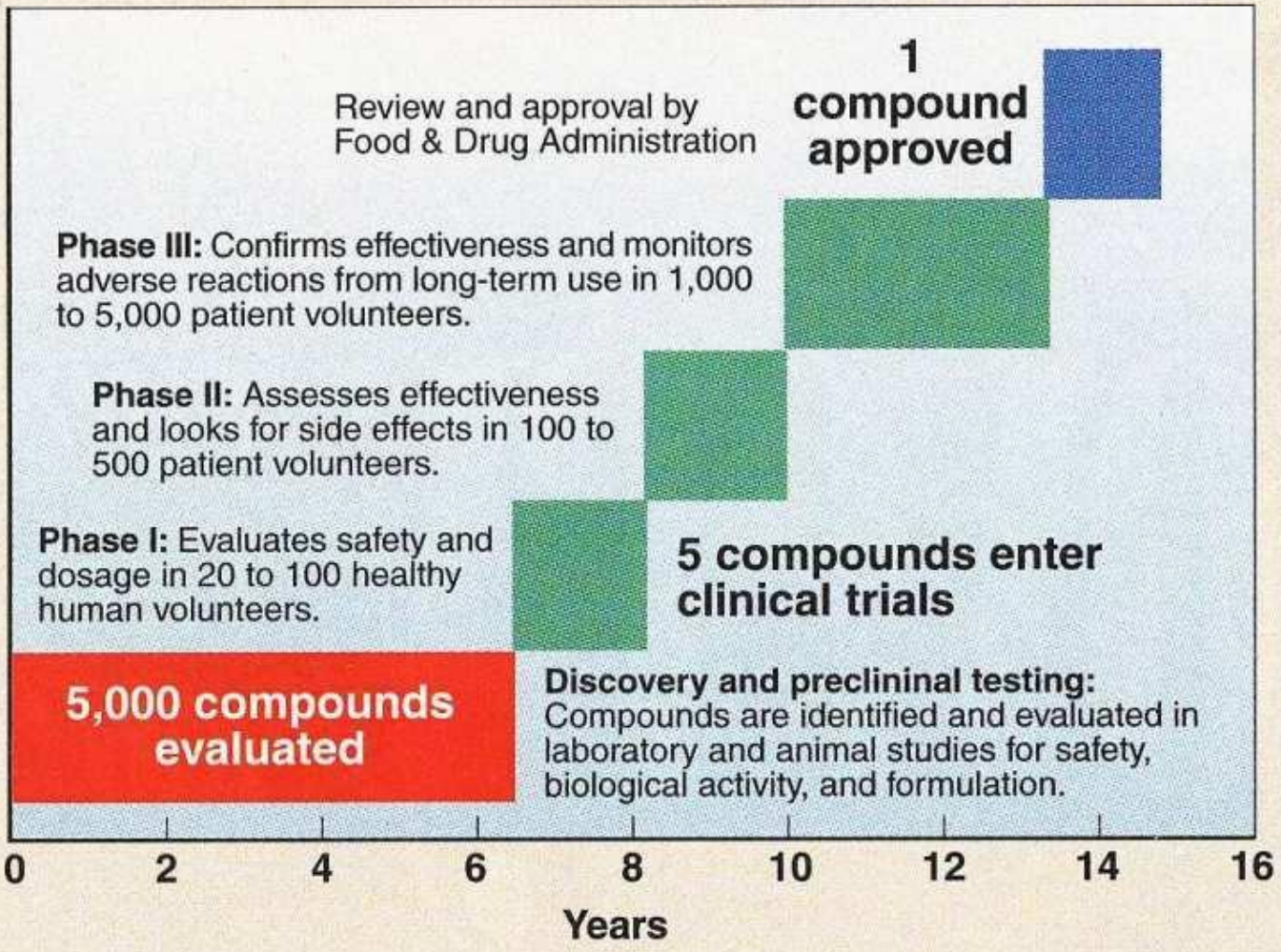
Drug Design and Discovery

Bringing a new drug to market can take 15 years



Source: Tufts Center for the Study of Drug Development

How are drugs created or discovered?

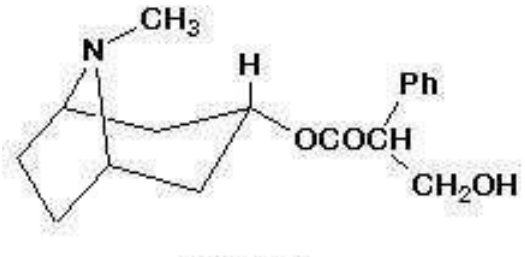
Natural drug products have been used for millenia

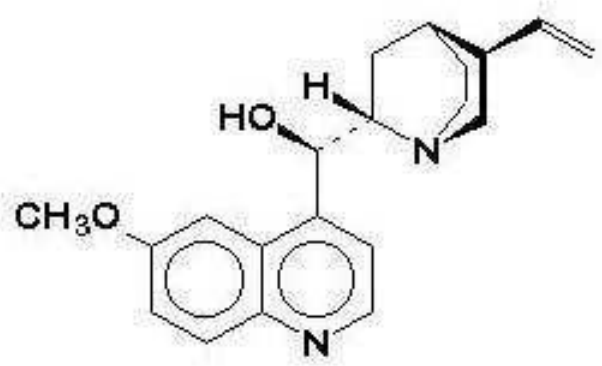
Synthetic drugs came into being during the 19th century

Today, drugs are still come from this two sources

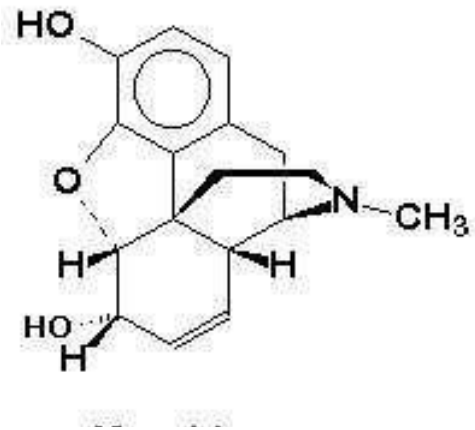
Chemicals found in nature or synthesized in labs are randomly screened for their therapeutic ability

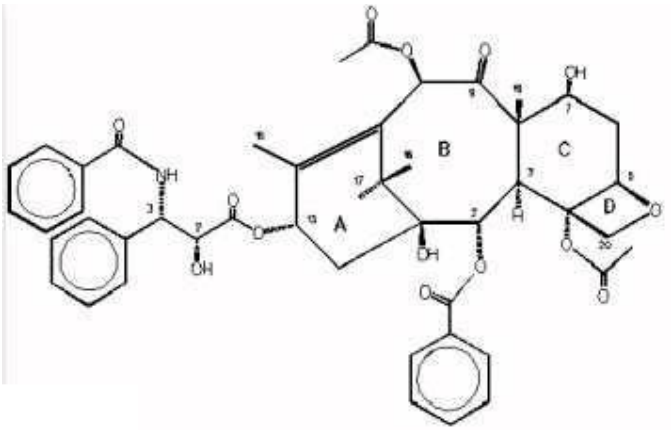
Drugs from Natural Sources





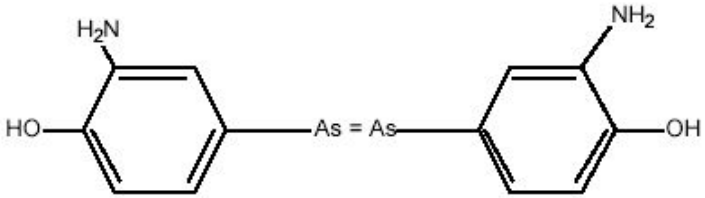
Atropine from Nightshade (Belladonna) Quinine from Cinchona bark



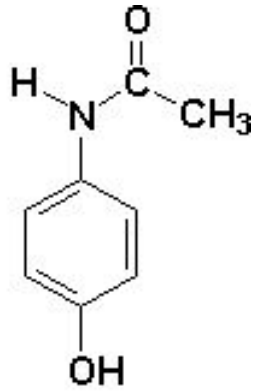


Morphine from Poppies Taxol from Yew Trees

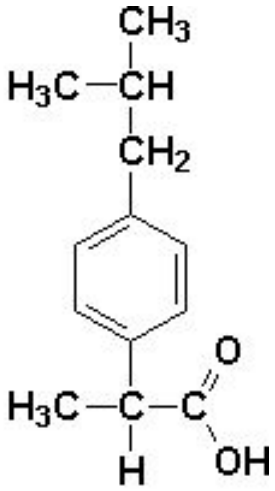
Drugs from Artificial Sources



Salvarsan



Acetaminophen



Ibuprophen

Problems are:

Long design cycle of 7-12 years

High cost approximately $350 million per marketed drug

Salvarsan, the first chemtherapeutic, was the 606^{th} compound tested by Dr. Ehrlich in over three years of study of syphilis

One way to increase the odds of finding a drug is through High Throughput Screening (HTS)

HTS seeks to increase the number of compounds tested at one time for drug-like properties

By testing 100s to 1000s of compounds at one time, HTS allows a drug company to search through many compounds in less time

Potential compounds are screened using plates capable of holding 96 to over 3000 different compounds

HTS relies on small samples rapidly tested usually by robot

The test or assay used depends on the type of drug required

The assay must be simple to perform and easily detected by a robot, the assay also must be able to be performed in a small volume 2 to 200µl

These assays often involve the measurement of luminescence, fluorescence, or absorbance, all of which are easily quantifiable

What are the targets of the drugs developed or what do they screen against?

Traditionally drugs were first tested against an animal or a human who had the disease the company is interested in creating a drug against

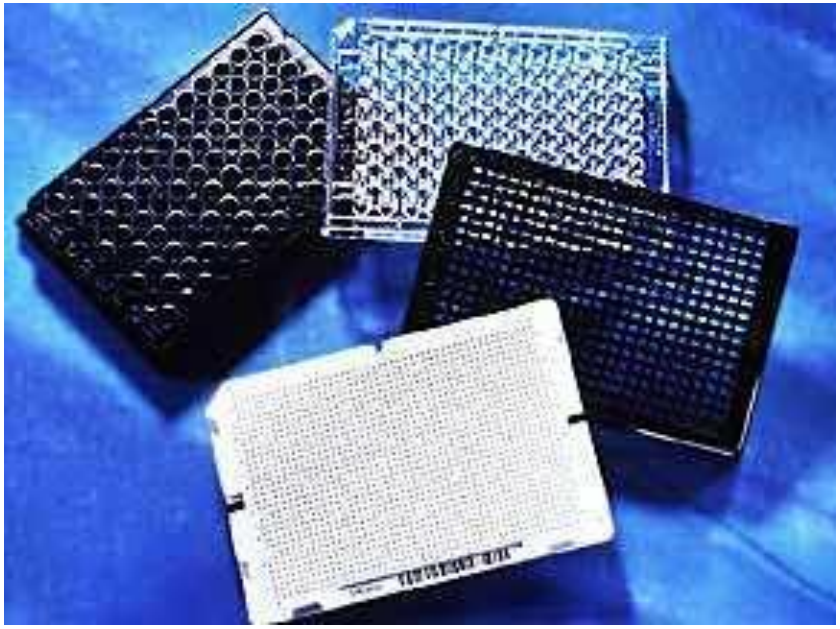
This is expensive, time consuming & can be dangerous

While this is still done, it is done at a much later stage in the drug development

Some HTS assays use cells, but many are cell-free or *in vitro*

There are some HTS assays though that use organisms, but these are mainly flies, worms, or fish

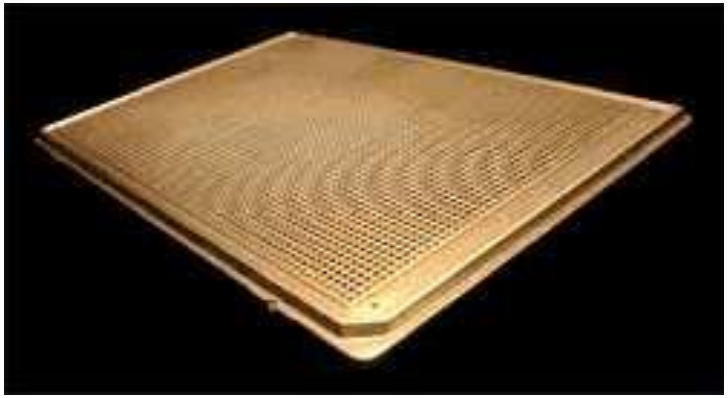
Assay Plates



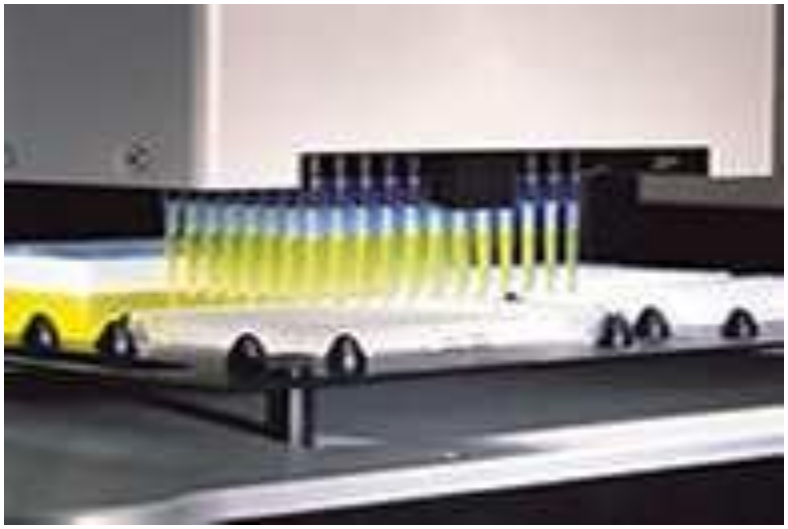
96, 384, & 1536 well plates

Hold 100, 20, 2 µl/well respectively

http://www.pcop.com/dd/techno/tech_hts.html



3456-well plate, each well holds 200nl



Robot pipetting samples into a 96 well plate

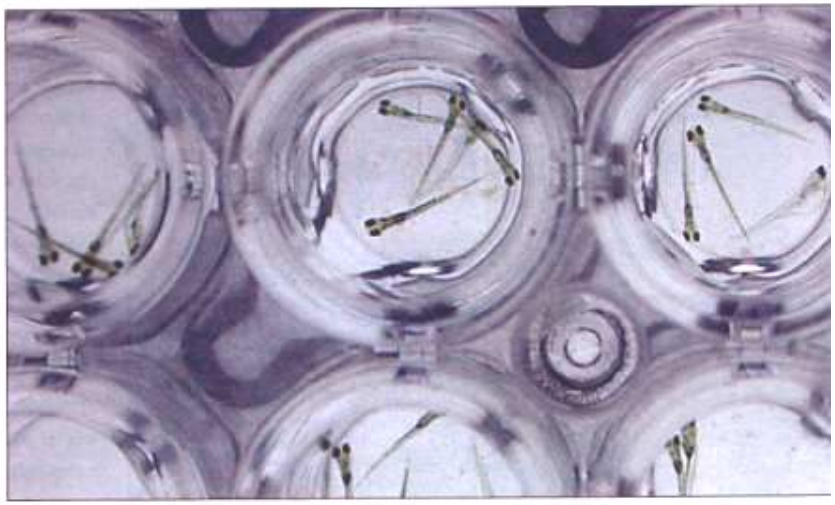
http://www.noabbiodiscoveries.com/hts.htm

Robot moving plates for screening

http://mango2.vtt.fi:84/bel/services/hts.htm

Large scale robotic screening area

http://www.thomasnewman.com/novartis/public/helping/txt_02.html



Zebrafish in the well of a 96-well plate

Current ultra-HTS (uHTS) systems are capable of screening 100,000 to 200,000 compounds per day

GlaxoSmithKline just opened a new center capable of screening 300,000 compounds against multiple targets per day

Where do companies get all these different compounds?

Combinatorial Libraries

Combinatorial libraries are large collections of randomly generated compounds usually based on a scaffold molecule

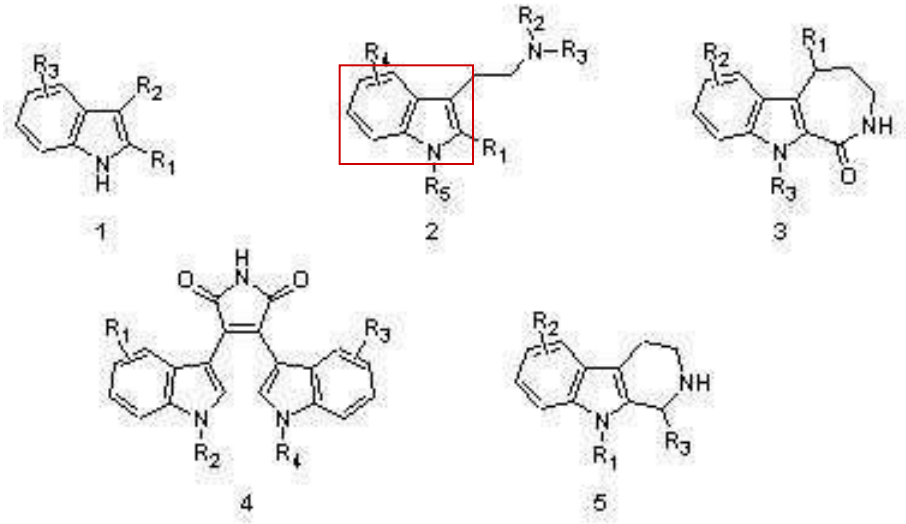
The scaffold molecule often is the skeleton of a known class of drugs or a random chemical structure

The scaffold molecule is modified by the addition of functional groups such as methyl, ethyl, amino, or carboxyl groups

Libraries can contain anywhere from 500 to 50,000 randomly generated members

These libraries are then screened for possible drug compounds

Examples of basic scaffolds of an indole library



R- groups represent regions which would be varied to create up to 40,000 discrete molecules

http://schultz.scripps.edu/Research/FunctionalGenomics/research.html

Libraries are screened to find hits

Hits are active samples that meet a defined success criteria

These criteria are determined by the company and are specific to the assay being used

Once these hits are validated, meaning the compounds nature is confirmed, they progress to lead compound status

A lead compound is a hit with sufficient potential to progress to full drug development

The lead compound then progresses to the next phase of drug development

Where other aspects of its physical nature are tested

The compound is assayed for toxicity, often this is done during HTS, but further tests are required in cells or whole organisms

This is also when it will be determined how the drug is to be delivered

It was originally thought that combination of chemistry, robotics, & computers would deliver blockbuster drugs

However, HTS of random compounds has not delivered a large number of new blockbuster drugs

Companies are now taking known drugs or compounds that have drug-like properties & using these as scaffolds to create libraries

These libraries are more focused in that they are tailored to the disease being targeted

Another option is rational or structure based drug design

Rational Drug Design

Engineering of a molecule or protein through specific changes such that it becomes drug-like

Often requires choosing a target molecule in the cell, such as a receptor or enzyme and designing a therapeutic that prevents the target from causing or contributing to a disease

Need to know the structure of the target usually obtained through X-ray crystallography or NMR

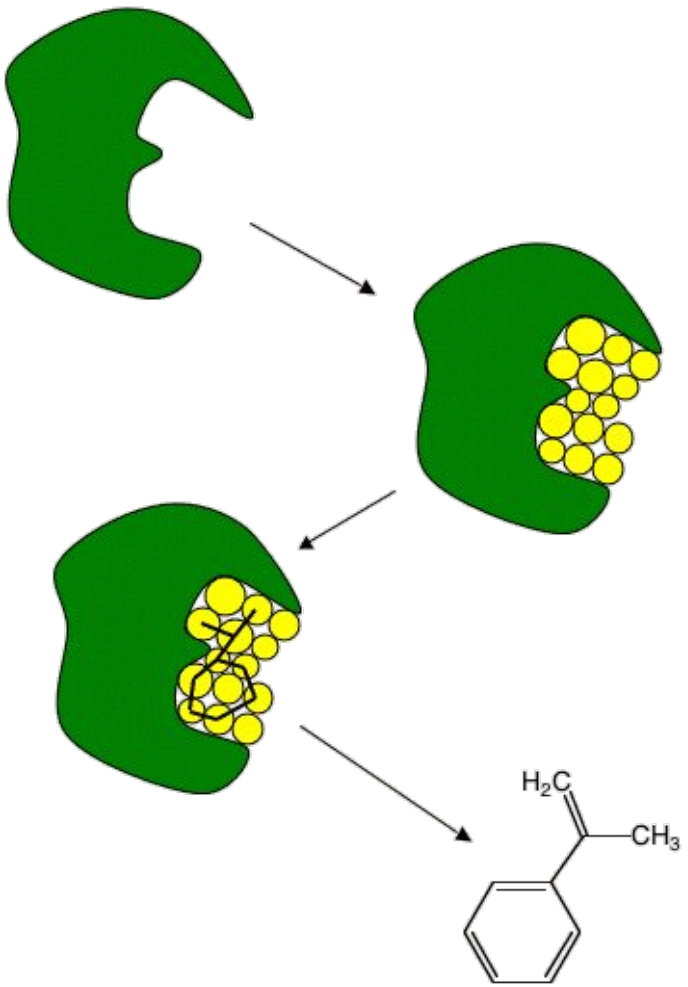
Also need a complete understanding of the thermodynamics factors involved in binding, which vary from interaction to interaction

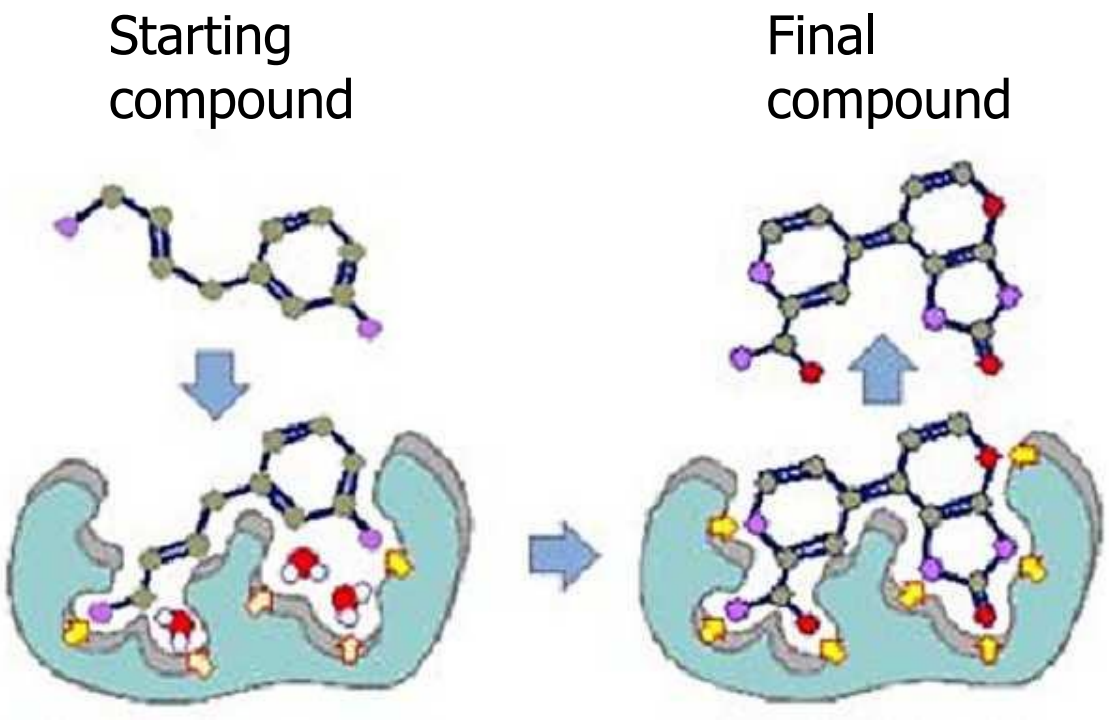
The target (green) has a very distinct shape to which the drug can bind

The molecule shown, has a shape which would allow it to fit into the binding site

Once a drug designer knows this, he can use this molecule as a base to build his drug

By attaching methyl groups, carboxyl groups, etc. he can change the action the drug will induce

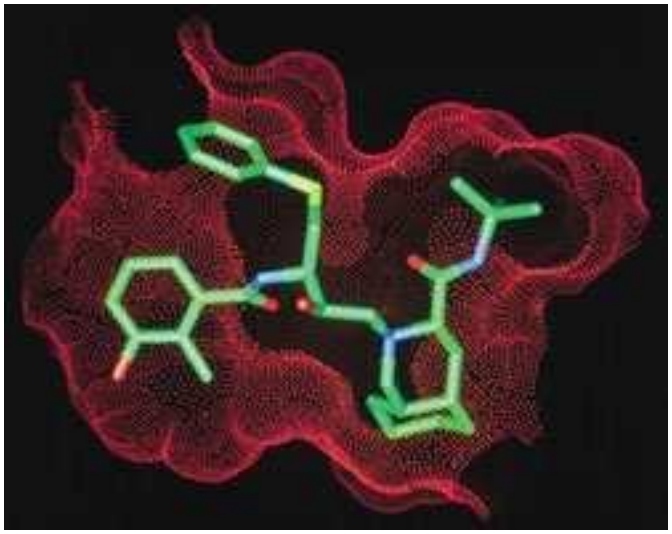




Starting molecule loosely binds to receptor

As the molecule is modified it binds tighter to the receptor

Eventually the designed molecule binds so tightly that it prevents the natural compound from binding



AIDS drug nelfinavir (Viracept) is one of the few drugs on the market that can be traced directly to structure-based methods

Here, the molecule is shown in the active site of HIV-1 protease

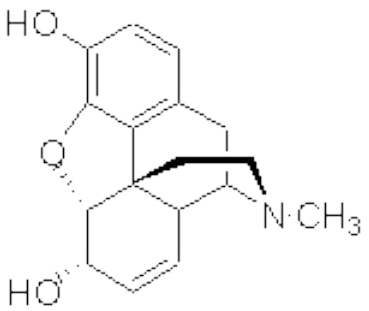
http://pubs.acs.org/cen/coverstory/7923/print/7923drugdesign.html

Other methods of drug design are based on taking known drugs & modifying their structure to make them better

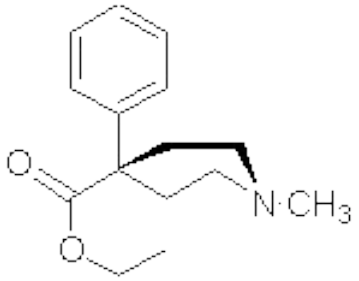
This requires one to know the structure of the drug

Alterations may:

- Cause the drug to be more potent
- Give the drug fewer side effects
- Increase its solubility, giving better absorption



morphine



meperidine
(pethidine, Demerol)

Meperidine has only 2 rings instead of 4, but it maintains strong analgesic activity

It has better oral absorption than morphine, and shows less GI side effects

Another method of drug design is to take a known molecule & design a drug mimic

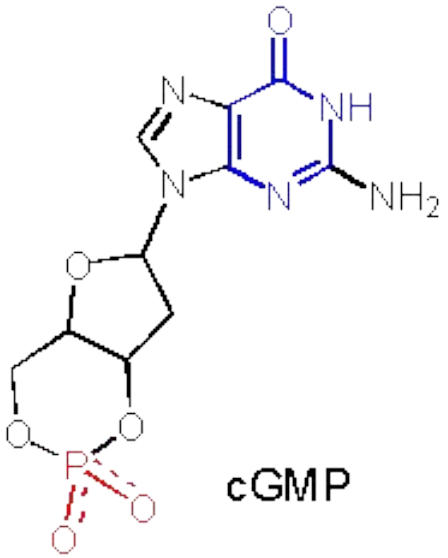
A mimic looks like the endogenous molecule, but is not processed by the cell the same way

These mimics work either as antagonists, that prevent cell functions

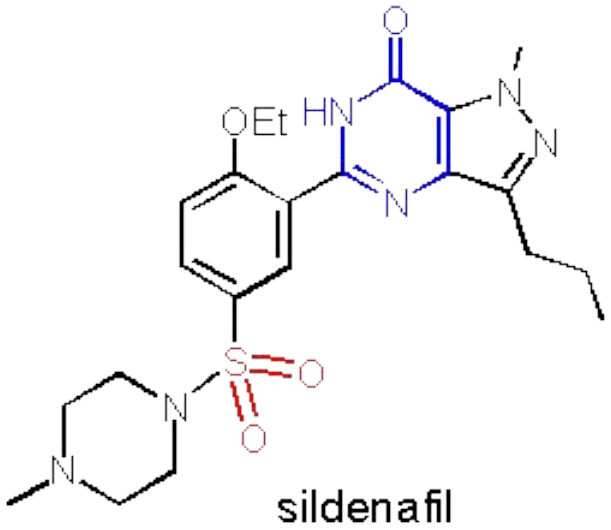
Or agonists that turn on cellular function in the absence of the normal signal

Sildenfil was designed to mimic cGMP & be an antihypertensive or an anti-angina

cGMP leads to, among other things, vascular relaxation which allows more blood to flow through vessels



cGMP



sildenafil

Phosphodiesterase (PDE), is the enzyme that converts cGMP to GMP

By blocking PDE-5, sildenafil prevents the breakdown of cGMP

Leading to more blood in the vessels

Unfortunately sildenafil did not work as well as the normal treatment, nitroglycerine

But its side effect was much more promising…

Rational Protein Modification

Involves taking a known therapeutic protein and optimizing it to function as a drug

Even though the endogenous protein functions well in the cell, there are properties unique to being a drug which can be added to improve its therapeutic nature

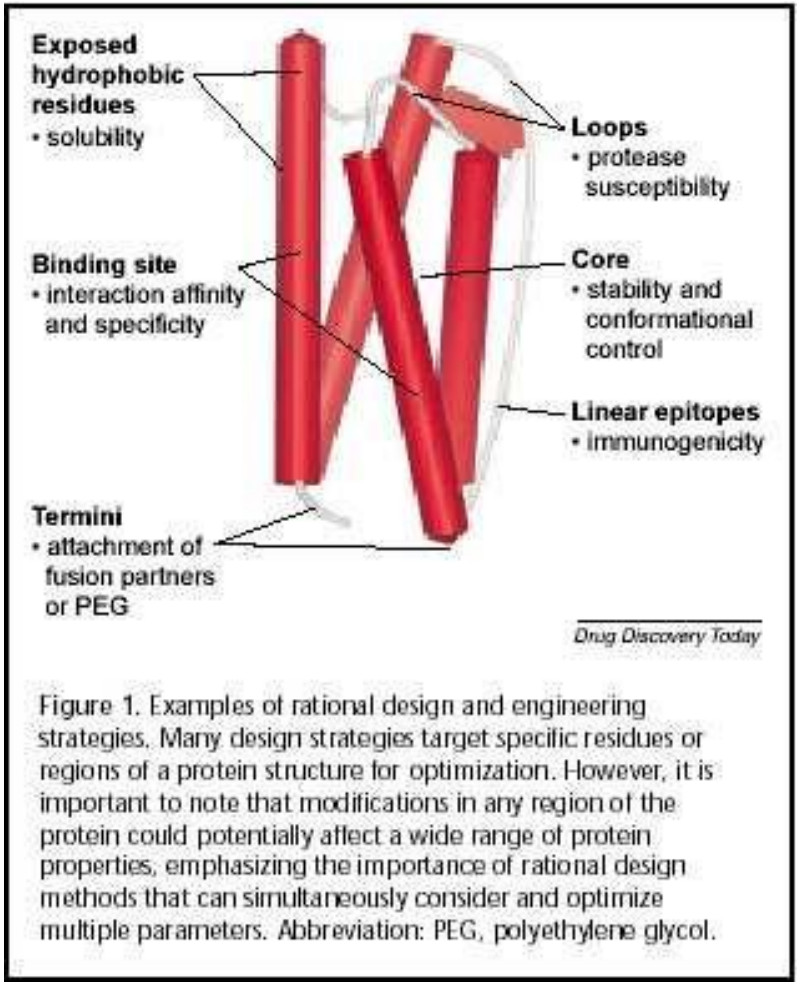


Figure 1. Examples of rational design and engineering strategies. Many design strategies target specific residues or regions of a protein structure for optimization. However, it is important to note that modifications in any region of the protein could potentially affect a wide range of protein properties, emphasizing the importance of rational design methods that can simultaneously consider and optimize multiple parameters. Abbreviation: PEG, polyethylene glycol.

Table 1. Engineered protein therapeutics on the market[a]

Name	Family	Company	Indication	Modification	Property
Proleukin® (aldesleukin)	IL-2	Chiron	Cancer	Mutated free cysteine	Decreased aggregation; improved bioavailability
Betaseron® (interferon beta-1b)	IFN-β	Berlex/Chiron	Multiple sclerosis	Mutated free cysteine	Decreased aggregation
Humalog® (insulin lispro)	Insulin	Eli Lilly	Diabetes	Monomer not hexamer	Fast acting
NovoLog® (insulin aspart)	Insulin	Novo Nordisk	Diabetes	Monomer not hexamer	Fast acting
Lantus® (insulin glargine)	Insulin	Aventis	Diabetes	Precipitates in dermis	Sustained release
Enbrel® (etanercept)	TNF receptor	Immunex/ Amgen/Wyeth	Rheumatoid arthritis	Fc fusion	Longer serum half-life; increased avidity
Ontak® (denileukin diftitox)	Diptheria toxin-IL-2	Seragen/Ligand	Cancer	Fusion	Targets cancer cells
PEG-Intron® (peginterferon alfa-2b)	IFN-α	Schering-Plough	Hepatitis	PEGylation	Increased serum half-life; weaker receptor binding
PEGasys® (peginterferon alfa-2a)	IFN-α	Roche	Hepatitis	PEGylation	Increased serum half-life; weaker receptor binding
Neulasta™ (pegfilgrastim)	G-CSF	Amgen	Leukopenia	PEGylation	Increased serum half-life
Oncaspar® (pegaspargase)	Asparaginase	Enzon	Cancer	PEGylation	Decreased immunogenicity; increased serum half-life
Aranesp® (darbepoetin alfa)	Epo	Amgen	Anemia	Additional glycosylation sites	Increased serum half-life; weaker receptor binding
Somavert® (pegvisomant)	Growth hormone	Genentech/ Seragen/ Pharmacia	Acromegaly	PEGylation; binding site mutations	Novel mode of action; increased serum half-life

PEG=polyethylene glycol