Lecture 2

- Drug Development an interdisciplinary effort
  - Various disciplines involved- who does what and when
- Preclinical Research and Development
  - NSAIDS and Statins
- Clinical Trials
  - Three phases
  - Regulatory agencies
- Post Marketing vigilance
  - **Black Box warnings**

Drug Development.

Requires a truly interdisciplinary effort involving the combined efforts of:

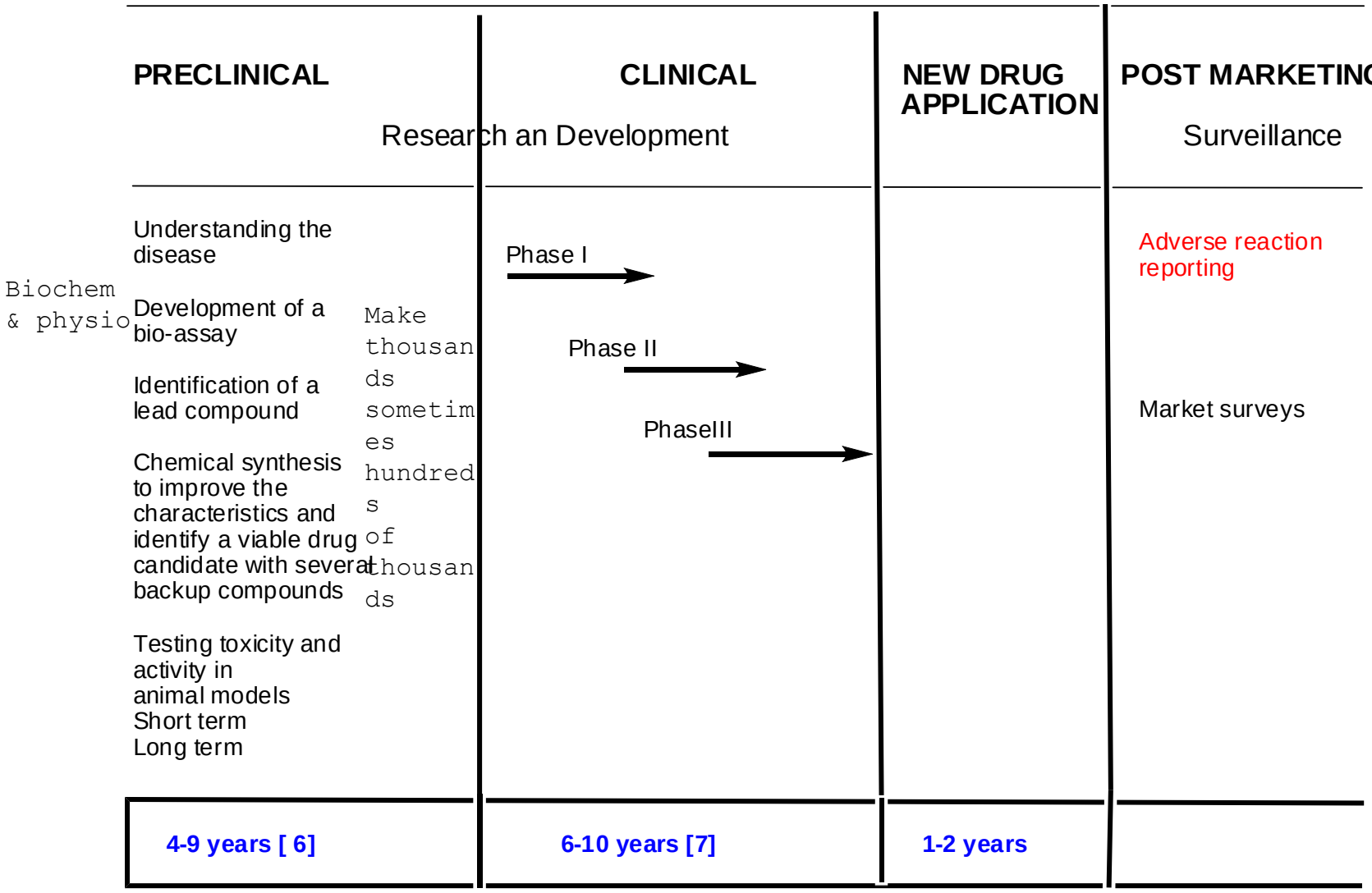
biochemists,
physiologists,
analytical chemists
toxicologists
medical doctors
chemical engineers
AND others

molecular biologists,
organic chemists,
pharmacologists
biostatisticians
pharmacists
legal experts

Drug Development. It is a long and costly road!!

- Typical Development Time
  - from the identification of a lead structure to approval for sales by Health Canada or the US Food and Drug Agency [FDA] is in the 10 to 15 year range.
- Four major time phases are recognized.
  - Preclinical Research and Development
    - 3-7 years
  - Clinical Trials
    - 5-8 years
  - New Drug Application Review
    - 1-2 years
  - Post Marketing sourveillance

Time course for drug development



Pre-clinical Research and Development

- Management makes a decision identifying the disease target.
  - Based on
    - Filling a need
    - New developments in the understanding of a disease either from an external or internal source
    - New bioassays , externally or internally generated
    - Ethnopharmacology
    - Competitor results

Clinical Trials.

- **Three Phases are recognized:**
- Phase I. Involves healthy patients [about 20 volunteers] and determines safety.
- Phase II. Determines efficacy on a small [<50] highly controlled group of patients.
- Phase III. Utilizes a large diverse group of patients that mimics to a considerable extent the type of people who would use the drug. Efficacy and safety.
- Time frame: 5-8 years
- Cost: Increases significantly from Phase I to III. Total cost of clinical trials can be in hundreds of millions of $

Typically young males are considered the guinea pigs. Paid, monitored...

Takes several months, maybe even 1-2 years.
Very controlled to give best possible results.
Safety is part of this phase, but efficacy is the focus.

Most long and costly.
Diverse grp of patients.

Cross section of typical population. Hundreds of mill $

If the males survive phase I, and it's considered safe, the compound can progress onto the next phase.

What are the odds that the compound the chemists and biochemists have made continue past this point?

Approximate success rate:
600-670 in every 1000 compounds entering phase 1 will pass.
Of the remaining, about 400 pass through phase 2.
Only about 50 will pass through phase 3. (5% overall)
The 50 remaining still need to wait potentially years for approval.

Regulatory Agency Approval

Key priorities: ensure the public is receiving a safe and useful drug.

- Dedicated effort requiring the company to convince Health Canada that the Indicated New Drug (IND) submission is complete and has covered all requirements
  - Material is manufactured under Good Manufacturing Procedures (GLP)
  - Knowledge of the metabolism and pharmaco-kinetics of the drug (ADME)
  - Safety (lack of toxicity and serious side effects) and efficacy as shown in Clinical Trials

Are the side effects worth it? Are there any side effects?

Anti-cancer drugs, severe obviously, more side effects can be tolerated. Non common side effects.

Post Marketing surveillance-side effects

- The genetic diversity in a large population makes it essentially impossible to test against all adverse effects
  - **. Not all side effects are discovered in clinical trials**

- If the drug treats a life threatening situation then more severe side effects are tolerated.

- For drug designed to treat a lifestyle situation, only minor side effects are tolerated.
- Listing of side effects: - Common:

Severe side effects : Black Box Warning:

The doctor has to assess if it is worth the patient taking the risk.

Pre clinical research & development:
Certain disease cures are based upon filling a need. (Void in the drug market).

Drug discovery

- Two case studies based on better understanding of the disease and biochemical processes
- The Search for a better Aspirin ® (anti-inflammatory drug based on selective inhibition of the COX-2 vs COX-1 enzyme:
  - The story of Celebrex and Vioxx

THE DEVELOPMENT OF COX-2 INHIBITORS

***Rod J. Flower* NATURE REVIEWS | DRUG DISCOVERY. VOLUME 2 | MARCH 2003 | p 179**

- The development of the statins (Cholesterol lowering drugs based on an understanding of the bio-synthesis of cholesterol.

"Neat biochem basis"

Derived from natural products.

  - Mevacor to Liptor

Very safe, could even be considered an over the counter drug.
May reduce heart attacks by a very significant amount.

Understanding Inflammation

- **From Willow bark to COX-2 Inhibitors**
- Ancient societies in many parts of the world used willow bark as a treatment for fevers and inflammation

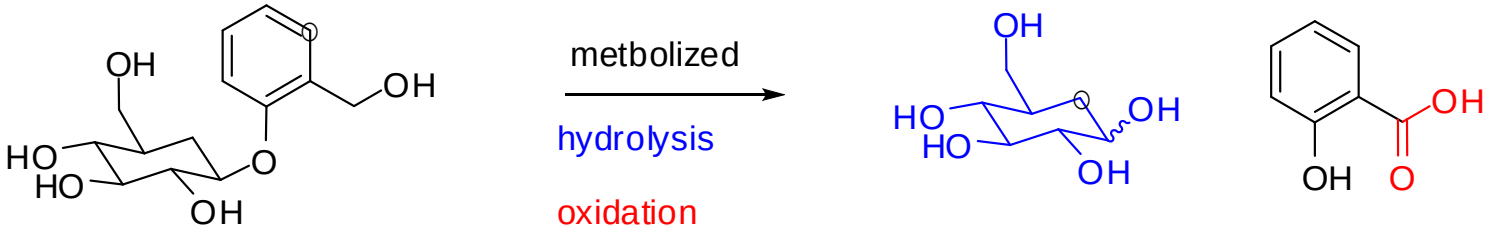
Chinese, greek, egyptian, even middle ages. First clinical trial was done with willow bark. Performed by the minister of an anglican church.

- By about 1840, the compound responsible for the activity had been identified as salicin

People had begun to work with chemicals. Scientists in France isolated the active chemical.

- By the 1860's the metabolism of salicin became known

Molecule is split into 2 parts, salicylic acid and a glucose molecule. Salycylic acid is an anti inflammatory agent.



salicin
(gucoside of 2-hydroxybenzyl alcohol)

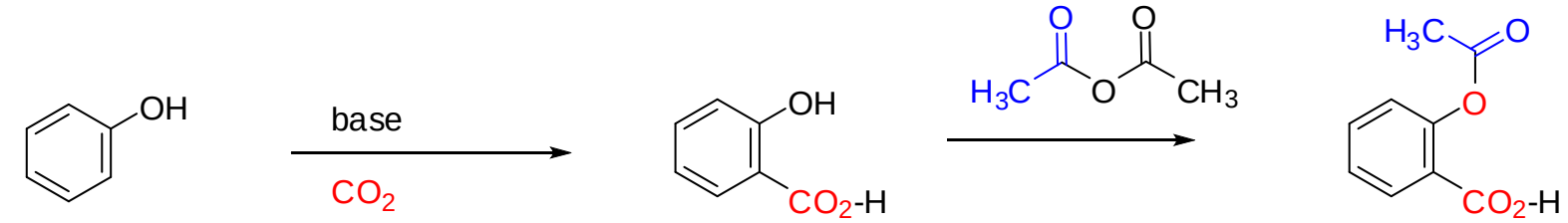
glucose

salicylic acid

Acetyl Salicylic Acid (Aspirin®)

- 1860-1890 Salicylic acid was produced in large quantities by synthetic procedures from phenol and CO2

Was used to treat the inflammation. Phenol was a readily available chemical. Base was likely potassium or sodium hydroxide.



phenol

1860s

1890s

- Salicylic acid reduced inflammation but was very unpleasant to the taste

Caused a lot of stomach irritation.

- 1890s –Felix Hoffmann (Bayer)

His father suffered from arthritis. His father insisted he make salicylic acid more palatable.

  - Reacted salicylic acid with acetic anhydride catalyzed by sulfuric acid to produce Acetyl salicylic acid – trade marked as Aspirin

Became available in 1899, and was the wonder drug of the last 100 years.

Aspirin – the wonder drug

- Many positive effects. Relieves:
  - Pain, (Analgesic)
  - Reduces fever (antipyretic)
  - Relieves inflammation
  - Reduces blood clotting
  - An enormous amount of good things that it causes.
- Causes gastric lesions Gastrotoxicity.

If this occurs long enough it can actually become fatal.

How much aspirin is used in Canada? The older generation uses a lot of aspirin for arthritis.

Typically, back in 2000, the data that was available, each person (from every age), on average consumes 120 tablets per year. One every three days. Each tablet contains 325 mg. Each person eats about 40g of aspirin per year.

1,320,000,000 g is consumed per year. Continues to be an effective drug.

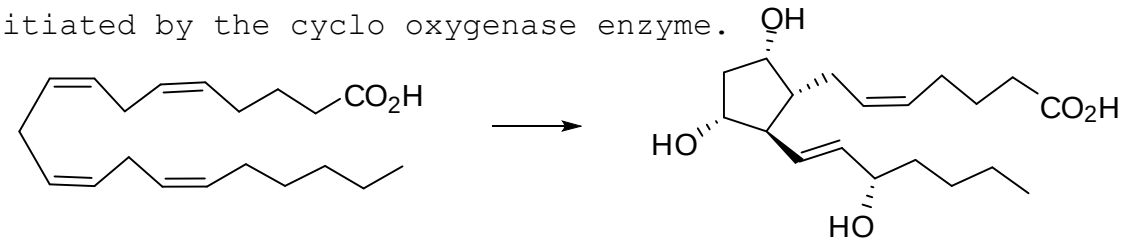
Cheap, no difference between bayer and the generic brand. One may dissolve faster than the other.

Understanding the mechanism of action of ASA

Biochemists tried to discover what exactly aspirin did.

- 1960s to 1980s (Started even earlier in the 1940s).
- It was shown that ASA inhibits the formation of **prostgalandins** from arachadonic acid – a poly-unsaturated fatty acid

Reaction is initiated by the cyclo oxygenase enzyme.



Arachadonic acid **a prostaglandin (PGFα)**

The precursor is a C-20 carboxylic acid, a simple chain compound (all cis).

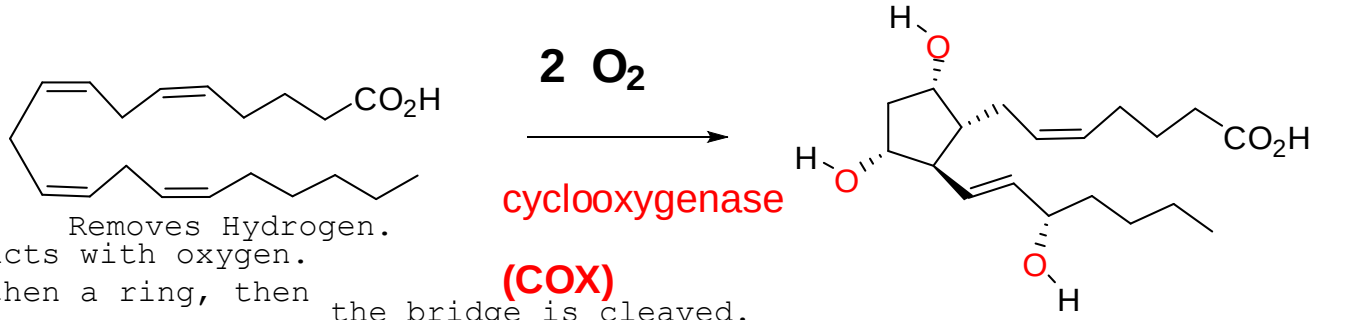
- **Prostaglandins**
  - Many structure variations mediate different biological processes

Control an enormous number of bodily functions. (Temperature - fever reduction, & inflamm.)
The structure may widely vary.

How does nature flatten the molcule with no chirality, and create a compound that has 3 more oxygens (from O2)and produces all kinds of stereochemistry.

Cyclo-oxygenase enzyme

- **Overall reaction**

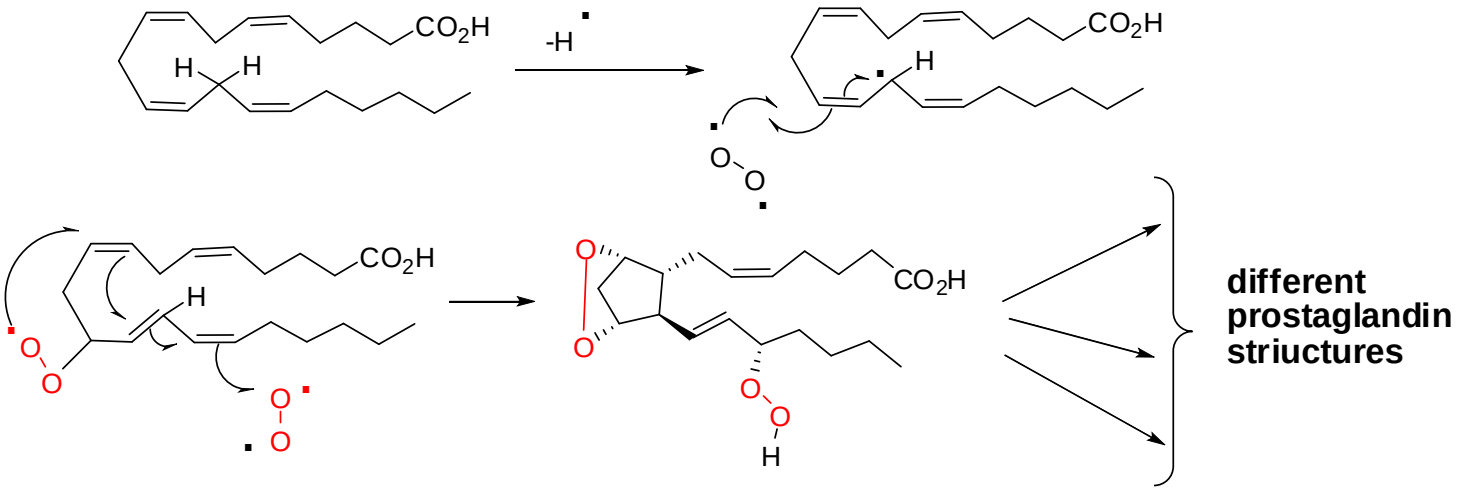


Removes Hydrogen.
Radical then reacts with oxygen.
makes a bring, then a ring, then the bridge is cleaved.

Arachadonic acid

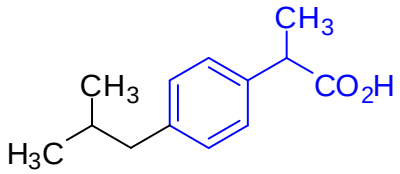
a prostaglandin (PGFα)

- **Mechanism**

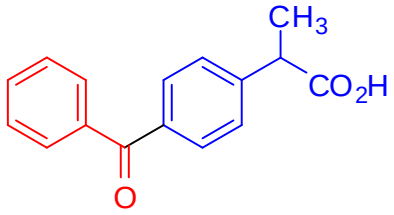


New anti-inflammatory drugs 1970>

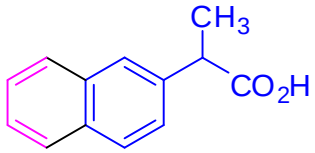
- Compounds that Inhibit COX
  - Non steroidal anti-inflammatory drugs



Ibuprofen



Ketoprofen



Naproxen

- Often more potent anti-inflammatory drugs than aspirin – more potent COX inhibitors than ASA
  - Do not reduce fevers! Are not cardio-protective!
- Major side effect: Gastric lesons and stomach bleeding

Aspirin and all of the other anti inflammatory drugs, ibuprofen (advil), motrin, etc...
All inhibit cyclo oxygenase, but to various extents. Some are more potent inhibitiors.

This is late ~1980s.

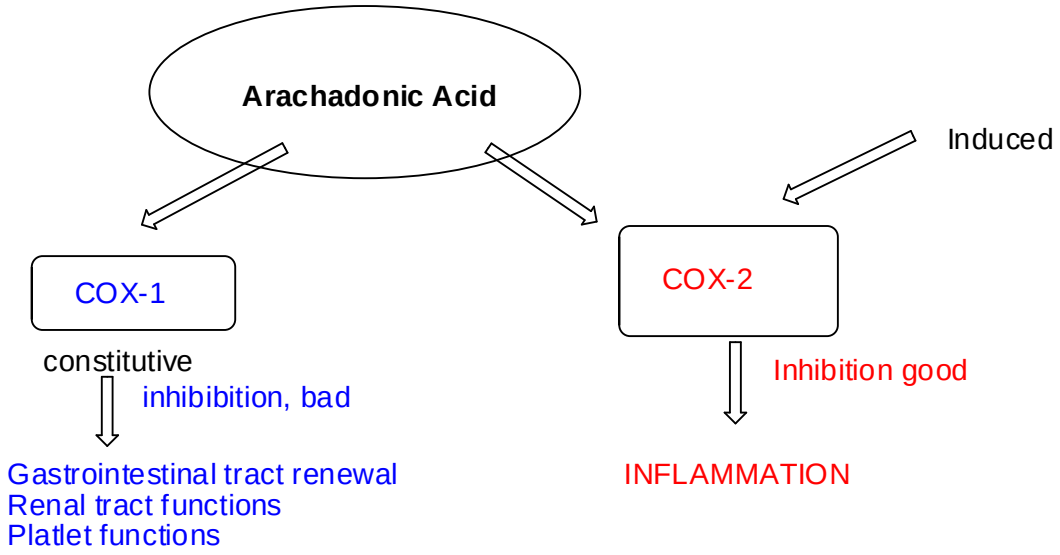
Major Breakthrough

- Academic researchers in the mid to late 1980s discover that there are two cyclo-oygenase enzymes- closely related!
- Both inhibit prostaglandin formation
  - One form of the enzyme inhibits prostaglandin formation associated with inflammation – COX-2
  - One form, designated as COX-1 mediates the formation of prostaglandins associated with important body functions including renewal of the stomach and esophagus lining This occurs often because the stomach is a harsh environment.

Effects of COX-1 and COX-2

If you could inhibit COX-2, you can stop the inflammation.
We don't want to inhibit COX-1.

- Simplified picture



- Opportunity!

We want to find:

- Molecules that selectively inhibit COX2 vs COX-1 should relieve inflammation but not be gastro-toxic

This would be a blockbuster drug.

The search for COX-2 selective compounds

All the current drugs are the variations of the same type of compound.

- First priority- Develop a bio-assay to determine the inhibition of both COX 1 and COX -2 by various compounds.
- Once available- test known anti-inflammatory compounds
- Results:

The smaller the number, the greater the inhibition.
IC50 is the concentration of the substance that inhibits 50% of the activ. of the enzyme.

	COX-1 IC50 umolar	COX-2 IC50 umolar	Inhibition Ratio COX-2/COX-1
ASA	1.7	7.5	0.23
Ibuprofen	7.6	20	0.38
Ketoprofen	0.047	0.24	0.23
Naproxen	9.3	35	0.27

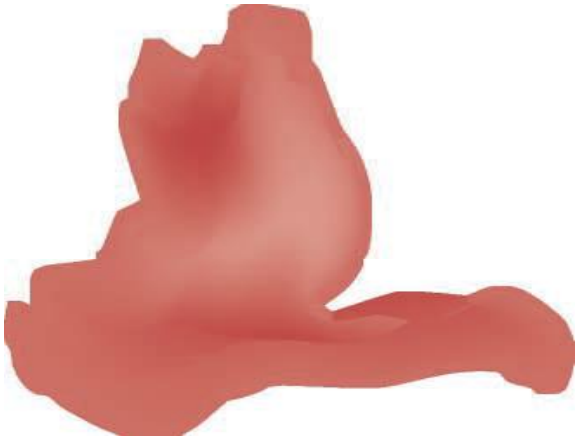
All of these compounds should cause problems with gastrotoxicity.

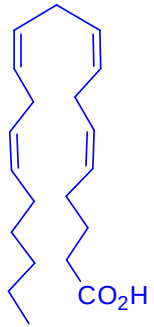
The receptors

Based upon x ray analysis. COX-1 is a smaller receptor site than COX-2. Therefore we want larger molecules.

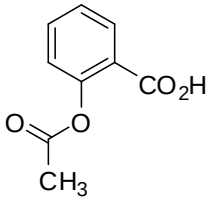
- COX-1

COX-2

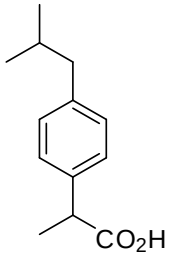




Arachadonic acid
(natural ligand)



ASA



Ibuprofen

These molecules all appear to fit inside COX-1. The Dupont compound has parts that are similiar to an ASA compound, and extra atoms that would fit into the COX-2 receptor, whilst not fitting into the COX-1 receptor.

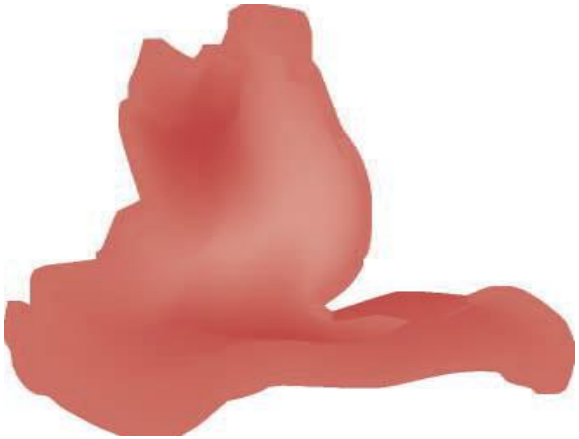
Conclusions

- ASA and the major NSAIDS inhibited both COX -1 and COX-2 but often show greater inhibition of COX-1 than COX-2
- General observations: Somewhat larger, similar molecules tended to inhibit CO2 to a slightly greater extend than COX-1.
- Large library screening gave the first breakthrough lead structure- published by Dupont ~1990. During this time they developed pictures of what the receptors looked like.

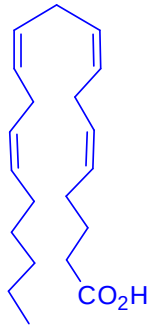
The first COX-2 selective compound

- COX-1

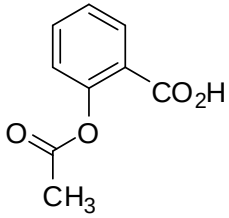
COX-2



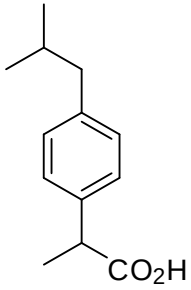
Aromatic ring, main structure with substituents on the carbon.



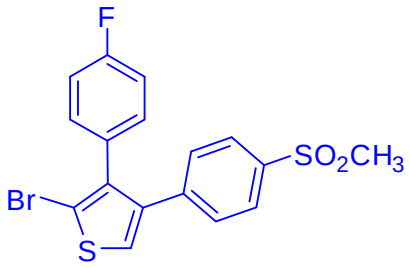
Arachadonic acid (natural ligand)



ASA



Ibuprofen



DuP697

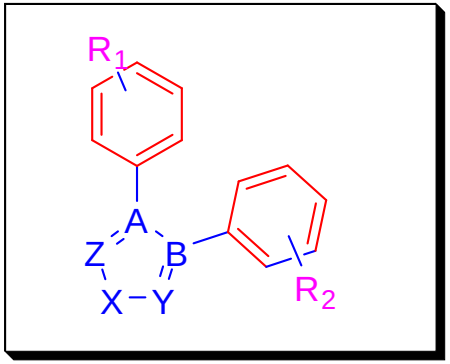
Optimizing the lead structure

- **Race to be first:** Synthesized hundreds to thousands of structures based on that variation. An organic chemist alters the dupont chemical slightly.
- Pfizer (US) – Merck Frosst (Montreal
  - Medicinal chemists synthesized hundreds of compounds with variations on the DuP697 structure

Probable structure requirements

1. Central 5-membered heterocylic ring; likely aromatic
2. Two aromatic rings ortho to each other; likely benzene rings but other 5-or 6-membered aromatic rings also plausible
3. Suitable substituents on the aromatic rings
4. Possible additional substituents on the central 5-membered ring
5. Can the central ring be a six membered ring?

=



  - Develop a synthetic method to prepare the compounds
  - Evaluate each and provide feedback to generate more potent structures

Relative COX-1 to COX-2 Inhibition

- **IC50: Concentration that inhibits 50% of the activity of a substance (enzyme)**

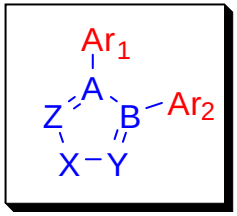
	COX-1 IC50μmolar	COX-2 IC50 μmolar	Inhibition Ratio COX-2/COX-1
ASA	1.7	7.5	0.23
Ibuprofen	7.6	20	0.38
Ketoprofen	0.047	0.24	0.23
Naproxen	9.3	35	0.27
Celebrex	1.2	0,83	1.4
Vioxx	63	0.84	75

- **Inhibition of COX-1 results in gastrointestinal toxicity**

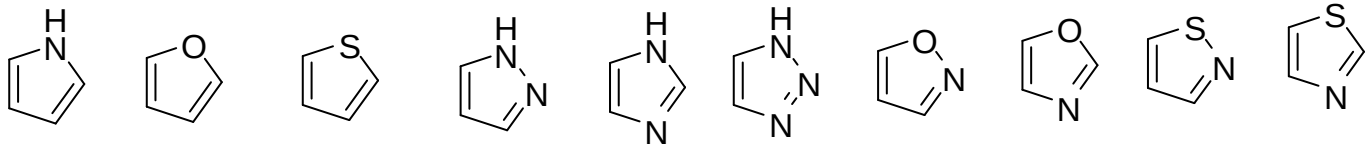
The synthetic chemistry challenge

- How many plausible structures are there?
  - Different central rings

Planar 5 membered rings. One of these 10, and start to add substituents, ..

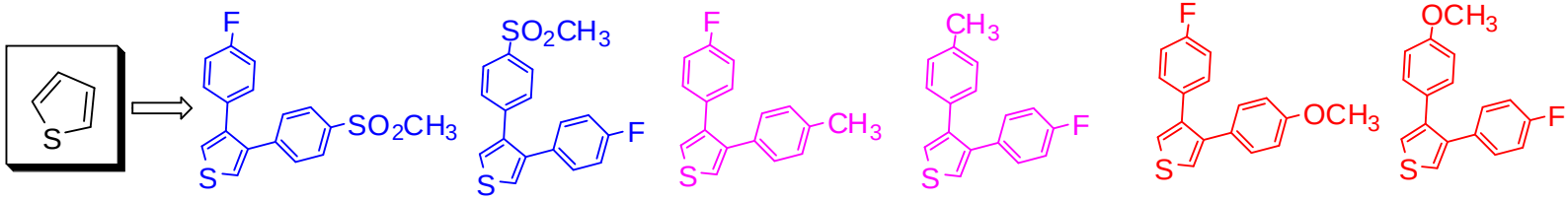


Common 5--membered heterocyclic ring systems - at least 10!!

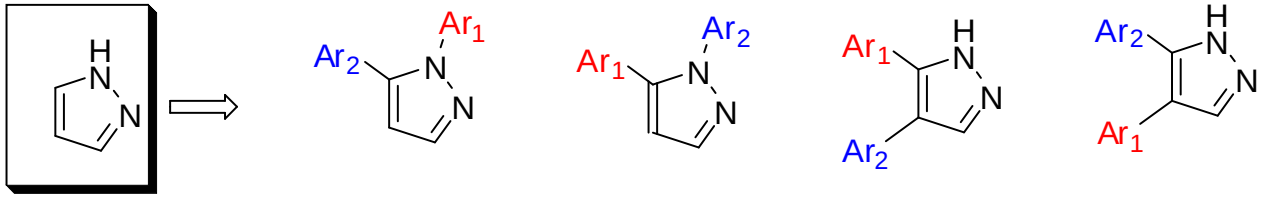


- Different Ar1 and Ar2, in different positions

Fluorine is a good compound to add to a ring because it makes the cmpd more metabolically stable.



Which do you use? Try to get feedback with the biochemistry and the bioassays.
Those that have modeled the enzyme will have further ideas of where to put atoms in the structure.



The synthetic chemistry challenge

Be as inclusive as possible so another company can't make a "me too" compound.

- How many plausible structures are there?
  - Different central rings ? 10
  - Each has a minimum of 2 and more likely 4 possible arrangements of two different Ar groups

  Potential simple substituents on the aromatic rings:

  F, C, Br,

  OH, OCH3, OC2H5, OC3H7,

  NH2, NHCH3; N(CH3)2, NHCOCH3, N(CH3)COCH3, NHCOAr , N(CH3)COAr; NO2, NHAr, N(CH3)Ar, NHCH2Ar, N(CH3)CH2Ar

  SCH3, S(O)CH3, SO2CH3, SCH2Ar, SAr, SO2Ar

  CH2OH, CH2OCH3, CH(O), COCH3, CO2H, CO2CH3, CO2C2H5

- **First estimate 10 X 3 X 33 ~ 1000!** Analysis of these compounds must be performed.
  - **Does not take into account possible di and trisubstitution on the aromatic rings**
  - **Does not count changing CH3 to C2H5 … to C6H11, branching in the chain or making rings. Final tally: MILIONS OF POSSIBLE STRUCTURES**

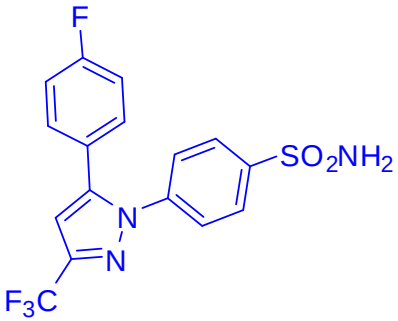
Merck & pfizer had a dispute between the number of compounds they've claimed. Pfizer claimed more cmpnds than there are atoms in the universe. 10^50th power of variations.

Bio-assay guidance and Computational guidance

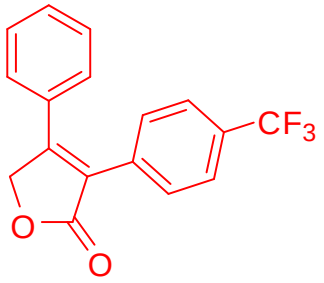
- Cuts down on the synthesis challenges –helps guide the choice of new structures to a manageable level.
- **Final Merck and Pfizer drug candidates. Pfizer is first! By a few months**

The relationship between the two compounds is noteable:

- Celebrex®



Vioxx®



- COX-2 vs COX-1 selectivity

A high number is desired.

- Celebrex® ~ 10-20

Mainly inhibits COX-2.

Vioxx® High> 50!!

Very selective inhibitor.
Only had to take 2 mg of the compnd.
Very inexpensive to make. Can be made in a day & a half.

Marketing of Celebrex and Vioxx

- Both were approved in 1999 in Canada and the US.

Both were approved in 7 years, good science behind both.
"The perfect aspirin" does all of the good things, none of the bad.
Vioxx was supposedly safer than celebrex.

- Heralded as miracle drugs- Excellent treatment for inflammation but not likely to cause stomach lesions.
- Key applications:
  - arthritis – long term
  - – Injuries from accidents and sports- short term.
- Sales: Within one or two years: several B $

Problems with Vioxx (and NSAIDS)

- Sept. 30, 2004 Merck announces withdrawal of Vioxx from the market. Did they anticipate it? Or did they brush it under the rug?
  - **A study concerning the use of Vioxx for the prevention of colon cancers showed that long term use of Vioxx increased the risk of heart attach or stroke by a factor of two.** Estimated 60-70,000 people died of heart attack, etc.. as a result of Vioxx.
- Other clinical data and post marketing surveillance also indicated significant concerns for Vioxx, other Cox-2 selective inhibitors and long term use of other NASIDs

ALL the inhibitors all start to show problems. They are not as safe as we thought they were.

Merck was sued for 10s of millions and maybe even billions of dollars.

Complicated situation

- After a review of all available data in 2005 both the US FDA and Health Canada approved /recommended the re-introduction of Vioxx 10:1 in favor of reintroducing it.
  Recommended more study of NSAIDS. Merck has never introduced the compound.
  - Benefits outweighed the risks for some patients
  - The cardiovascular risks for Vioxx seemed no worse than those for Ibuprofen
  - Recommended further studies for all NSAIDs to fully understand the risk/ benefit profiles
- Merck has not re-introduced Vioxx- fear of litigation?
  Fear if there was misuse when taking the drug.
- Pfizer stopped advertising and strongly marketing Celebrex
  - Resumed again three or four years later.

What is the difference? Vioxx is much more selective. COX-2 must do more than simply mediate inflammation.

COX-2 is present in heart tissues. People are speculative that COX might have more functions that we don't know about. Involved in the production of a prostaglandin known as prosta cyclin. Potent inhibitor of platelet aggregation, there can be an effect on cardiovascular disease.

Vioxx: Problems with long term use

- **Vioxx: lessons for Health Canada and the FDA**
- *CMAJ January 4, 2005 vol. 172 no. 1*

Development of Cholesterol Lowering Drugs : The Statins

- Lipitor – a member of the "statin" family of drugs - the best selling drug in history.
  - Lowers blood cholesterol levels
  - Stabilizes plaque; prevents strokes
    - Total sales since 1996 > 125$ Billion
    - Patent ended ~2011
- Baycol Introduced by Bayer, about 1998, as a competitor to Lipitor
  - Many serious side effects (deaths) including wasting of skeletal muscles and resulting kidney failure
  - Withdrawn in 2001.

Development of Cholesterol Lowering Drugs : The statins

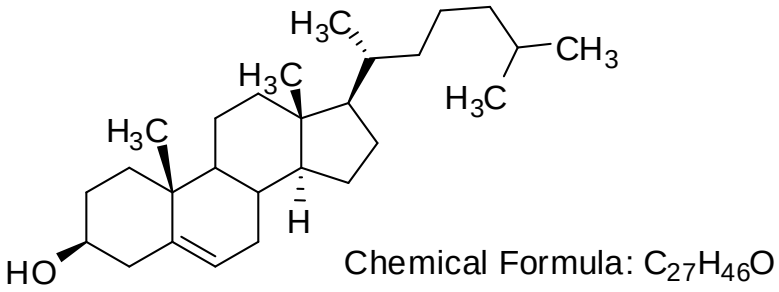
- Cholesterol: its importance to life and how it can cause cardiovascular disease.
- Sources of cholesterol
- Biosynthesis of cholesterol: rate determining step. Discovering the enzyme that facilitates that step.
- Discovering compounds that inhibit the function of that enzyme requires a bio-assay

Cholesterol: its importance to life .

- Cholesterol
- a vital substituent of cell membranes
  - Brain and spinal chord
- Precursor to all steroid hormones including testosterone, estrone, cortisol,
- Precursor to bile acids which are crucial to digestion
- Deposition in the arteries is associated with atherosclerosis and heart disease.

Sources of cholesterol

- Foods of animal origin
  - Meat, butter, lard
    - Commercial source spinal cord of cattle

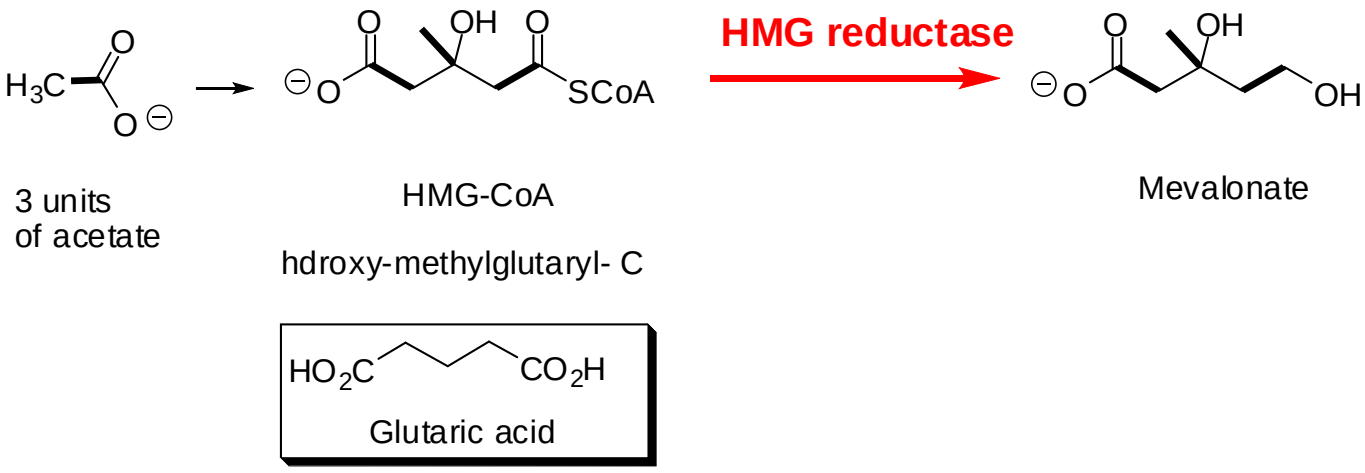


Chemical Formula: $C_{27}H_{46}O$

- Not in vegetables or plant derived food
-

Biosynthesis of cholesterol -1

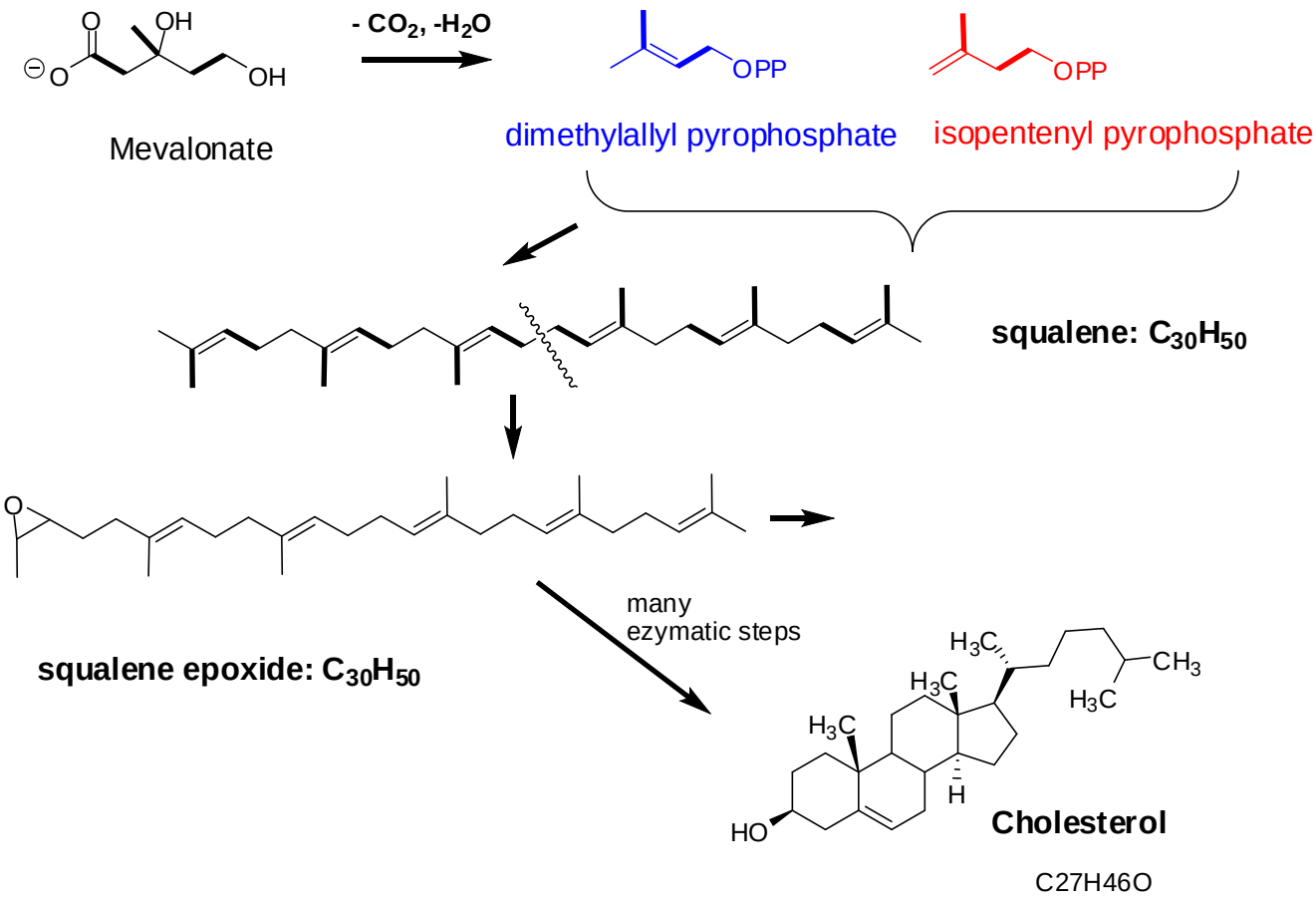
- Well studied-multi-step involving many different enzyme
- Carbon source: Acetate [C2 –building block]
- First key intermediate –Mevalonic Acid
-



HMG reductase - mediates the redate determining step in cholesterol biosynthesis

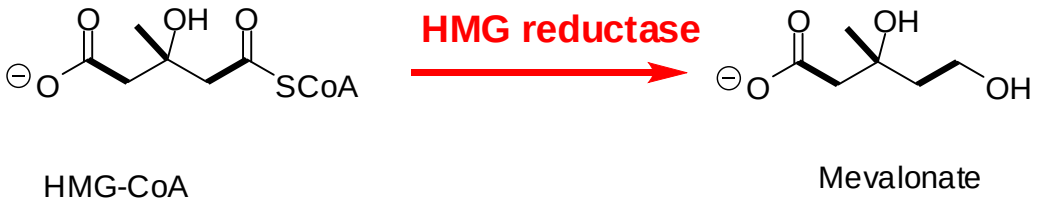
Biosynthesis of cholesterol -1

- Mevalonate is converted into two C5 units {isoprene}



Inhibiting Cholesterol Biosynthesis

- Interfere in the rate determining step by inhibiting the enzyme, HMG reductase, that carries out that step.
- How does one approach this?
  - The natural ligand for this enzyme is HMG-Co

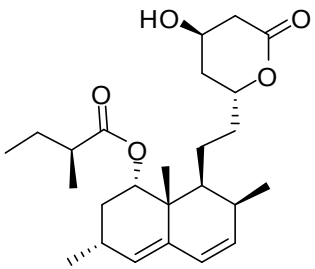


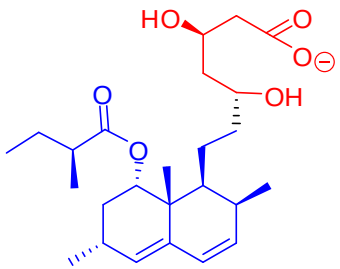
HMG-CoA Mevalonate

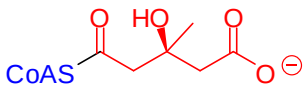
  - A molecule with a similar structure would be expected to bind to HMG-reductase and thus prevent it from converting HMG-CoA into mevlonate and eventually cholesterol

HMG-reductase inhibitors- natural and synthetic

- **Mevastatin {Lovostatin}**



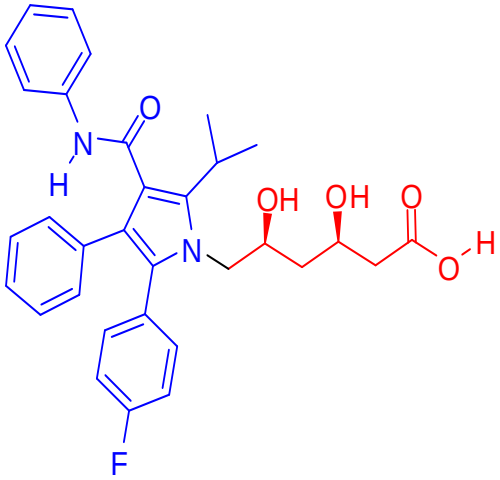




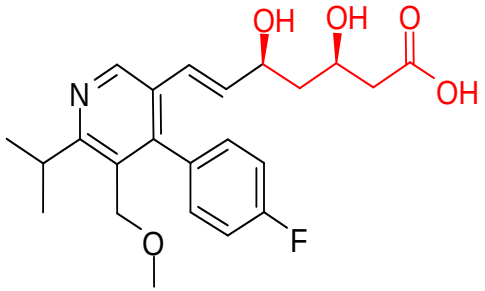
HMG-CoA

mevastatin
- a potent inhibitor of HMG reductase,
-a natural product produced by a mushroom called oyster mushroom (3%) of dry wieght)

- **Lipitor**



Baycol



Lipitor vs Baycol

- Lipitor – a member of the "statin" family of drugs - the best selling drug in history.
  - Lowers blood cholesterol levels
  - Stabilizes plaque; prevents strokes
    - Total sales since 1996 > 125$ Billion
    - Patent ended ~2011
- Baycol Introduced by Bayer, about 1998, as a competitor to Lipitor
  - Many more serious side effects (deaths) including wasting of skeletal muscles and resulting kidney failure
  - Withdrawn in 2001.

Lecture 3. The drug discovery process

- Choice of Projects
  - Ethnopharmacology
- Lead structures
  - Natural product, via ethnopharmacology
  - Natural product libraries obtained via classical isolation