Discovery of Selective OX2 Antagonists and Study of Orexin 1 and 2 GPCR Receptors
Agenda

- Introduction
- Medicinal Chemistry
- Computational Chemistry
Agenda

- Introduction

- Medicinal Chemistry
  - HTS Campaign
  - Indole-sulfone Profile
  - Indole-sulfone Optimisation
  - Conclusions

- Computational Chemistry
Orexin Receptor Antagonists

Insomnia

- Clinically validated new mechanism for insomnia
  - Almorexant, SB-649868, Suvorexant (MK-4305)
- All 3 compounds are dual OX1/2 Antagonists
- An OX2 selective compound has been reported to have hypnotic activity*

Goal: discover a selective OX2 antagonist for insomnia

* Dugovic et al, JPET 2009, 330(1), 142-151
Orexin Antagonist Programme

Screening Campaigns

250K Evotec Library

- Primary screen: Ca$^{2+}$ flux FLIPR in CHO cells transfected with hOX1 or hOX2
- 514 Primary hits
- Counter-screen: activity against CHO-endogenous ATP receptor

70 confirmed, specific hits

- Synthetic tractability
- Med Chem assessment

5 series, 7 singletons

1 ‘series’ selected

OX1 HTS also produced hits (EP2161266):

EP-109-0092

EP-009-0049
## Hit Profiles

**Indole-3-sulfones**

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<tbody>
<tr>
<td>OX1 / OX2 (nM)</td>
<td>3989 / 103</td>
<td>3704 / 50</td>
</tr>
<tr>
<td>Aq Sol (μM)</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>MW / tPSA</td>
<td>433 / 59</td>
<td>447 / 59</td>
</tr>
<tr>
<td>cLogP / LipE</td>
<td>3.9 / 3.1</td>
<td>4.2 / 3.1</td>
</tr>
<tr>
<td>Microsomal Clearance</td>
<td>High / High</td>
<td>High / High</td>
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**Potent, OX2 selective**

Metabolically unstable
Indole-3-Sulfones

Optimisation

Goals:
- Establish SAR
- Increase stability
- Increase aqueous solubility

Strategy:
- Block (or remove) potentially labile positions
  - Most on benzylamine fragment
- Globally reduce lipophilicity
Indole-3-sulfones

Synthetic Route

- Core and Sulfone introduced early
- Late-stage variation of benzylamine
**Indole-3-sulfone Optimisation**

**Screening Cascade**

- hOX1 and hOX2 (FLIPR)

- P2Y1 (endogenous CHO receptor Gq), rOX1 and rOX2

- Non-radioactive GTPγS assay

- Schild plot / MOA

- Human and rat microsomes, aq solubility

- P450, PPB, Caco-2

- IV / PO PK, human and rat hepatocytes

- No OX1 or OX2 species differences noted

- Good correlation between FLIPR and GTPγS assays
Indole-Sulfone SAR

Which changes are tolerated?

- Benzylamine Substitution
- Benzylsulfone Substitution
- Electron-withdrawing indole substituents
- N-methyl deletion

- Fluoro and chloro best
Indole-Sulfone SAR

Which changes aren’t beneficial?

- Conformational restriction
- Amide replacement
- Sulfone replacement (+ indole inversion)
- Electron-donating indole substituents
- Shortened linkers

Early SAR established
## Indole-3-sulfone Optimisation

### Blockade of labile positions

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<tr>
<td><strong>OX1 / OX2 (nM)</strong> FLIPR</td>
<td>3989 / 103</td>
<td>1221 / 23</td>
<td>971 / 46</td>
<td>&gt;20 µM / 33</td>
<td>1576 / 179</td>
</tr>
<tr>
<td><strong>Aq Sol (µM)</strong></td>
<td>&lt;20</td>
<td>-</td>
<td>33</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td><strong>MW / tPSA</strong></td>
<td>433 / 59</td>
<td>451 / 59</td>
<td>477 / 59</td>
<td>485 / 59</td>
<td>465 / 59</td>
</tr>
<tr>
<td><strong>cLogP / LipE</strong></td>
<td>3.9 / 3.1</td>
<td>4.0 / 3.6</td>
<td>4.5 / 2.9</td>
<td>4.6 / 2.9</td>
<td>4.4 / 2.3</td>
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<tr>
<td><strong>Microsomal Clearance Human / Rat</strong></td>
<td>High / High</td>
<td>High / High</td>
<td>High / High</td>
<td>High / High</td>
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Blocking Strategy

Summary

- Microsomal clearance remains high

- Blocking strategy unsuccessful

- Would lipophilicity reduction fare any better?
Reduced Lipophilicity

Benzylamine Modification

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<td>3989 / 103</td>
<td>&gt;20 µM / &gt;20 µM</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>MW / tPSA</td>
<td>433 / 59</td>
<td>438 / 85</td>
<td>434 / 72</td>
<td>434 / 72</td>
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<tr>
<td>cLogP / LipE</td>
<td>3.9 / 3.1</td>
<td>2.5 / -</td>
<td>2.7 / 2.7</td>
<td>2.6 / 2.9</td>
</tr>
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</table>

- Loss of potency on changing the benzylamine group
- Revised synthetic routes needed to target different cores
Indolizine Synthetic Route

- Core and sulfone properties fixed early on
- 2-Methyl group introduced for synthetic reasons
Core properties fixed early on

Sulfone introduced later
Indole-3-sulfone Optimisation

Reduction of Lipophilicity – core changes

Two orders of magnitude potency increase for 2-methylindolizine!
Core Replacement Follow up
Indazole and 2-Methylindole

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<tr>
<td><strong>OX1 / OX2 (nM) FLIPR</strong></td>
<td>998 / 14</td>
<td>&gt;20 µM / 123</td>
<td>278 / 1.1</td>
<td>&gt;20 µM / &gt;20 µM</td>
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<tr>
<td><strong>Aq Sol (µM)</strong></td>
<td>37</td>
<td>-</td>
<td>&lt;20</td>
<td>-</td>
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<td><strong>MW / tPSA</strong></td>
<td>434 / 72</td>
<td>445 / 105</td>
<td>465 / 59</td>
<td>428 / 90</td>
</tr>
<tr>
<td><strong>cLogP / LipE ChemAxon</strong></td>
<td>3.6 / 4.3</td>
<td>3.2 / 3.7</td>
<td>4.2 / 4.8</td>
<td>2.0 / -</td>
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LipE Progression Summary

From Ep-009-0236
Electrostatic Complementarity

Useful tool for structure-base drug discovery

- We developed a method\textsuperscript{1} to predict regions of the ligand that are electrostatically attracted to or repelled by the protein, and to map / visualize it on the ligand surface.
- Modifications in these regions usually have a significant effect on binding affinity.
- We called these important regions of the ligand “hot spots”.

\textsuperscript{1} Davenport and Heifetz et al. Assay Drug Dev Technol. 2010 Dec;8(6):781
Agenda

- Introduction

- Medicinal Chemistry

- Computational Chemistry
  - Receptors selectivity study
  - Conclusions
hOX1 vs. hOX2 Selectivity

Study Motivation

- Precise determinants of antagonist binding and selectivity were neither fully known nor rationalised
  - Site-directed mutagenesis and domain exchange (chimera) studies have provided important insight on key features of the OX1/2 binding sites\textsuperscript{1,2,3}
  - 11 mutations were performed for OX1 and 18 for OX2

- To explain the role of each mutated residue for binding and selectivity of a set of OX1/2 antagonists

- Support discovery of novel OX1/2 antagonists

\textsuperscript{3} Heifetz et al Biochemistry. 2012 Apr 17;51(15):3178
hOX1 vs. hOX2
What we can learn from sequence alignment?

- **TMD:**
  - **Identity:** 69.3%
  - **Similarity:** 80.5%
Intriguing SDM data

Small differences in sequence can lead to large changes in IC$_{50}$

<table>
<thead>
<tr>
<th>Position</th>
<th>hOX1</th>
<th>Almorexant</th>
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<th>hOX2</th>
<th>Almorexant</th>
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<tbody>
<tr>
<td>3.33</td>
<td>A127T</td>
<td>↓↓ ?</td>
<td>3.33</td>
<td>T135A</td>
<td>= ?</td>
</tr>
<tr>
<td>7.43</td>
<td>Y348A</td>
<td>↓↓ ?</td>
<td>7.43</td>
<td>Y354A</td>
<td>= ?</td>
</tr>
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</table>

- Why does the A127$^{3.33}$T mutation significantly reduce binding of Almorexant to OX1, while the T135$^{3.33}$A mutation in OX2 has no effect?

- Why does mutating the conserved tyrosine located in the 7.43 position give opposite effects in OX1 vs OX2?
  
  - mutation of Y348$^{7.43}$ in OX1 eliminated Almorexant binding, the same mutation Y354$^{7.43}$ in OX2 had no effect

Almorexant, Dual OX1/2

hOX1 IC$_{50}^+$ = 6.7 – 19 nM
hOX2 IC$_{50}^+$ = 1.3 – 2.5 nM
Exploration of OX1/2 Receptors

Method validation

OX1 and OX2 Receptor Homology Modelling

Molecular Dynamics Refinement

Analysis of models & antagonist binding

Site-directed mutagenesis study (Biology)

Discovery of Novel OX1, -2 Selective Antagonists (Medicinal Chemistry)
GPCR Modeling Protocol

The optimized similarity routines

- Each tier of the modeling process assesses the need for the next stage

- GPCR specific seq. alignment matrix
- Helical rotation alignment
- Addition of SDM data
- MD kink detection & formation
- Molecular Dynamics Simulations
- Loop remodeling
- MC side-chain rotamer library sim.

- Homology Modeling based on single template
- Hybridized Homology Modeling
- Ab-initio Decoy Template Modeling

- Protein packing & complementary score
- Fit to sequence alignment
- Individual optimization routine scoring
- Fit to SAR and SDM data
MD Simulations

Established Protocol for GPCR Modelling

Aims

• Optimise homology models
• Explore structural behaviour -
  ➢ OX1/OX2 and mutants/wild-types

**System size**

- ~117k atoms
- ~31k waters
- ~327 residues
- ~5200 protein atoms
- ~350 lipids

**GROMACS**

OPLS-AA forcefield

Performance = ~1ns/day 8 cores
Focus on TM3

Focus on TM3 – important for selectivity of OX antagonists

Amino-acid sequence differences

Different inter-helical interactions formed with neighbouring TMs

Conformational Change
Focus on TM3

- Different inter-helical interactions force the core of TM3^{OX1} and TM3^{OX2} to adopt slightly different conformations.

- In OX2, there appeared to be a tilting of the TM3^{OX2} core away from its original position in OX1.
The residue $Y_{348}^{7.43}$ does not form a direct interaction with Almorexant but it still plays a key role by stabilising $Q_{126}^{3.32}$ in a conformation in which it interacts with Almorexant.

The mutation of $A_{127}^{3.33}$ into the larger residue threonine limits the approach of the antagonists into the OX1 sub-pocket between TM3, 4 and 5.
Intriguing SDM data

Solving the mystery

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- We showed that different inter-helical interactions force the conformations of TM3OX1 and TM3OX2 to be different

- A127 and T135 are located in the same position in the sequences but in different locations in the structures

- The mutation of A1273.33 into the larger residue threonine limits the approach of antagonists into the OX1 sub-pocket between TM3, 4 and 5, resulting in a significant decrease in the binding of certain antagonists

- The OX1 residue Y3487.43 does not form a direct interaction but it still plays a key role by stabilising Q1263.32 in a conformation in which it interacts with antagonists – so mutating it into Alanine will affect antagonist binding

- The OX2 residue Y3547.43 (in contrast to Y3487.43 of OX1) is “unemployed”, it neither interacts directly with antagonists nor does it stabilise Q1343.32 – so mutating it to Alanine will not have any effect
GPCRs Modelling Conclusions

- Minor differences in sequences can lead to significant differences in the tertiary structure of GPCRs, their ability to bind ligands, and their selectivity

- GPCR models based solely on homology modelling might not be sufficient to rationalise potency and selectivity

- MD simulations allow refinement of GPCR models to a degree that is not possible with static homology modelling alone

- The structural insights gained from this process are critical for rationalising the SDM data, and for the design of new GPCR ligands
Discovery of Novel OX1, -2 Selective Antagonists

A: hOX1R with EP-009-0049 (OX1 selective)

B: hOX2R with EP-009-0513 (OX2 Selective)
### Acknowledgements

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<tr>
<th>Evotec (UK)</th>
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<th>University of Oxford</th>
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<tr>
<td>Tara Fryatt</td>
<td>Dominique Manikowski</td>
<td>G. Benjamin Morris</td>
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<td>Oliver Barker</td>
<td>Rita Reifegerste</td>
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<td>Gurubaran Raju</td>
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<td>Sandeep Pal</td>
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<td>Richard Law</td>
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- *In vitro* DMPK Group (UK)
- Evotec (India)
- Royal Society, UK for Industry award
Appendix
• TMD Cα atom RMSDs fluctuate within the same narrow range of 1.7 to 2.4 Å, which is comparable to the values typically obtained for MD simulations of GPCR crystal structures.

• TM3 Cα atom RMSDs fluctuate within a narrow range between 1.0 and 1.5 Å for the OX1WT, OX1A127T and OX2WT indicates that the TM3 conformation of receptors is almost “frozen” AND is not affected by the A127T3.33 mutation.
OX1 vs. OX2 Receptor

Final Models

- TMD conformational differences observed in structures and binding site topology of the OX1 vs. OX2 structures
- OX1 is orange and OX2 is green
Electrostatic Complementarity

Useful tool for structure-base drug discovery

- We developed a method\(^1\) to predict regions of ligand that are electrostatically attracted to or repelled by the protein, and to map / visualise it on the ligand surface.
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