Paths to drug discovery and the role of computational chemistry

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Munich
My research lines

From enzymatic function to drug discovery
My research lines

- From basic to applied science -

☑ Computational Enzymology – Reaction mechanism

☑ From enzymatic function to mechanism of inhibition

☑ Structure-Based Drug Discovery
Topics of the day

1. The process of Drug Discovery & Development

2. Computational Drug Design
   Structure-based Drug Design
"Is there really a case where a drug that’s on the market was designed by a computer?"

When asked this, I invoke the professorial mantra ("All questions are good questions."), while sensing that the desired answer is "no". Then, the inquisitor could go back to the lab with the reassurance that his or her choice to avoid learning about computational chemistry remains wise. The reality is that the use of computers and computational methods permeates all aspects of drug discovery today. Those who are most proficient with the computational tools have the advantage for delivering new drug candidates more quickly and at lower cost than their competitors.
“Is there really a case where a drug that’s on the market was designed by a computer?”

However, the phrasing of the question suggests misunderstanding and oversimplification of the drug discovery process (..) It is inconceivable that a human with or without computational tools could propose a single chemical structure that ends up as a drug; there are far too many hurdles and subtleties along the way.
What is a Target: any bio-macromolecule (e.g., enzymes or channels)

**Target Identification** aims to determine a biological macromolecule that is related to the development or to the life cycle of a certain pathology.

**Target validation** aims to establish the link between inhibition/modulation of the target activity and the potential cure of the disease.

- Tricky issue: different “levels” of validation.
Reliability of ‘new drug target’ claims called into question

Bayer halts nearly two-thirds of its target-validation projects because in-house experimental findings fail to match up with published literature claims, finds a first-of-a-kind analysis on data irreproducibility.

Asher Mullard
Drug Discovery & Development

Drug Discovery process

What is a Hit: small-molecule inhibitor with low affinity for the target

Affinity:

\[ P + I \rightleftharpoons PI \]

\[ K_i = \frac{[P][I]}{[PI]} \]

Competitive mechanism of inhibition:

\[ P + I \rightleftharpoons PI \]

\[ S + PI \rightleftharpoons PS \]

\[ K_i \sim 10 \text{ nM} \]
Drug Discovery & Development

Drug Discovery process

Target Discovery ➔ Hit Identification ➔ Hit-to-Lead ➔ Lead Optimization ➔ Preclinical Development ➔ Drug Candidate

What is a Hit: small-molecule inhibitor with low affinity for the target

Inhibitory Concentration (IC)\textsubscript{50}: The half maximal inhibitory concentration
Drug Discovery & Development

Drug Discovery process

What is a Hit: small-molecule with low (but promising!) affinity for the target

Relationship between Ki and IC\(_{50}\) (Cheng-Prusoff equation):

\[
Ki = \frac{IC_{50}}{1 + \left[\frac{[S]}{K_M}\right]}
\]

For competitive inhibitors

\([S] = \text{Substrate concentration}\)

When \([S] \sim 0 => Ki \sim IC_{50}\)

Hit compounds have low affinity (or IC50) - ~10-1 \(\mu\)M
Drug Discovery & Development

Drug Discovery process

Target Discovery \hspace{1cm} Hit Identification \hspace{1cm} Hit-to-Lead \hspace{1cm} Lead Optimization \hspace{1cm} Preclinical Development \hspace{1cm} Drug Candidate

Methods for hit identification:

- High Throughput Screening (HTS)
- NMR screening
- Crystallography
- Computational methods

Required:
Assay development

Goal: identify possible active “scaffolds”
What is a scaffold: the simplest substructural element of a molecule

The scaffold often represents the “core” of the molecule, and it greatly affects the physicochemical properties of the molecule (for instance, solubility).

For this reason, **DIVERSITY** in screening is the **KEY**.

Source: Scaffold composition and biological relevance of screening libraries
Drug Discovery & Development

Drug Discovery process

Target Discovery → Hit Identification → Hit-to-Lead → Lead Optimization → Preclinical Development → Drug Candidate

Example, a good hit:

Melanocortin 4 receptor (MC4R)
Millennium Pharmaceuticals & Abbot

Ki = 2.7 μM

Vos, TJ et al. 2004 J Med Chem 47 1602 - 04
Drug Discovery & Development

Drug Discovery process

- Target Discovery
- Hit Identification
- Hit-to-Lead
- Lead Optimization
- Preclinical Development

Drug Candidate

What is a Lead: a compound with improved affinity for the target

Methods for hit-to-lead:
- Medicinal chemistry
- Computational methods
- Crystallography
- In vitro testing

Lead compounds have high affinity (or IC50) - < 1 μM or better

Goals: 1. improve compound activity (better affinity)
2. create series of active compounds

Series of compounds: compounds sharing the same scaffold.
Drug Discovery & Development

Drug Discovery process

Target Discovery > Hit Identification > Hit-to-Lead > Lead Optimization > Preclinical Development → Drug Candidate

What is a Lead: a compound with improved affinity for the target

A must be aromatic; optimal substitution is 2-methoxy-5-bromo-phenyl

Y = CH2, S are equivalent; two atoms linker is optimal

In B, substitutions are tolerated only in 3’

In C, cyclic amidine is necessary; R = H, alkyl are equivalent; n = 1,0 are equivalent

Vos, TJ et al. 2004 J Med Chem 47 1602 - 04
Drug Discovery & Development

Drug Discovery process

Target Discovery → Hit Identification → Hit-to-Lead → Lead Optimization → Preclinical Development → Drug Candidate

What is a Lead: a compound with improved affinity for the target

MC4R Antagonist Lead

\[ \text{Ki} = 0.04 \ \mu M \]

Potency improved almost two orders of magnitude

\[ \text{Ki} = 2.7 \ \mu M \]

Vos, TJ et al. 2004 J Med Chem 47 1602 - 04
Drug Discovery & Development

Drug Discovery process

Example: non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs).

Optimization of Azoles as Anti-Human Immunodeficiency Virus Agents Guided by Free-Energy Calculations
Jorgensen W et al
J. AM. CHEM. SOC. 2008, 130, 9492–9499
Best leads are optimized in terms of their drug-likeness: Improved affinity is coupled with a promising pharmacokinetic (PK) profile.

PK includes **ADMET** characterization:

- **Absorption** (e.g., bioavailability, F)
- **Distribution** (e.g., binding to serum proteins)
- **Metabolism** (metabolites, e.g., cytochrome P450)
- **Excretion** (kidneys system)
- **Toxicity** (e.g., Affinity towards h-ERG)

The route of administration (e.g., intravenous, oral) and the site of action of the drug (e.g., brain, skin, organs) critically influence the desired ADMET profile.
Methods for lead optimization:
- Computational methods
- Medicinal chemistry
- Crystallography
- In vitro testing
- In vivo testing (key for lead optimization)

Additional issue:
- Target specificity – Assays development involved.

Ultimately:
Lead optimization aims to develop and identify promising compounds within a given series of compounds suitable for preclinical development.
**Drug Discovery process**

- **Target Discovery**
- **Hit Identification**
- **Hit-to-Lead**
- **Lead Optimization**
- **Preclinical Development**

---

**MC4R Antagonist Lead**

\[ \text{Ki} = 0.04 \, \mu M \]  

\[ \text{Ki} = 0.16 \, \mu M \text{ but BETTER PK profile!} \]

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**ML00253674**

Vos, TJ et al. 2004 J Med Chem 47 1602 - 04

Marsilje, T et al. 2004 Bioorg Med Chem Letters 14 3721 - 3725
Drug Discovery & Development

Drug Discovery process

Goals:
1. determination of the lead’s ultimate safety profile.
2. determination of a safe starting dose of the drug for clinical trials

Methods:
- Synthetic chemistry (scale up)
- Further in vivo pharmacology
- Further Tox/safety Pharmacology
Drug Discovery & Development

Drug Discovery process

Target Discovery → Hit Identification → Hit-to-Lead → Lead Optimization → Preclinical Development → Drug Candidate

**Drug Candidate:**
Winner molecule.
Molecule that will be moved into clinical trials. *(tough decision... big gamble...)*

**Note:**
For those scientists involved in drug discovery research, to contribute to the creation of a drug candidate is a great achievement.

Although getting a drug out if it... would make it better ... 😊
Computational Drug Design

Two approaches:

🔹 **Structure-Based Drug Design - SBDD**
- Drug design based on the interaction of the **ligand** with the 3D dimensional structure of the **receptor**

🔹 **Ligand-Based Drug Design - LBDD**
- Unknown structure of the receptor.
- Drug design based on the key features of active **compounds**.
- **Hypothesis:**
  Ligands similar to an active ligand are more likely to be active than random ligands. (pharmacophore models)
Computational Drug Design

Approaches and methods:

✧ Structure-Based Drug Design - SBDD

Docking (Glide, Dock, Autodock, ICM... etc)
Kitchen D., Nat. Review Drug Discovery Vol. 3 Nov. 2004

De novo design (BOMB, SMoG, BREED.. etc)

✧ Ligand-Based Drug Design - LBDD

Quantitative Structure-Activity Relationship (QSAR)

Ligand similarity approaches (2D or 3D)
Structure-Based Drug Design

**MUST:** 3-dimensional structure of the target.

Sources of structures:
1. Crystallography
2. NMR structures
3. Homology structures

![Diagram showing the process of structure-based drug design](Image)
Structure-Based Drug Design

**MUST:** 3-dimensional structure of the target.

Sources of structures:
1. Crystallography
2. NMR structures
3. Homology structures

Average of multiple structures
**MUST:** 3-dimensional structure of the target.

Sources of structures:
1. Crystallography
2. NMR structures
3. Homology structures
Docking molecules...

An attempt to predict the structure(s) of the complex formed between the ligand and the macromolecule.
Docking

Target

Molecule
Ligand Docking

Two problems to solve:
1. Posing (the easy part)
2. Scoring (the tough part)
Ligand Docking - Posing

The posing problem (*the easy part*):

**The posing is a “solved” problem!**

Docking methods can place a ligand with great accuracy in a binding pocket, reproducing crystallographic data.

The challenging issue is to rank different poses, according to their score and identify the “best one”.

Docking and scoring in virtual screening for drug discovery: methods and applications
Douglas B. Kitchen et al
*Drug Discovery, Vol. 3, Nov 2004*
Ligand Docking - Scoring

Ligand Binding

The quantitative modeling of receptor–ligand interactions can be achieved by determining the equilibrium binding constant $K_{eq}$. The binding constant $K_{eq}$ is directly related to the Gibbs free energy:

$$\Delta G_{bind} = -RT \ln K_{eq}$$

$$\Delta G_{bind} = \Delta H - T \Delta S = G_{complex} - (G_{receptor} + G_{ligand}).$$

Why it is so difficult to score compounds:

**Experimental range of binding affinities:** from $10^{-2}$ M (mM) to $10^{-12}$ M (pM)

At $T=298$K the enthalpic contribution to the $\Delta G_{binding}$ is between -2.4 kcal/mol and -16.7 kcal/mol

In other words, a change in binding (free) energy of ~1.5 kcal/mol alters the binding affinity of one order of magnitude ($T=298$) !!!!
Ligand Docking - Scoring

Scoring Functions FOR DOCKING CALCULATIONS:

• Force-Field Based

• Empirical

• Knowledge-based

Consensus Scoring

Consensus scoring are more often applied in Virtual Screening
An exercise carried out by computational means aimed at predicting which molecules from an ensemble will likely display some activity against a target.

VLS is usually implemented as an iterative docking simulation at the target binding site.

<table>
<thead>
<tr>
<th></th>
<th>1st -37.9 kcal/mol</th>
<th>2nd -29.7 kcal/mol</th>
<th>3rd -27.3 kcal/mol</th>
<th>4th -9.8 kcal/mol</th>
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<tr>
<td><img src="image1.png" alt="Molecule 1" /></td>
<td><img src="image2.png" alt="Molecule 2" /></td>
<td><img src="image3.png" alt="Molecule 3" /></td>
<td><img src="image4.png" alt="Molecule 4" /></td>
<td><img src="image5.png" alt="Molecule 5" /></td>
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</table>
Structure-based drug design
From hit to lead

Example: non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs).

HIT

EC$_{50}$ = 5 μM

<table>
<thead>
<tr>
<th>compd</th>
<th>R$^1$</th>
<th>X</th>
<th>Y</th>
<th>R$^2$</th>
<th>EC$_{50}$</th>
<th>CC$_{50}$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4-Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4.8</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>4-Cl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1.2</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>4-Cl</td>
<td>2-Cl</td>
<td>H</td>
<td>H</td>
<td>0.62</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>4-Cl</td>
<td>H</td>
<td>3-Cl</td>
<td>H</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>4-Cl</td>
<td>2-Cl</td>
<td>4-Cl</td>
<td>H</td>
<td>2.9</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>4-Cl</td>
<td>H</td>
<td>3-Cl</td>
<td>5-Cl</td>
<td>1.3</td>
<td>10</td>
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<tr>
<td>10</td>
<td>4-Cl</td>
<td>2-Cl</td>
<td>5-Cl</td>
<td>H</td>
<td>0.38</td>
<td>15</td>
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<td>12</td>
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<td>Me</td>
<td>2.4</td>
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<tr>
<td>13</td>
<td>4-Cl</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>2.5</td>
<td>27</td>
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<tr>
<td>14</td>
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<td>Cl</td>
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<td>21</td>
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<td>15</td>
<td>5-Cl</td>
<td>2-Cl</td>
<td>H</td>
<td>H</td>
<td>0.41</td>
<td>12</td>
</tr>
</tbody>
</table>

Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents
Jorgensen W et al
J. MED. CHEM. 2011, 54, 8582–8591
Structure-based drug design
From hit to lead

Example: non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs).

HIT

\[
\text{EC}_{50} = 5 \, \mu\text{M}
\]

Lead

\[
\text{EC}_{50} = 55 \, \text{pM}
\]

Computationally-Guided Optimization of a Docking Hit to Yield Catechol Diethers as Potent Anti-HIV Agents
Jørgensen W et al
J. MED. CHEM. 2011, 54, 8582–8591
Crystal Structures of HIV-1 Reverse Transcriptase with Picomolar Inhibitors Reveal Key Interactions for Drug Design

Kathleen M. Frey,‡ Mariela Bollini,† Andrea C. Mislak,‡ José A. Cisneros,‡ Ricardo Gallardo-Macias,† William L. Jorgensen*,† and Karen S. Anderson*‡

†Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States
‡Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520-8066, United States

Crystal Structures of HIV-1 Reverse Transcriptase with Picomolar Inhibitors Reveal Key Interactions for Drug Design

Jorgensen W et al
J. Am. Chem. Soc. 2012, 134, 19501
De novo drug design

**DE NOVO DESIGN** produces novel molecular structures FROM SCRATCH, by incremental construction of a ligand model within a model of the receptor or enzyme active site.

**Procedure:**
1. Identify catalytic site
2. Place a starting seed (small fragment)
3. Build compounds using fragments as building blocks
4. Score final molecule
De novo drug design

**DE NOVO DESIGN** produces novel molecular structures FROM SCRATCH, by incremental construction of a ligand model within a model of the receptor or enzyme active site.

**Major advantages:**
1. Investigation of chemical space outside the chemical space contained in a chemical collection/dataset (docking).
2. Optimization of the receptor/ligand interaction with ad-hoc modification of the ligand.
De novo drug design

DE NOVO DESIGN produces novel molecular structures FROM SCRATCH, by incremental construction of a ligand model within a model of the receptor or enzyme active site.

Major limitations:
1. The number of chemically feasible, drug-like molecules has been estimated to be in the order of $10^{60} - 10^{100}$. Huge chemical space!
2. Is there an available route for synthesis to make the promising compounds?
De novo drug design

Modus operandi:
1. Individuation of key interactions in the binding pocket (binding hypothesis).
2. Evaluations of starting seeds and their interaction with key residues in the pocket.
3. Incremental construction of compounds by screening of fragments as building blocks.
4. CONSTANT interaction with synthetic chemists, in order to build feasible compounds. (for instance, avoid stereo-centers!)
Drug-likeness (‘druggability’)  

When is a compound drug-like?

Lipinski's Rule of Five
1. Not more than 5 hydrogen bond donors (NH, OH groups)
2. Not more than 10 hydrogen bond acceptors (N and O)
3. A molecular weight under 500
4. An octanol-water partition coefficient less than 5 (logP < 5)

\[
\log P_{\text{oct/wat}} = \log \left( \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}} \right)
\]

- \( \log P_{\text{oct/wat}} = 0 \)  
  ration 1:1 oct/wat
- \( \log P_{\text{oct/wat}} = 1 \)  
  ration 10:1 oct/wat
- \( \log P_{\text{oct/wat}} = -1 \)  
  ration 1:10 oct/wat

Drug-likeliness (‘druggability’)

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\[
\text{Log } P_{\text{oct/wat}} = \log \left( \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}} \right)
\]

\[
\text{Log } P_{\text{oct/wat}} = 0 \quad \text{ration } 1:1 \text{ oct/wat}
\]
\[
1 \quad \text{ration } 10:1 \text{ oct/wat}
\]
\[-1 \quad \text{ration } 1:10 \text{ oct/wat}
\]

Several exceptions!
For instance: Macrolides (antibiotics) are good drugs, but do not comply with the Lipinski’s Rule of five!

Drug-likeness (‘druggability’)
Structure-Based Drug Design

**Step 1**

- Binding design – new inhibitors
  - X-ray Structure
    - X-ray Structure refinement
      - Compound (De novo) Design
        - Synthesis
          - In vitro test

**Step 2**

- From inhibitors to drugs
  - Step 1 outputs
    - Computed ADME profile
      - SAR with experiments
        - Hypothesis generation & Determination of key descriptors
          - Predictive statistical model
            - Compound design (optimization)
              - Synthesis and test
Inflammation and pain

- Tens of millions of people afflicted worldwide
- Current therapies have major limitations (gastric lesions, kidney damage, vascular events, CNS penetration related issues)
- A number of inflammatory/pain pathways exist
- Yet an unmet medical need

I. Melnikova, Nature Reviews Drug Discovery 2010, 9, 589
The arachidonic acid pathway

- Among the oldest medicines ever
- Still widely used
- Not always effective
- Several side-effects

The cannabinoid pathway

- Relatively new
- Beneficial effects of CBs stimulation without severe side effects

D. Piomelli, A. Giuffrida, A. Calignano, F.R. de Fonseca, Trends in Pharmacological Sciences, 2000, 21, 218
Multitarget FAAH-Cyclooxygenases (COXs)

Dual-target Single Compound → Dual-binding & Inhibition → COX → Synergistic effect → Biological response → Neuropathic and Inflammatory pain treatment
Dual FAAH-COX inhibition

- Recent studies have shown promising data about the simultaneous inhibition of COX and FAAH
- FAAH product is COX substrate
- Anandamide can be metabolised by COX2

CJ. Fowler, PS. Naidu, A. Lichtman and V. Onnis, British Journal of Pharmacology, 2006, 156, 412
Getting the best from both worlds

- A dual-inhibition could:
  - Increase efficacy
  - Lower side-effects
- Single compound better than cocktails of drugs
- Probably the most effective drugs are multitarget

R. Morphy, Z. Rankovic, Drug Discovery Today, 2007, 12, 156
POC: in vivo studies – synergistic effect

Carrageenan-induced inflammation

Mechanical hyperalgesia

There is a synergistic effect of co-administration of ARN 354 and Indomethacin
POC: in vivo studies – side effects

**Gastric Toxicity**

- Indomethacin (3 mg/Kg, os.)
- Indomethacin (10 mg/Kg, os.)

**ARN 354 has a protective effect on Indomethacin-induced gastric ulcers**

---

O Sasso, R Bertorelli, A Reggiani, D Piomelli, Pharmacological research 65, 553-63 (2012)
Multitarget FAAH-Cyclooxygenases (COXs)

Similarity in binding pockets

Hydrophilic (light blue) and hydrophobic (orange) isocontour surfaces
Looking for multitarget inhibitors

- Framework Combination

- Selective Ligands

- Linked

- Fused

- Merged

- High MW

Adapted from “Lead Generation Approaches in Drug Discovery”
From single to dual target inhibitor

Docking COX inhibitors in FAAH

~380 COXs inhibitors from DrugBank and DUD were docked into the structure of FAAH

25 compounds purchased and tested

Topological distance and physico-chemical similarity
In vitro Assays

**FAAH radiometric assay**

The assay directly measures PGF$_{2\alpha}$ by SnCl$_2$ reduction of PGH$_2$ generated by *ovine* COX1 or recombinant human COX2 via an immunoenzymatic method with a specific antiserum that binds all major PG subtypes (Cayman Chemicals Co., MI – USA).

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC50± SD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URB597</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>URB937</td>
<td>3.1±1.0</td>
</tr>
<tr>
<td>PF-4457845</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

**COX1 and COX2 assay**

- **URB937**
  - IC50 = 1.1 ± 0.1 nM

- **PF-4457845**
  - IC50 = 3.1 ± 1.0 nM

- **DUPE897**
  - IC50 = 5.6 nM
From single to dual target inhibitor

Carprofen, ARN0120

- Known COX inhibitor
- Marketed as Rimadyl® for the treatment of arthritic symptoms in geriatric dogs (available as a racemate)
- Used in humans from 1988 to 1998 (withdrawn after Advil® launch)

<table>
<thead>
<tr>
<th>FAAH IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>COX-1 IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>COX-2 IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>22</td>
<td>4</td>
</tr>
</tbody>
</table>
Unique non-covalent FAAH binding site
Resolution: 2.3 Å

“A binding site for non-steroidal anti-inflammatory drugs in Fatty Acid Amide Hydrolase”
Bertolacci L, ..., De Vivo M, Piomelli D, Garau G
J Am Chem Soc, ASAP Article
Improving the affinity

- De novo design using BOMB
  - Starting point for step-wise growth and decorations

BOMB 2.7 and MCPRO 2.1, Prof. W. Jorgensen, Yale
SAR exploration around ARN0120

Cl essential for COX activity

Most chemical functions are tolerated on FAAH
COX activity is highly dependant on the substitution

COOH essential for COX activity

esters, amides

alkyls, acyls, ureas, carbamates, sulfonamides

**ARDS**

**ARN0120**

**ARN0426**

FAAH: IC$_{50}$ 22 µM

COX-1: IC$_{50}$ 74 µM

COX-2: IC$_{50}$ 72 µM
SAR exploration around ARN0426

- Change the position of Cl on the pendant phenyl ring
- Replace Cl by another atom/group
- Identify alternative pendant rings

**ARN0426**
- FAAH: $IC_{50}$ 22 µM
- COX-1: $IC_{50}$ 74 µM
- COX-2: $IC_{50}$ 72 µM

**ARN1406**
- FAAH: $IC_{50}$ 20 µM
- COX-1: inactive
- COX-2: inactive

**ARN1313**
- FAAH: $IC_{50}$ 60 µM
- COX-1: inactive
- COX-2: inactive

**ARN2579**
- FAAH: inactive
- COX-1: inactive
- COX-2: inactive
SAR exploration around ARN0426

- Change the position of Cl on the pendant phenyl ring
- Replace Cl by another atom/group
- Identify alternative pendant rings

**ARN0426**

- FAAH : IC$_{50}$ 22 µM
- COX-1 : IC$_{50}$ 74 µM
- COX-2 : IC$_{50}$ 72 µM

**ARN2485**

- FAAH : IC$_{50}$ 31 µM
- COX-1 : inactive
- COX-2 : inactive

**ARN1324**

- FAAH : IC$_{50}$ 11 µM
- COX-1 : inactive
- COX-2 : inactive

**ARN2578**

- FAAH : IC$_{50}$ 25 µM
- COX-1 : inactive
- COX-2 : inactive
SAR exploration around ARN0426

- Change the position of Cl on the pendant phenyl ring
- Replace Cl by another atom/group
- Identify alternative pendant rings

**ARN0426**
- FAAH: IC$_{50}$ 22 µM
- COX-1: IC$_{50}$ 74 µM
- COX-2: IC$_{50}$ 72 µM

**ARN1421**
- FAAH: IC$_{50}$ 85 µM
- COX-1: IC$_{50}$ 30 µM
- COX-2: IC$_{50}$ 28 µM

**ARN2606**
- FAAH: IC$_{50}$ 6 µM
- COX-1: IC$_{50}$ 13 µM
- COX-2: inactive

**ARN14065**
- FAAH: inactive
- COX-1: inactive
- COX-2: inactive
From single to dual target inhibitor

- We identified multitarget inhibitors that block simultaneously FAAH, COX-1 and COX-2 activities, which are among the most active dual FAAH/COX inhibitors so far reported.

**Arn0120**
- FAAH: IC\(_{50}\) 79 µM
- COX-1: IC\(_{50}\) 22 µM
- COX-2: IC\(_{50}\) 4 µM

**Arn0426**
- FAAH: IC\(_{50}\) 22 µM
- COX-1: IC\(_{50}\) 74 µM
- COX-2: IC\(_{50}\) 72 µM

**Arn1421**
- FAAH: IC\(_{50}\) 85 µM
- COX-1: IC\(_{50}\) 30µM
- COX-2: IC\(_{50}\) 28µM

*Identification and characterization of carprofen as a multitarget FAAH/COX inhibitor*
Favia AD, .., and De Vivo M
Drug development process

IND:
Investigational New Drug application to the FDA (Food&drug Administration) containing results of preclinical studies, results of chemical manufacturing controls (including details of active ingredients, stability and purity) and other laboratory results requesting permission to conduct studies in humans
Drug Discovery & Development

Drug development process

Drug Candidate → Phase I → Phase II → Phase III → FDA review and approval → Market

IND → CLINICAL TRIALS → NDA

Phase I Clinical Trials:
20-100 healthy subjects; First-in-man safety studies.

SAFETY!
Drug Discovery & Development

Drug development process

Drug Candidate → Phase I → Phase II → Phase III → FDA review and approval → Market

IND → CLINICAL TRIALS → NDA

Phase 2 Clinical Trials:
100-500 subjects; Primary focus is efficacy and includes additional safety and side effect analysis. Dosage guidelines formulated.

EFFICACY ! (& SAFETY)
Drug Discovery & Development

Drug development process

Phase 1
IND

Phase II

Phase III
NDA

FDA review and approval

Market

Drug Candidate

CLINICAL TRIALS

Phase 3 Clinical Trials:
1,000-5,000 subjects; Pivotal trials. Drugs that complete Phase III have ~60% chance of FDA approval.
Safety profiles and side effects further studied to establish the benefit-risk relationship.

EFFICACY & SAFETY ON LARGER NUMBER OF PATIENTS
Drug Discovery & Development

Drug development process

Drug Candidate → Phase I → Phase II → Phase III → FDA review and approval → Market

IND → CLINICAL TRIALS → NDA

NDA:
Submission of New Drug Application to the FDA Center for Drug Evaluation and Research requesting clearance to market the drug. This application contains information on every patient from the clinical trials, and information on the company’s drug production.
Drug development process

Drug Candidate → Phase I → Phase II → Phase III → FDA review and approval → Market

IND → CLINICAL TRIALS → NDA

FDA approval for a NME (New Molecular entity)
A new small-molecule drug can be referred to as a NME.
Drug Discovery & Development

Drug development process

Drug Candidate > Phase I > Phase II > Phase III > FDA review and approval > Market

IND > CLINICAL TRIALS > NDA

Example
Drug on the market (ZYVOX)

1 linezolid
Drug on the market (Zyvox)

1993: first synthesis
1995: clinical trials – Phase I
**mid-1996**: phase II
1998: Phase III
**April, 18 2000**: FDA approval

**Impact on patients:**
About 2 million patients in hospitals in U.S. every year find that they have contracted a hospital-acquired or so-called nosocomial infection (including: methicillin-resistant *Staphylococcus aureus* (MRSA)).

It is estimated that nosocomial infections lead to 90,000 deaths per year in the U.S. and that 70% of these infections are caused by bacterial pathogens.

About 3.7 million patients have been cured with Zyvox since 2000.

**Economic return:**
**Zyvox**: ~1 Bn $ only in 2008, (Pfizer).
Drug on the market (Zyvox)

1993: first synthesis
1995: clinical trials – Phase I
mid-1996: phase II
1998: Phase III
April, 18 2000: FDA approval

Impact on patients:
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It is estimated that nosocomial infections lead to 90 000 deaths per year in the U.S. and that 70% of these infections are caused by bacterial pathogens.

Linezolid (ZYVOX), the First Member of a Completely New Class of Antibacterial Agents for Treatment of Serious Gram-Positive Infections
Drug Discovery & Development

Drug development process

Drug Candidate → Phase I → Phase II → Phase III → FDA review and approval → Market

IND → CLINICAL TRIALS → NDA

After the drug is approved and reaches the market:

Phase 4 Clinical Trials
Ongoing surveillance for side effects in routine use for as long as the drug remains on the market.

Example:
Vioxx (Merck product for pain relief) was approved by FDA in 1999 and then withdrawn from the market in 2004, for safety concerns.
Drug Discovery & Development

Average time requested for it

Time zero

Target Discovery

Hit Identification

Hit-to-Lead

Lead Optimization

Preclinical Development

Drug Candidate

IND

CLINICAL TRIALS

NDA

Drug Candidate

Phase I

Phase II

Phase III

FDA review and approval

Market

1.5

1.5

2

1

(~6 years for discovery)

1.5

2.5

2.5

1

(~7.5 years for development)

Total time (on average) = ~13.5 years
Drug Discovery & Development

Average cost requested for it

Target Discovery  Hit Identification  Hit-to-Lead  Lead Optimization  Preclinical Development

Drug Candidate

(~820 $ Million for discovery)

Drug Candidate

IND  CLINICAL TRIALS  NDA

Phase I  Phase II  Phase III

Market

(~960 $ Million for development)

Total cost on average = ~1.78 $ Billion for one NME

Source: How to improve R&D productivity: the pharmaceutical industry’s grand challenge
Steven M. Paul, Nat. Review Drug Discovery March Vol. 9 2010
In the period 1991 –2000, the highest % of attrition of drug in clinical development was due to sub-optimal PK/bioavailability properties.

Success rates from first-in-man to registration.

The overall clinical success rate is 11% (2004 estimate)

Patent:
FDA approved drug patent lasts 20 (+extension) years. Time start from patent approval, which means that life of protection for an on-the-market drug varies a lot.

Best 3 blockbusters on the market (2007):
Lipitor (Pfizer) treats high cholesterol - \(~13.7\) bl \$\ per year
Plavix (BMS/Sanofi-Aventis) used for heart disease - \(~8.1\) bl \$\ per year
Advair (GSK) used for Asthma - \(~7.0\) bl \$\ per year

FDA drug approvals
19 NMEs approved in 2009.

Source: 2009 FDA drug approval
Bethan Hughes,
Nat. Review Drug Discovery Feb Vol. 9 2010
Drug Discovery & Development

- Some facts -

Table 1 | Selected drugs facing patent expiry in the United States

<table>
<thead>
<tr>
<th>Branded drug (INN drug name; company)</th>
<th>Indication</th>
<th>Worldwide 2009 sales (billion)*</th>
<th>Expected patent expiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aricept (donepezil; Eisai/Pfizer)</td>
<td>Alzheimer’s-type dementia</td>
<td>¥303.8 (US$3.61)</td>
<td>Nov 2010</td>
</tr>
<tr>
<td>Lipitor (atorvastatin; Pfizer)</td>
<td>High cholesterol</td>
<td>US$11.43</td>
<td>2011</td>
</tr>
<tr>
<td>Zyprexa (olanzapine; Eli Lilly &amp; Company)</td>
<td>Schizophrenia, bipolar I disorder</td>
<td>US$4.92</td>
<td>2011</td>
</tr>
<tr>
<td>Lexapro (escitalopram; Forest Laboratories/Lundbeck)</td>
<td>Depression and anxiety</td>
<td>DKK 7.77 (US$1.37)</td>
<td>2012</td>
</tr>
<tr>
<td>Actos (pioglitazone; Takeda)</td>
<td>Type 2 diabetes</td>
<td>¥334.5 (US$3.98)*</td>
<td>2012</td>
</tr>
<tr>
<td>Plavix (clopidogrel; Sanofi–Aventis/Bristol-Myers Squibb)</td>
<td>Clot-related cardiovascular events</td>
<td>US$6.15</td>
<td>2012</td>
</tr>
<tr>
<td>Lovenox (enoxaparin; Sanofi–Aventis)</td>
<td>Acute deep vein thrombosis</td>
<td>€3.04 ($4.03)</td>
<td>2012</td>
</tr>
<tr>
<td>Seroquel (quetiapine; AstraZeneca)</td>
<td>Schizophrenia, bipolar disorder, major depressive disorder</td>
<td>US$4.87</td>
<td>2012</td>
</tr>
</tbody>
</table>

*Data from company annual reports. *Europe and the Americas. INN, international nonproprietary name.

Source: The patent cliff steepens
Harrison C., Nat. Review Drug Discovery Jan Vol. 10 2011

Dangling from the patent cliff
Harrison C., Nat. Review Drug Discovery Jan Vol. 12 2013

"The impending patent cliff is anticipated to erode US$78 billion in worldwide sales from branded drugs that are facing patent expiry between 2010 and 2014."
Drug discovery is interdisciplinary!
At the end of the Preclinical Development phase, the clinical candidate is selected, and all the data that have been generated are gathered to prepare the documents to be submitted to the regulatory agencies for approval of the clinical program – the Investigational New Drug (IND) package for the Food and Drug Administration (FDA) in the US; the Investigational Medicinal Product Dossier (IMPD) and the Investigator’s Brochure (IB) for the Ethics Committees of the EU sites where clinical trials will be conducted.