derivatives

Target-based evaluations
- IC₅₀ at different incubation times
- Measurements of Thermal Shifts
- Mass spec analysis
- SDS Gel Electrophoresis
- Single point mutations and verifications
NMR based screening of POS combinatorial libraries (HTS by NMR) can be powerful in the identification and optimization of potent and selective peptide mimetics and lead agents.

Expanding the method to focused POS combinatorial libraries using an anchoring moiety (fHTS by NMR) allows rapid identification of low micromolar to nanomolar hits.

Introduction of proper aryl-sulfonyl-fluorides or aryl-fluoro-sulfates can lead to potent, selective, cell permeable (pharmacologically viable) covalent agents targeting Lys and Tyr residues (expanding the target space for covalent drugs).

**covHTS by NMR with fluorosulfates? pY as a probe for Lys?**
HTS by NMR – new sulfonamide based library

Examples of elements of the library:

- MW: 400 +/- 100
- cLogP: 1 +/- 3
- HB donors and acceptors: < 10

Possible combinations: 96 x 36 x 36 = ~124,000 agents

To be tested: 96 MIXTURES (36 x 36 = 1,296 agents)

Baggio, Alboreggia, et al. Pellecchia, under investigation
Thank you!

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