Molecular Modeling, Spr 2014
Lecture 24
Rational drug design
VIOXX (rofecoxib)

- Used to treat pain, arthritis.
- Vioxx was voluntarily taken off the market after reports that it caused increased risk of heart attacks.
  - Estimated resulting in 88,000-140,000 heart attacks, 1/3 of them fatal.
  - $970M spent in legal settlements.
  - $2.5B total sales revenue
  - Never banned by the FDA.
- Non-steroidal anti-inflammatory drug (NSAID)
- Specific for cyclooxygenase Cox-2, not Cox-1
Prostaglandins
First found in **prostate gland** (seminal) fluid
20-carbon fatty acid + oxygen -->

**arachidonic acid**

**COX = cyclooxygenase**

GPCR

**Prostaglandins**
Prostaglandin (eicosanoid) synthesis

blocking cyclooxygenase activity blocks all prostaglandins

Diagram:
- Prostaglandin (eicosanoid) synthesis pathway:
  - Arachidonic acid
    - Phospholipase C
    - Phospholipase A₂
- HPETE (hydroperoxyeicosatetraenoic acid)
- Lipoxygenase (FLAP, Alox5)
- LTB₄
- Leukotriene A₄
- Leukotriene C₄
- Leukotriene D₄
- Leukotriene E₄
- Glutathione
- Glutathione-S-transferase
- Glutamic acid
- Prostaglandin H₂ (PGH₂)
  - PGD₂
  - PGE₂
  - PGF₂
  - 6-keto-PGF₁₀
  - Prostacyclin (PGI₂)
  - Prostacyclin synthase
  - Prostacyclin synthase
  - Thromboxane synthase
  - Thromboxane synthase
  - Thromboxane (TXA₂)
  - Platelets
GPCRs

- G-protein coupled receptors
- Transmembrane, 7 helices
- ~800 genes in human
- ligand-specific signal transduction -- olfactory, light, neurotransmitters, inflammation
Effects of aspirin and other pain killers?

Aspirin works on both COX-1 and COX-2 to inhibit arachidonic acid’s entry into the active site of the enzyme--acetyl group of aspirin binds to serine in COX--by blocking the activity of the COX enzymes, this relieves some of the effects of pain and fever--”nonselective”--many side effects

Tylenol—thought to have effects through inhibiting the activity of COX-3, an alternatively spliced form of COX-1
The difference between cox-1 and cox-2

<table>
<thead>
<tr>
<th>name</th>
<th>expression</th>
<th>function</th>
<th>Position 523</th>
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<tbody>
<tr>
<td>cox-1</td>
<td>constitutive</td>
<td>housekeeping enzyme</td>
<td>Ile</td>
</tr>
<tr>
<td>cox-2</td>
<td>induced</td>
<td>associated with inflammation</td>
<td>Val</td>
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</tbody>
</table>

paralogous

65% identical

A drug that binds cox-2 but not cox-1 would specifically address inflammation without gastrointestinal side-effects.
Short History of COX-2 Inhibitors (2)

- **mid 1990’s** - X-ray crystal structures showed that COX-2 active site has a side pocket that is absent in COX-1.

- **late 1990’s** - inhibitors designed that preferentially bind COX-2, are equally effective as NSAIDs, but are reported to cause less GI damage.

- **1998/1999** - Celebrex and Vioxx approved by FDA for osteoarthritis and rheumatoid arthritis.

- **2000’s** - next generation of drugs developed with even higher selectivity for COX-2 over COX-1.

**Triumph for rational drug design**
Rational Drug Design (1)

- Based on molecular and structural studies, informed by cell biology, physiology, pharmacology, etc.
Requirements

• Lipinski rule of 5
• Tight binding (nanomolar or better)
• Mode of binding creates specificity (Val509 vs Ile509)
• No other modes of binding.
Exercise 24

- Open PDB ID = 3ln1, COX-2 bound to celebrex (related to VIOXX)
- Delete all waters and all ligands except one copy of celebrex (CEL) and one COX-2 enzyme. Delete all extra copies of enzyme and CEL.
- Open PDB ID = 1ddx
- Delete all waters and all ligands except one copy of prostglandin (PGX) and one COX-2 enzyme. Delete all extra copies of enzyme and CEL.
- Superpose the two COX-2 structures, making sure to synchronize by Tag. Delete 1ddx protein chain.
- Show CEL and PGX with electrostatic surface of 3ln1.
A drug in action

Celebrex (celecoxib)

prostaglandin
H2 (product)

Drug sterically blocks arachadonic acid binding, preventing enzyme activity.
Rationally design

- Delete PGX
- Select CEL.
- Calculate ligand interactions around CEL.
- Compare to COX-1/COX-2 sequence alignment (next slide). What residues are mutated between cox-1 and cox-2?
<table>
<thead>
<tr>
<th>COX-1</th>
<th>COX-2</th>
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<tr>
<td>32 VNPCYYPCQHQGIICVRFGLDRYQCDCRTGYSGPNCTIPGLWTWLNRNSLPSPSTHFL 91</td>
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<td>18 ANPCCCSPCQNGVCMSVGFDQIKCDCTRGTGFYGCNCTSTPEFLTRIKLFKPTPNTVHYI 77</td>
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<td>558 VKGCPFTSFVDP 570</td>
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Where is V509?
Docking

- Run Dock on CEL, using “induced fit” with “flex”
- What is the lowest energy pose?
- Run Dock again. (give output file a different name)
- What is the lowest energy pose?
- What is the difference in energy between COX-2 and “COX-1”
Drug design

- Use Builder to change CEL to Vioxx
- Repeat docking exercise, docking Vioxx to cox-1 and then cox-2.
- What is the energy difference?
CEL converted to Vioxx
<table>
<thead>
<tr>
<th>mol</th>
<th>recep...</th>
<th>rseq</th>
<th>ms...</th>
<th>S</th>
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**CEL • cox-2**

Celebrex binds better to cox-2

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**Vox • cox-1**

Vioxx binds better to cox-2

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**Vox • cox-2**

Specificity is better for Vioxx

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