PERSPECTIVE ON DRUG DISCOVERY & DESIGN

What is a drug ?

FDA Definition of a Drug

“ An active ingredient that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of a disease, or to affect the structure of any function of the human body, but does not include intermediates used in the synthesis of such ingredient. ”

Over-the-counter (OTC) drugs: sold without a doctor’s prescription

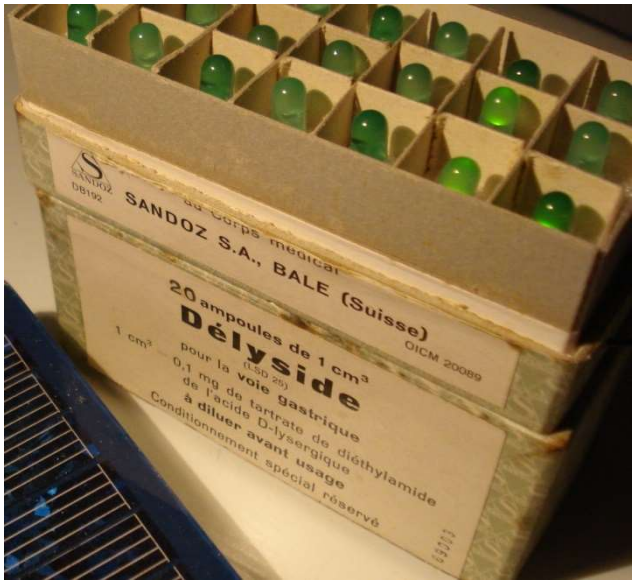
Ethical drugs: prescription drugs

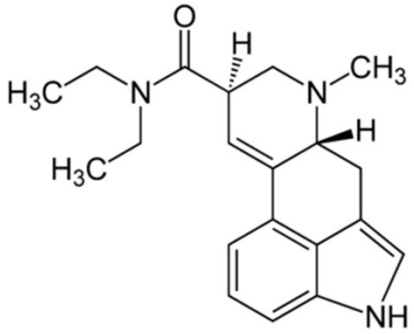
Biologicals: drugs that are biomolecules like antibodies, proteins, peptides, nucleic acids, etc…

Ilegal drugs: possession, use or commerce may be restricted or forbidden

FDA: Food and Drug Administration of the USA

What is a drug ?





LSD 25

What is drug design & discovery ?

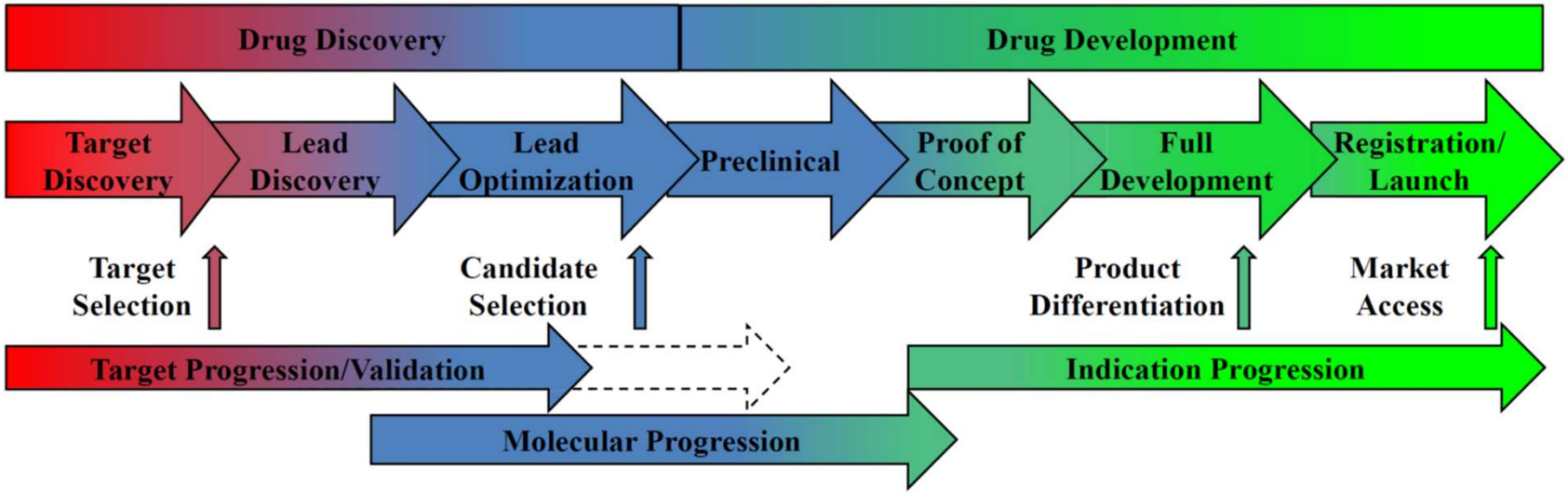
- **Drug discovery** – a generic term the encompasses all activities leading to new substances with pharmacological activity, be it natural substances, synthetic compounds, found by chance, search or design

- **Drug design** – a more specific term that refers to the process of creating new drugs, through a combination of biological, chemical and computational techniques

Drug Discovery *versus* Drug Development

Drug discovery - all of the experimentation and studies designed to move a program from the initial identification of a biological target and associated disease state to the identification of single compound with the potential to be clinically relevant.

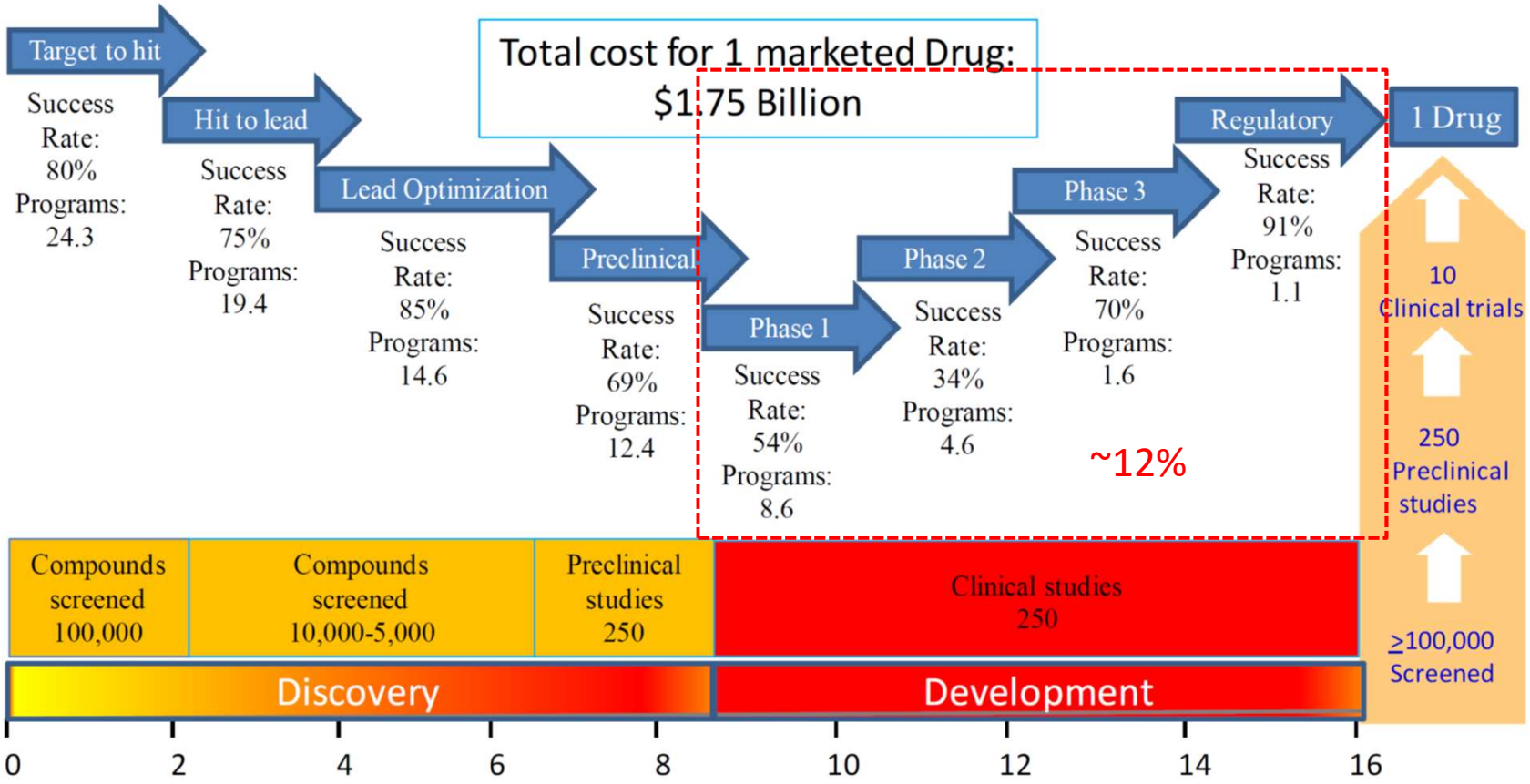
Drug Development - typically begins once a single compound has been identified, which is then progressed through various studies designed to support its approval for sale by the appropriate regulatory bodies.


Drug Discovery
Drug Development
Target Discovery
Lead Discovery
Lead Optimization
Preclinical
Proof of Concept
Full Development
Registration/ Launch
Target Selection
Candidate Selection
Product Differentiation
Market Access
Target Progression/Validation
Indication Progression
Molecular Progression

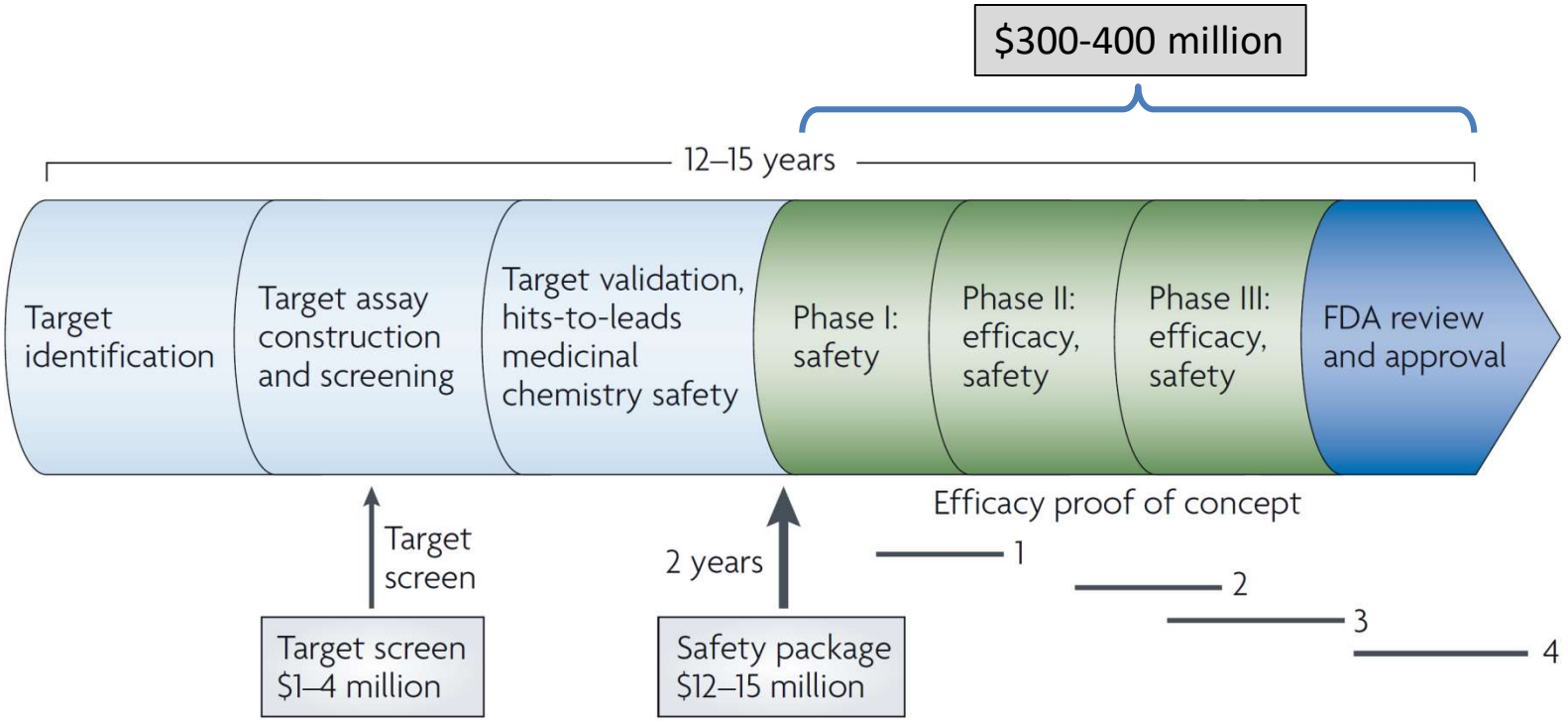
The challenge of DD

- The task of discovering new drugs is hard, expensive, lengthy and dependent on a very large number of scientific disciplines, techniques and expertise.
- Millions of compounds may have to be screened in activity tests to select but a few candidates (hits), of which only a few show promise as drug candidate (leads).
- Lengthy and thorough clinical testing in both animals and humans is required, without guarantee of approval by the regulatory entities.
- Millions (or billions) of dollars and ~5-15 years are required for the whole process.
- A large share of the profit generate by the pharmaceutical industries comes from only a few drugs.
- Patent expiry narrows the profitability range of drugs and pushes the “me too” drug concept

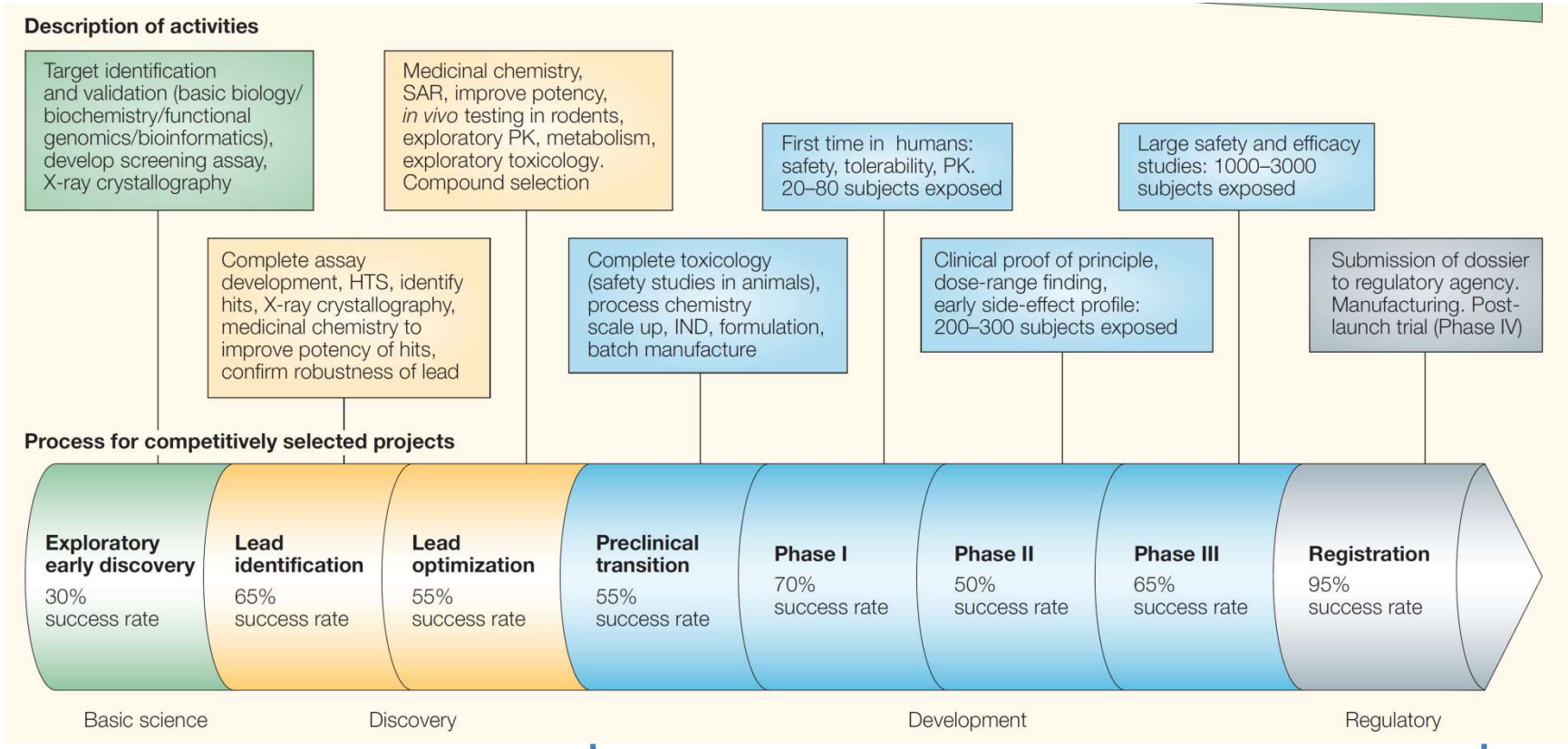
The path to a new drug



The Drug Discovery Pipeline



Only ~10% of the drugs that start phase I trials are eventually approved for marketing.
The total cost of developping a single drug often surpasses $1 billion.



Overall sucess rate: 12%

Table 1. Clinical Trial Success Rates by Phase (on Aggregate and by Therapeutic Area)[a]

Source	Phase 1 to Approval, %[b]	Phase 2 to Approval, %[c]	Phase 3 to Approval, %[d]	FDA Submission to Approval, %[e]
Aggregate rates				
Wong et al[18]	13.8	35.1	59.0	83.2
Thomas et al[19]	9.6	15.3	49.6	85.3
Hay et al[20]	10.4	16.2	50.0	83.2
Therapeutic-area-specific rates[18]				
Oncology	3.4	6.7	35.5	81.7
Metabolism and endocrinology	19.6	24.1	51.6	80.4
Cardiovascular	25.5	32.3	62.2	84.5
Central nervous system	15.0	19.5	51.1	82.2
Autoimmune and inflammation	15.1	21.2	63.7	80.3
Ophthalmology[f]	32.6	33.6	74.9	80.4
Infectious disease	25.2	35.1	75.3	84.9
Other[g]	20.9	27.3	63.6	80.4

Abbreviation: FDA, US Food and Drug Administration.

[a] Rates across all indications for individual therapeutic agents (as opposed to rates for lead indications, which were higher in all phases). Only the success rates used in this analysis were reported.

[b] Phase 1 trials, which usually include as many as 100 healthy volunteers and may take several months to conduct, are primarily used to assess the tolerability and safety of a therapeutic agent in different doses; these are sometimes referred to as first-in-human trials.

[c] Phase 2 trials, which can involve as many as a few hundred patients with a disease or condition and take several months to 2 years to complete, are typically used to gather data on the efficacy and safety of a therapeutic agent in different doses.

[d] Phase 3 trials, which can involve several thousand participants with a disease or condition and may take 1 to 4 years to run, are generally used to confirm the efficacy and safety of the dose of the therapeutic agent believed to provide the best risk-benefit ratio.[23]

[e] Indicates the proportion of new drug applications and biologics license applications approved by the FDA. Wong et al[18] reported aggregate and therapeutic-area–specific rates through phase 3. These data were supplemented with estimates of FDA submission to approval rates from Hay et al; if a particular category from the study by Wong et al was not reported by Hay et al, the category *Other* was used.[20]

[f] This category was applied to therapeutic agents classified as treating sensory organ diseases, ie, anatomical therapeutic chemical classification system code *S*.

[g] Values in this category were based on the rates for "all [agents] without oncology" reported by Wong et al.[18] These rates were applied to therapeutic agents that were outside the other categories.

Wouters(2020) *J.Am.Medical Assoc.*, 323:884

Estimated cost of a drug 2009-2018

JAMA | Original Investigation

Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018

Olivier J. Wouters, PhD; Martin McKee, MD, DSc; Jeroen Luyten, PhD

Mean Cost:
$1.3 billion

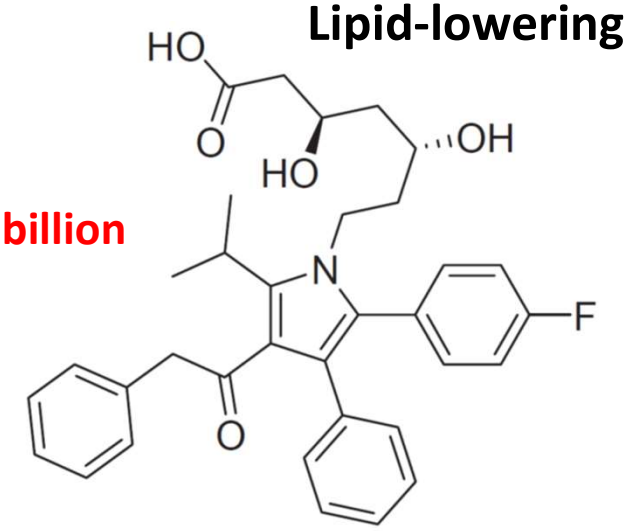
Table 4. Mean And Median Expected Research and Development Expenditure on New Therapeutic Agents Approved by the US Food and Drug Administration (2009-2018) by Therapeutic Area

Therapeutic Area[a]	Sample Size	Expenditure in US$, Millions (95% CI)[b]	
		Median	Mean
Antineoplastic and immunomodulating agents	20	2771.6 (2051.8-5366.2)	4461.2 (3114.0-6001.3)
Alimentary tract and metabolism	15	1217.6 (613.9-1792.4)	1430.3 (920.8-2078.7)
Nervous system	8	765.9 (323.0-1473.5)	1076.9 (508.7-1847.1)
Antiinfectives for systemic use	5	1259.9 (265.9-2128.3)	1297.2 (672.5-1858.5)
Dermatologicals	4	747.4	1998.3
Cardiovascular system	3	339.4	1152.4
Musculoskeletal system	3	1052.6	937.3
Blood and blood-forming organs	2	793.0	793.0
Sensory organs	2	1302.8	1302.8
Other[c]	1	1121.0	1121.0

Wouters(2020) *J.Am.Medical Assoc.,* 323:884

Commercially successful drugs

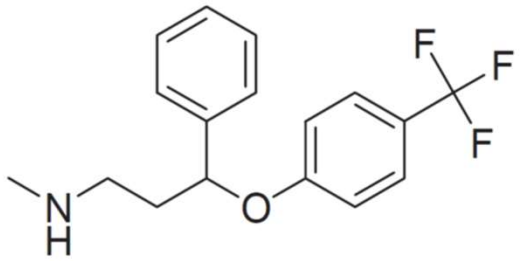
Lipid-lowering



Lipitor®
(Atorvastatin)

$13 billion

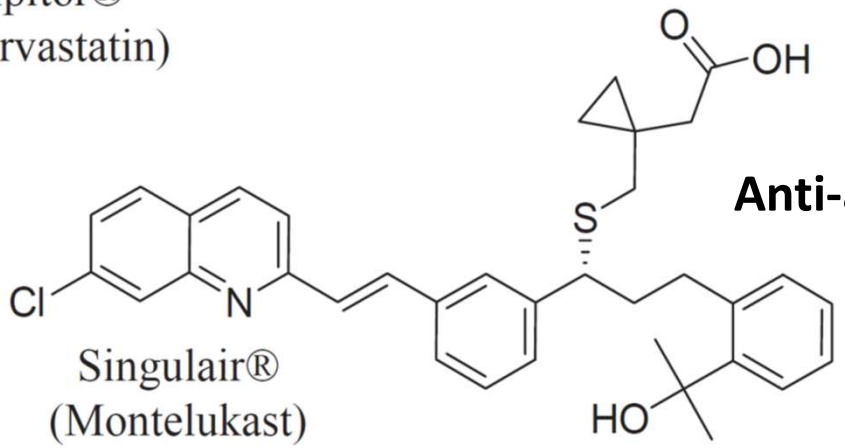
Anti-depressant



Prozac®
(Fluoxetine)

$2.8 billion

Anti-asthma

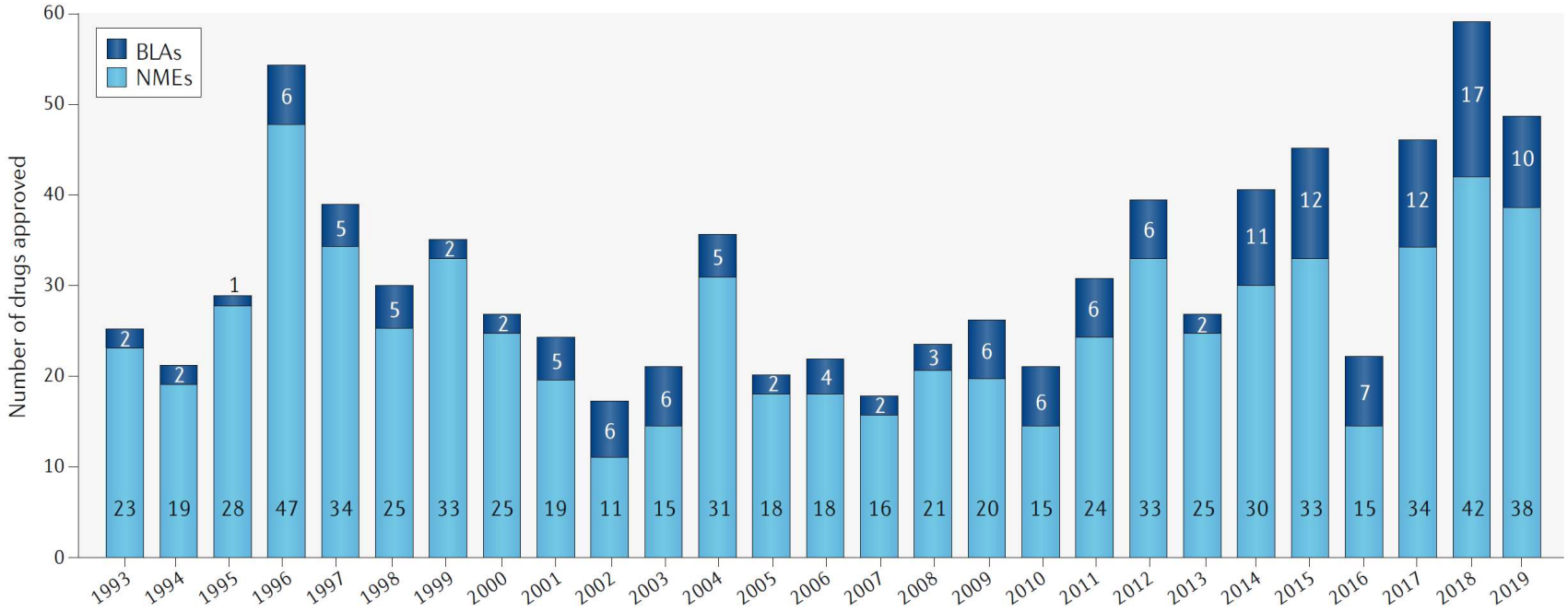


Singulair®
(Montelukast)

$5.5 billion

Peak annual sales

FDA drug approvals 1993-2019



NME- new molecular entities

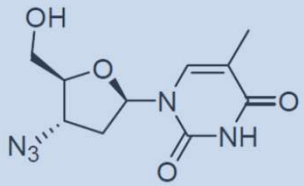
BLA – Biologics license applications

NME – a drug that contains an active moioety that has never been aproved by the FDA or marketed in the US.

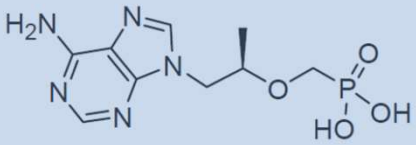
Mullard(2020) *Nature Rev. Drug Disc,* 19:226

Anti-HIV drugs

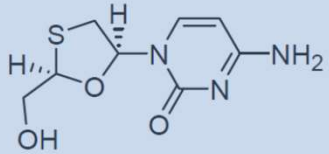
Reverse transcriptase inhibitors



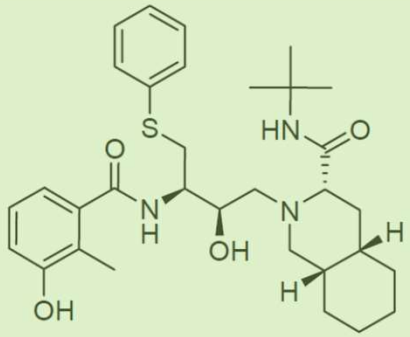
AZT®
(Retrovir)



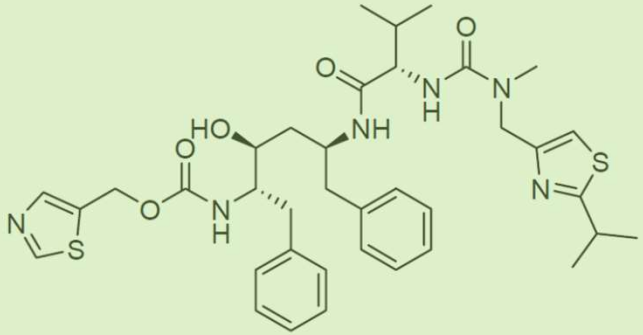
Viread®
(Tenofovir)



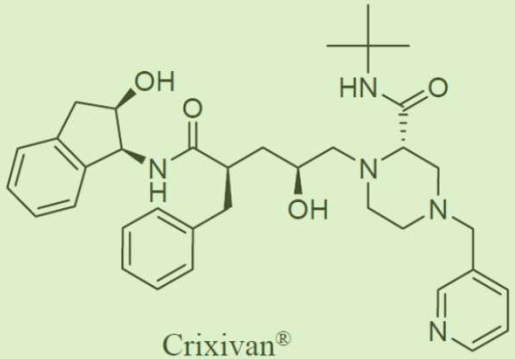
Zeffix®
(Lamivudine)



Viracept®
(Nelfinavir)



Norvir®
(Ritonavir)

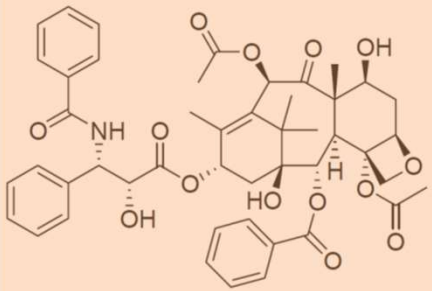


Crixivan®
(Indinavir)

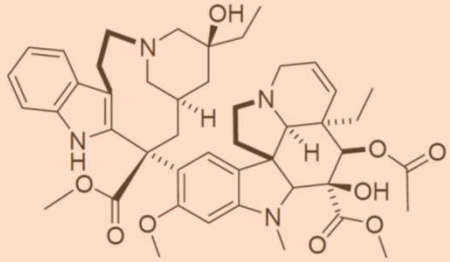
Protease inhibitors

Anti-cancer drugs

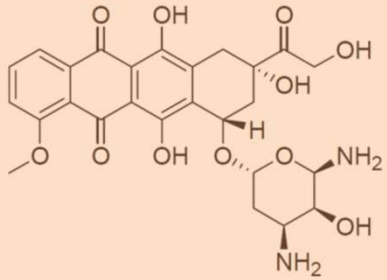
Natural Products (classical DD)



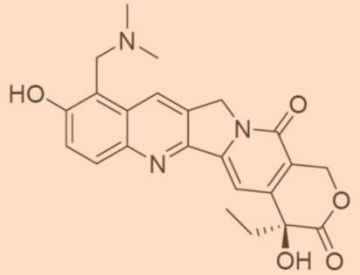
Taxol®
(Paclitaxel)



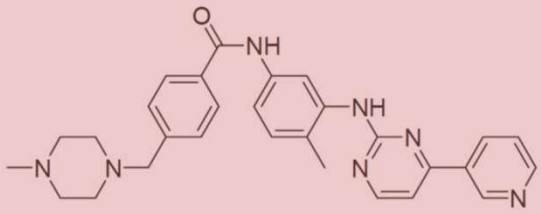
Velban®
(Vinblastine)



Adriamycin®
(Doxorubicin)

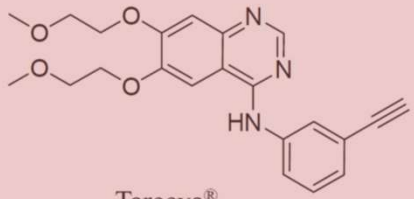


Hycamtin®
(Topotecan)



Gleevac®
(Imatinib)

Tasigna®
(Nilotinib)

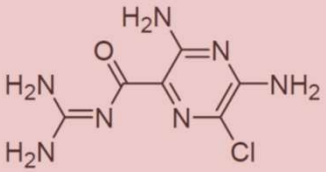


Tarceva®
(Erlotinib)

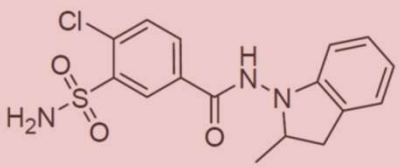
Synthetic Drugs (modern DD)

Cardiovascular drugs

Diuretics

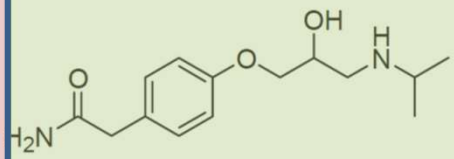


Midamor®
(Amiloride)

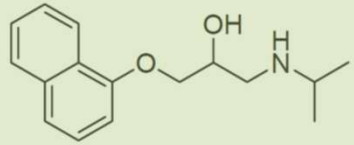


Lozol®
(Indapamide)

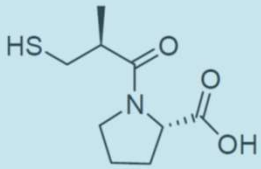
β-blockers



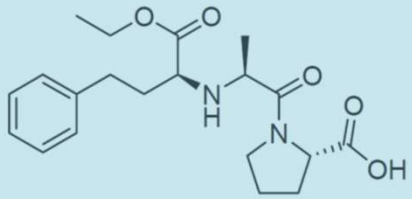
Tenoretic®
(Atenolol)



Inderal®
(Propranolol)

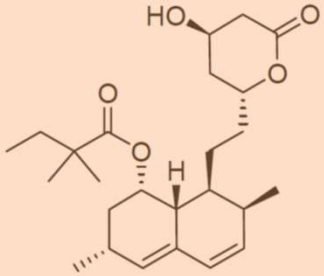


Capoten®
(Captopril)

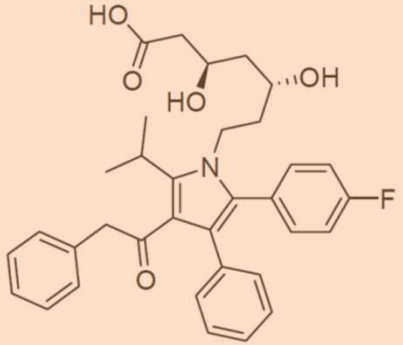


Vasotec®
(Enalapril)

ACE inhibitors



Zocor®
(Simvastatin)

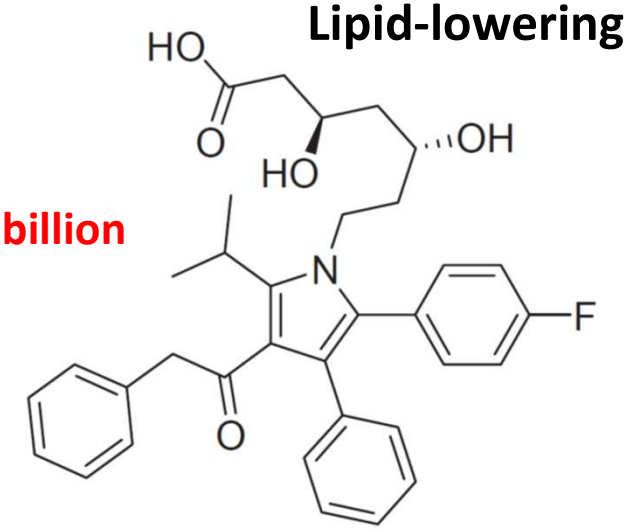


Lipitor®
(Atorvastatin)

HMG-CoA reductase inhibitors

Commercially successful drugs

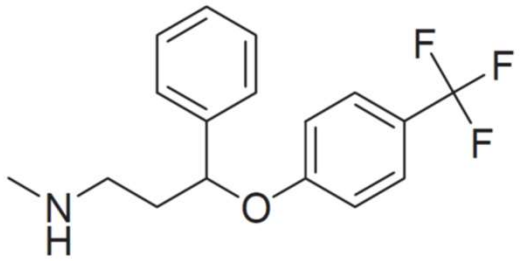
Lipid-lowering



Lipitor®
(Atorvastatin)

$13 billion

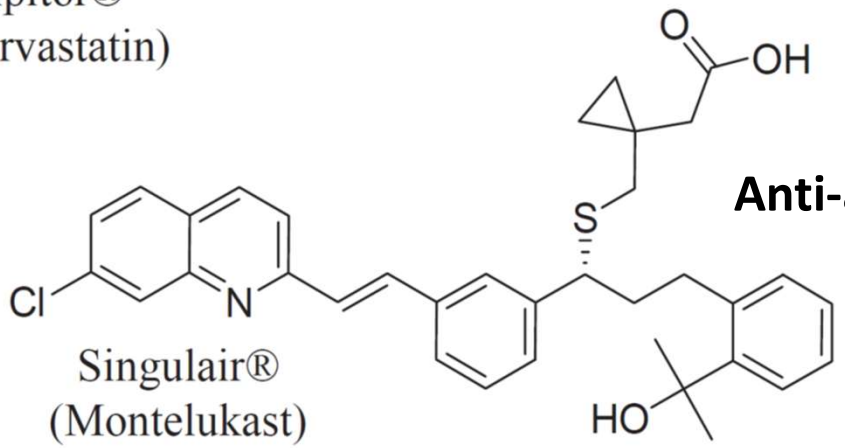
Anti-depressant



Prozac®
(Fluoxetine)

$2.8 billion

Anti-asthma



Singulair®
(Montelukast)

$5.5 billion

Peak annual sales

Tight regulation and approval

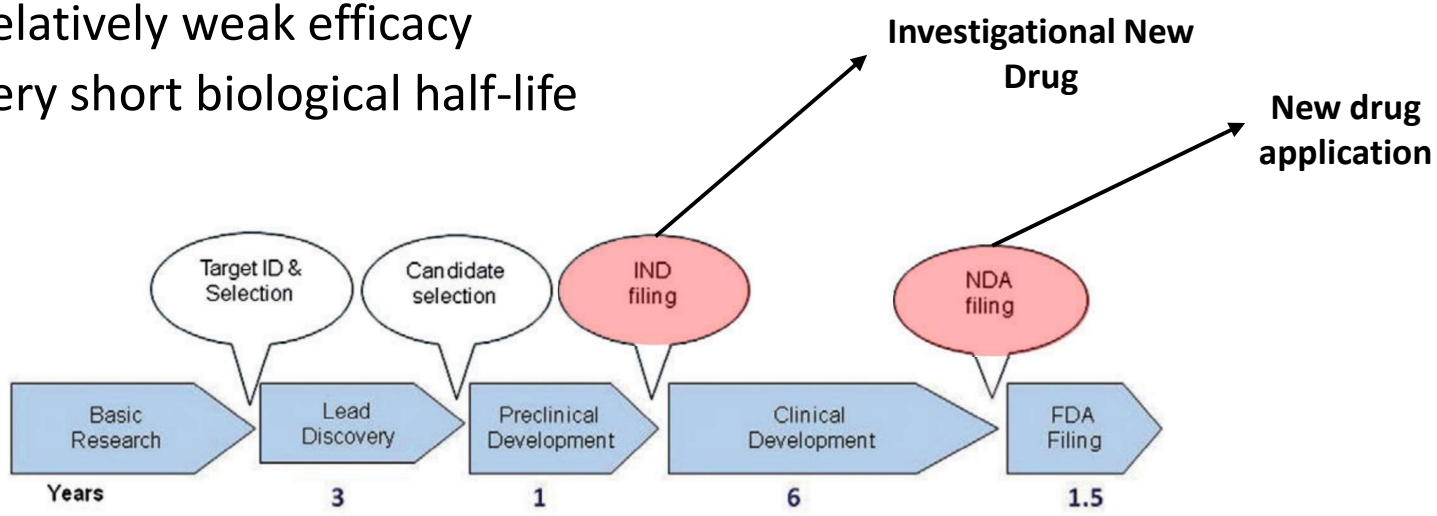
- Elixir of Sulfanilamide disaster (1937)
- Thalidomid disaster (1956-62)
- The Vioxx (rofecoxib) case (2004)
- … and others…

Pure Food and Drug Act (1906)
Food, Drug and Cosmetic Act (1938)
Durham–Humphrey Amendment (1951)
Kefauver–Harris Amendment (1962)
Hatch–Waxman Act (1984)

Due to various mishappenings, drug manufacturing is probably the most regulated human activity!

By today's standards, Aspirin© wouldn't make it into the market:

- Causes gastric bleeding
- It is an irreversible inhibitor
- Relatively weak efficacy
- Very short biological half-life

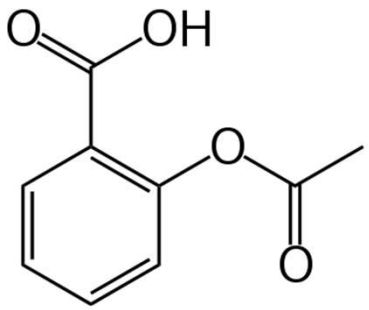


The Evolution of Drug Research

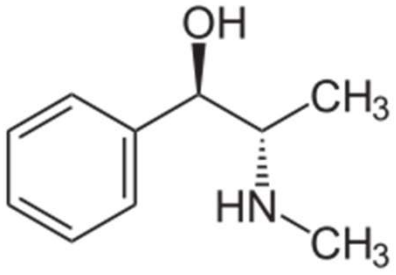
1. Empirical methods were the only source of medicines
2. Targeted isolation of active compounds from plants
3. Beginning of a systematic search for new synthetic materials with biological effects and the introduction of **animal models**
4. Use of molecular and other *in vitro* test systems as precise models replacing animal experiments (**screening**).
5. Introduction of theoretical and experimental methods: X-ray crystallography, QSAR, molecular modelling for the targeted **structure-based** and **computer-assisted** design of drugs
6. Discovery and therapeutic validation of targets through genomic, proteomic and transcriptomic analysis, knock-in and knock-out animal models and siRNA gene silencing

Drugs known by the end of the XIX century

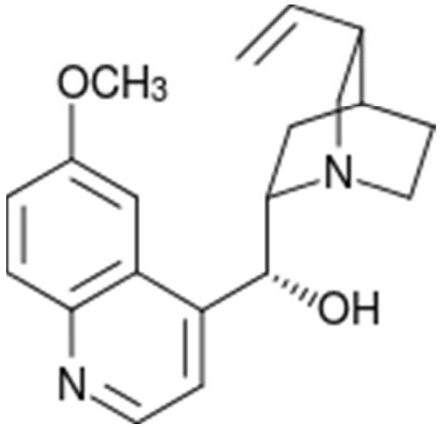
- Digitalin (heart stimulant)
- Quinine (anti-malarial)
- Ipecac (emetic)
- Aspirin (anti-inflammatory)
- Ephedrine (antiasthmatic and stimulant)
- Mercury (syphilis)



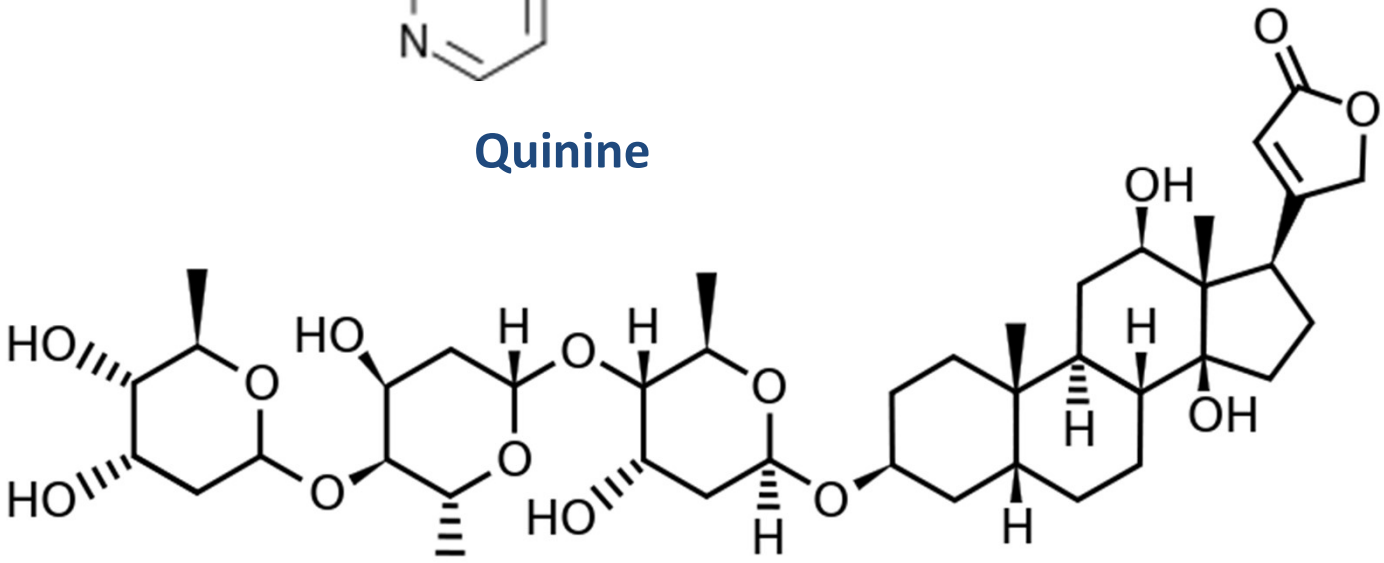
Aspirin



Ephedrine

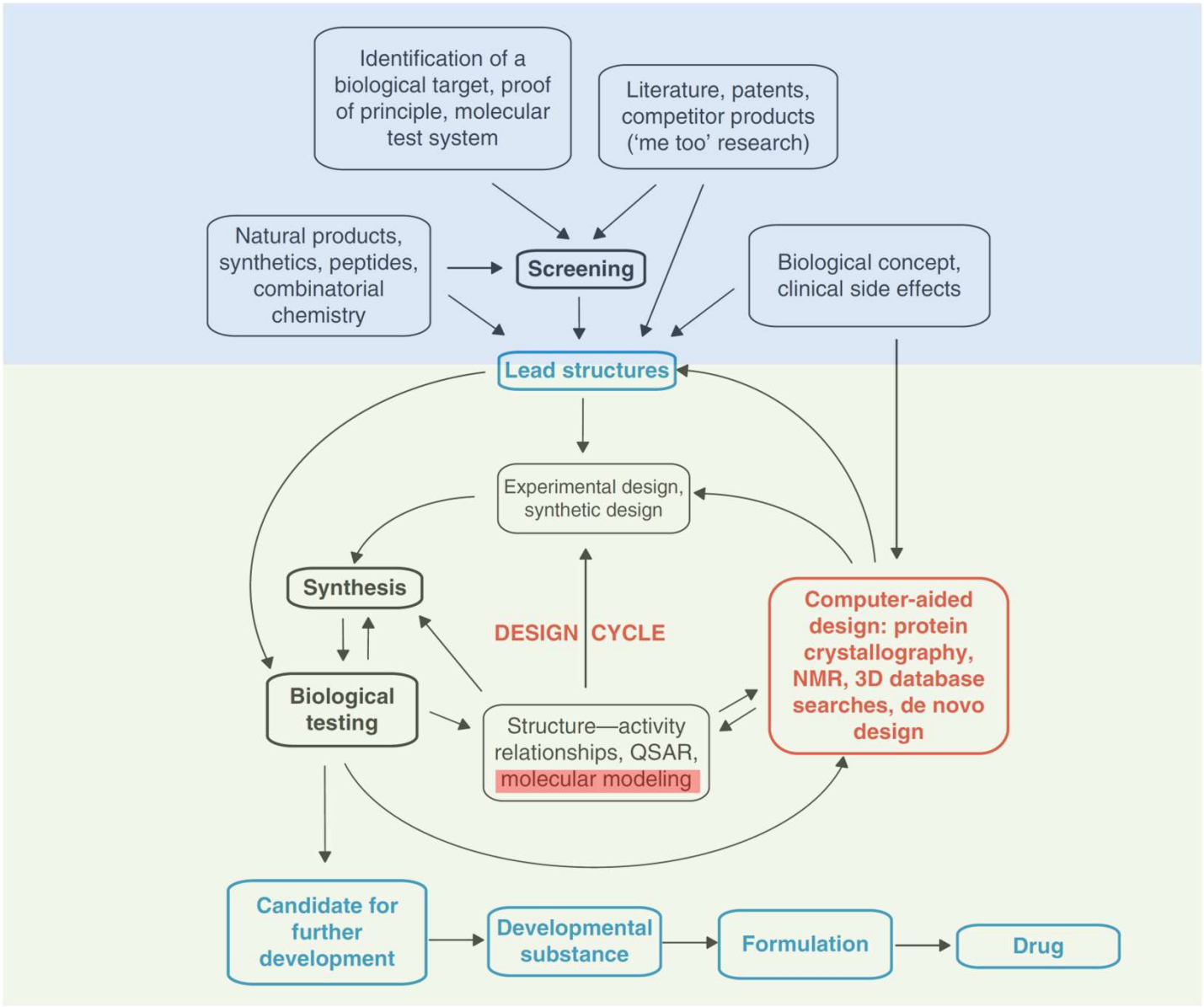


Quinine



Digitalin

The drug design cycle and CADD



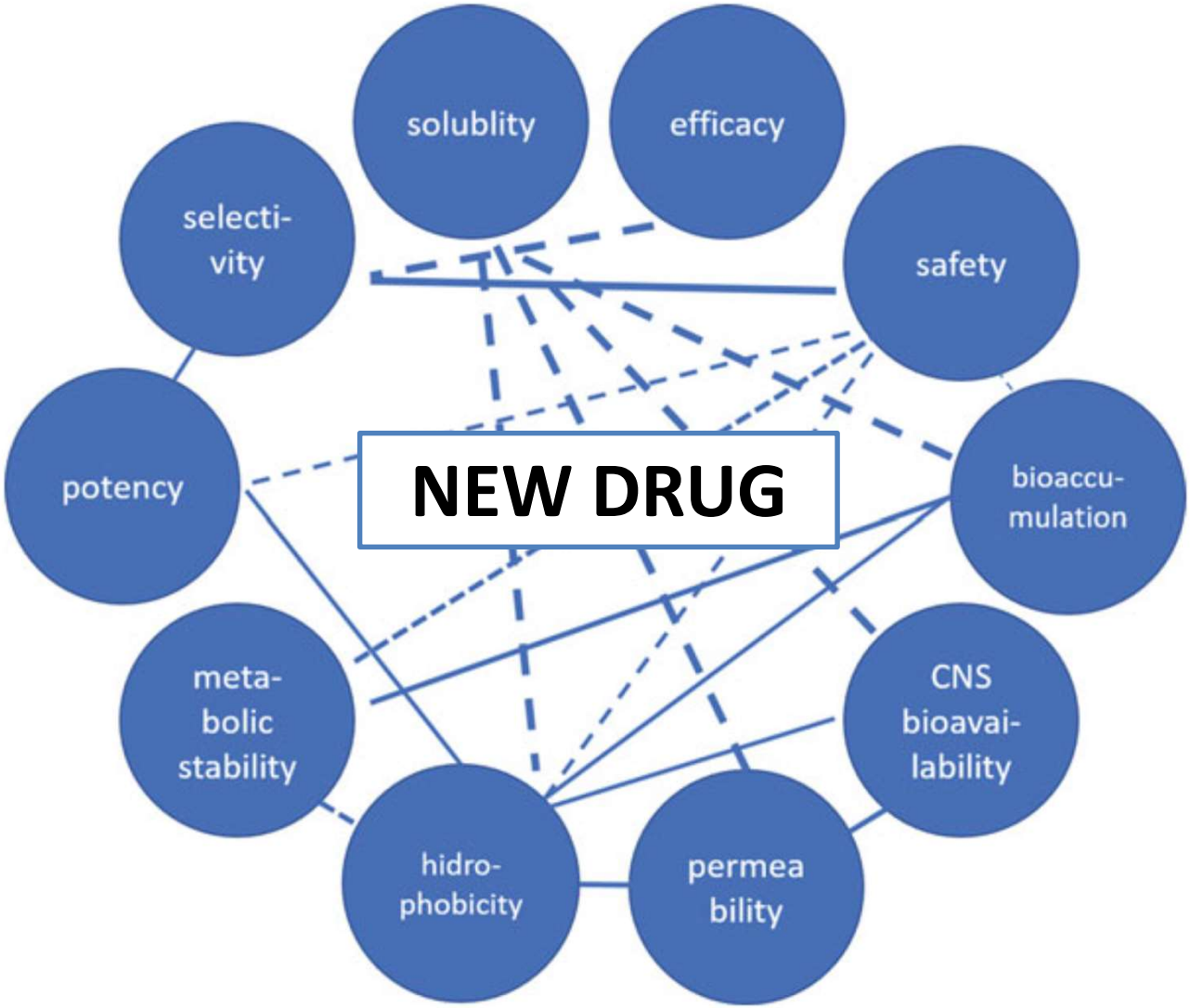
Disciplines for Drug Design

- Biochemistry
- Molecular Biology
- Medicinal Chemistry
- Pharmacology
- Genetics
- Physiology

- Biophysics
- Molecular Modelling
- Computational Biochemistry
- Bioinformatics
- Genomics
- Systems Biology

What makes a good drug?

- Potency
- Selectivity
- Few side effects
- Good bioavailability
- Ease of synthesis
- No drug-drug interactions
- High therapeutic index


solublity
efficacy
selecti-
vity
safety
potency
NEW DRUG
bioaccu-
mulation
meta-
bolic
stability
CNS
bioavai-
lability
hidro-
phobicity
permea
bility

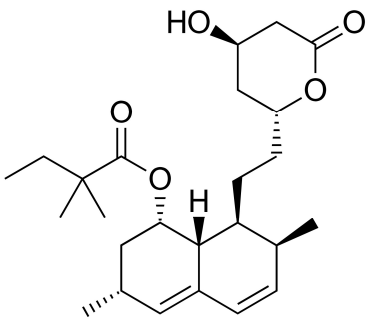
Requirements for drug candidates

- Efficacy data:
  - Enzyme activity
  - Whole organism activity
  - Animal models
- Metabolism:
  - *In vitro* metabolism
  - *In vivo* pharmacokinetics
- Safety:
  - *In vitro* selectivity
  - *In vitro* mutagenecity
  - *In vitro* cardiac
  - Animal toxicology
- Chemistry:
  - Physical form
  - Manufacture related
  - Back-up strategy
  - Objectives

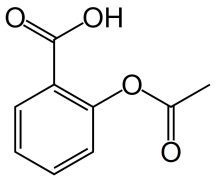
How are new drugs found ?

- Natural products (e.g. Aspirin)
- Screening assays
- Synthetic chemistry
- Combinatorial chemistry
- Similarity with know drugs (“Me too” drugs)
- Re-purposing (searching known drugs for a new effect)
- Serendipity:
  - drugs found by chance (*e.g.* Penicilin)
  - Unforeseen side-effect of a drug or candidate

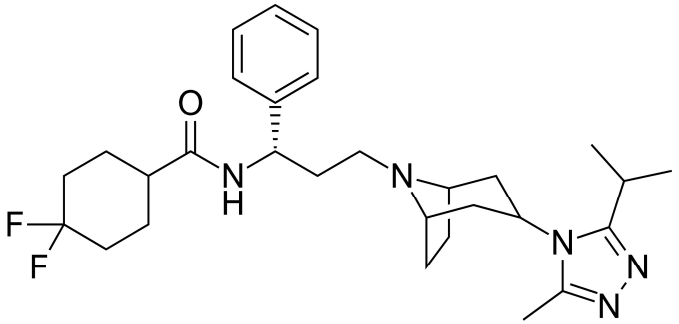
Drugs found by different methods



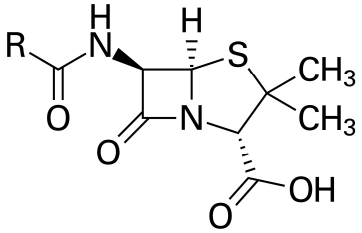
Simvastatin
(“me too”)



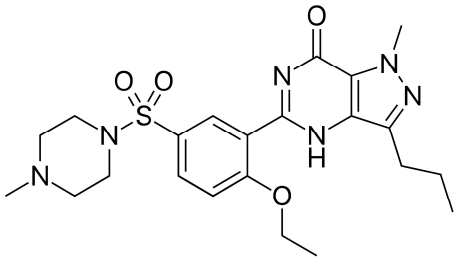
Aspirin
(natural product)



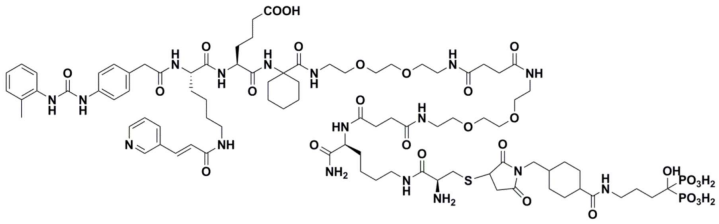
Maraviroc
(HTS assay)



Penicillin
(serendipity)

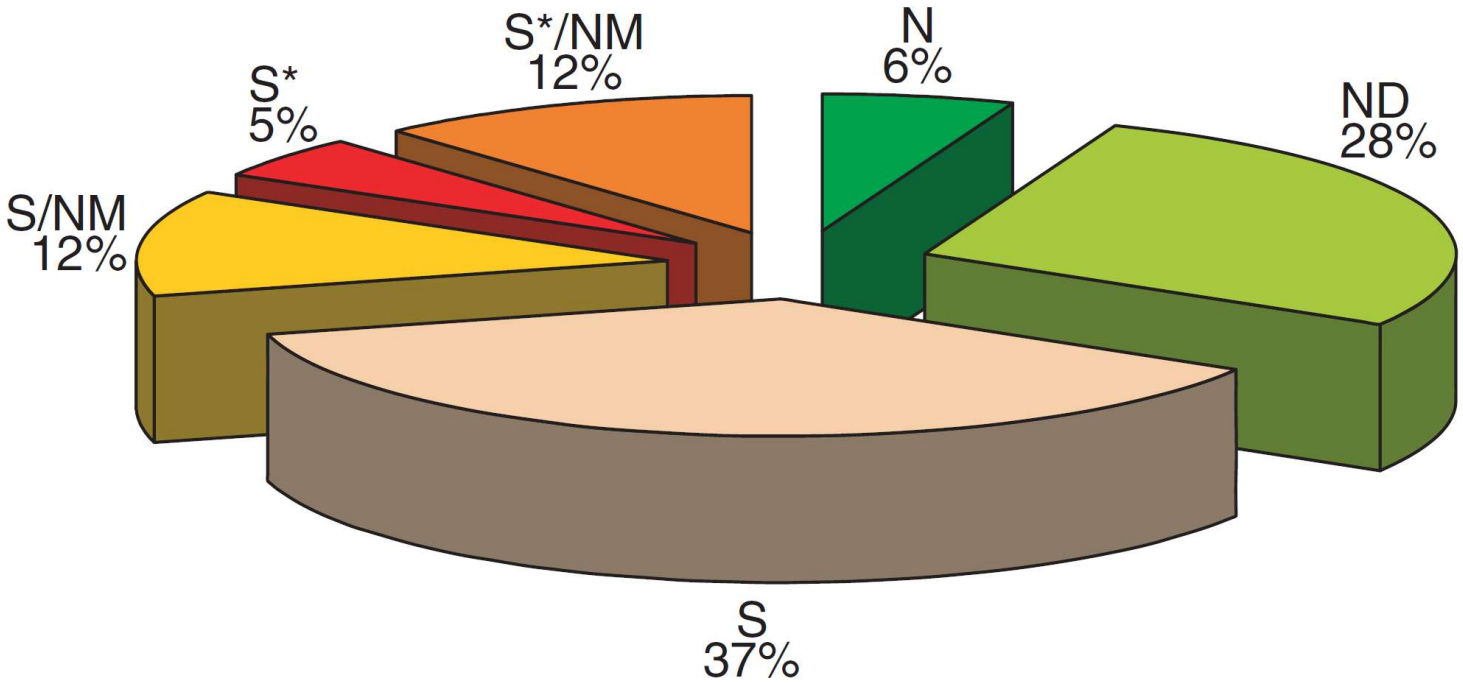


Sildenafil
(repurposing)



LLP2A-Ale
(combinatorial chemistry)

Where do drugs come from ? (1981-2006)



N – unmodified natural product

ND – modified natural product

S – synthetic compound

S* - synthetic compound with natural product pharmacophore

S/NM – synthetic compound showing competitive inhibition of the natural product substrate

Only 37% truly synthetic

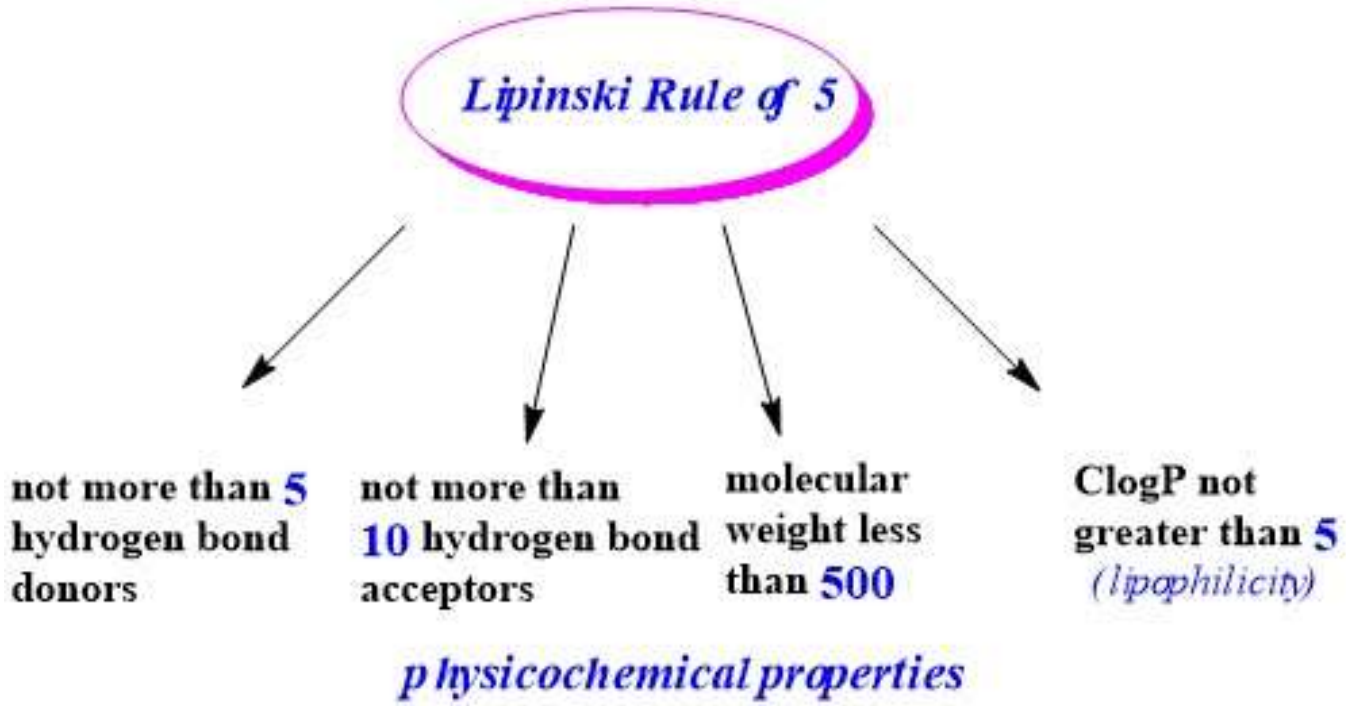
What makes a compound drug-like ?

Typical pharmaceutical compounds have:

- Molecular weight in the range 160<MW<480
- Number of atoms between 20 and 70
- Lipophilicity in the range -0.4 < logP < +.56
- Molar refractivity in the range 40<MR<130
- Few H-bond donors (<5)
- Few H-bond acceptors (<10)
- At least one –OH group (except CNS-active drugs)

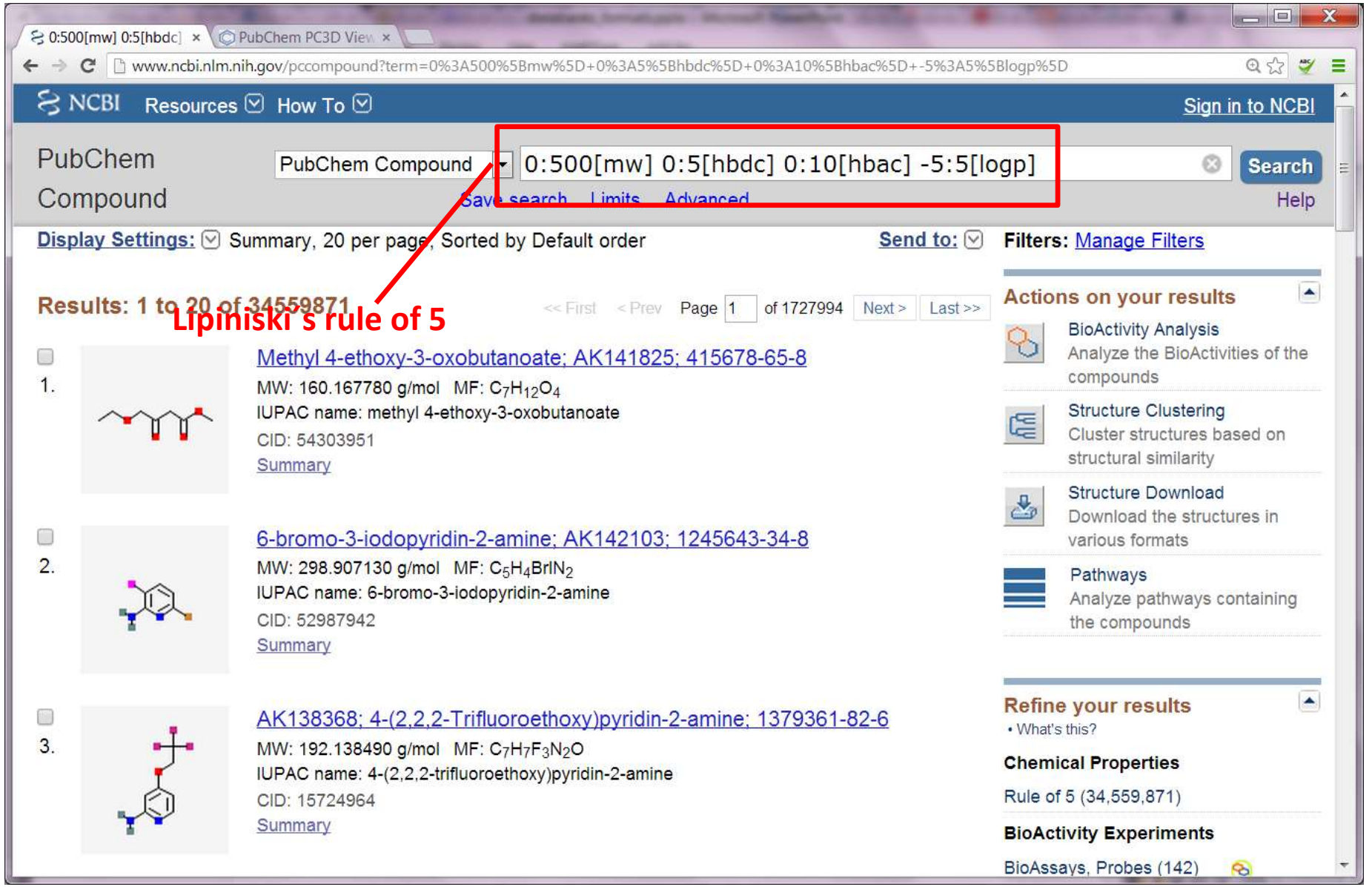
Lipinski's rule of 5

Christopher Lipinski formulated this rule of thumb to determine if a pharmacologically active substance is likely to work as an *oral drug.*



Lipinski, CA (2000) "Drug-like properties and the causes of poor solubility and permeability" *J Pharm Tox Meth* **44**:235-239

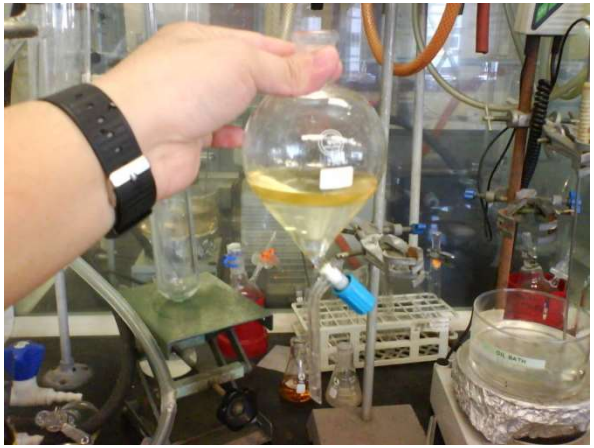
Rule of 5 in PubChem



logP

logP is the logarithm of the partition coeficient of a substante between octanol and aqueous phases. It is a measure of the **lipophilicity**.

A drug must be lipophilic enough to cross cell membranes, but no so much it can't dissolve in the plasma.



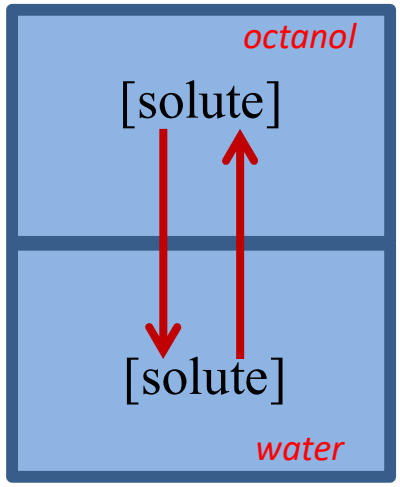
$$\log P = \log\left(\frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}}\right)$$

hydrophilic -4.0 < logP < +8.0 lipophilic

Citric acid –1.72

Iodobenzene +3.25

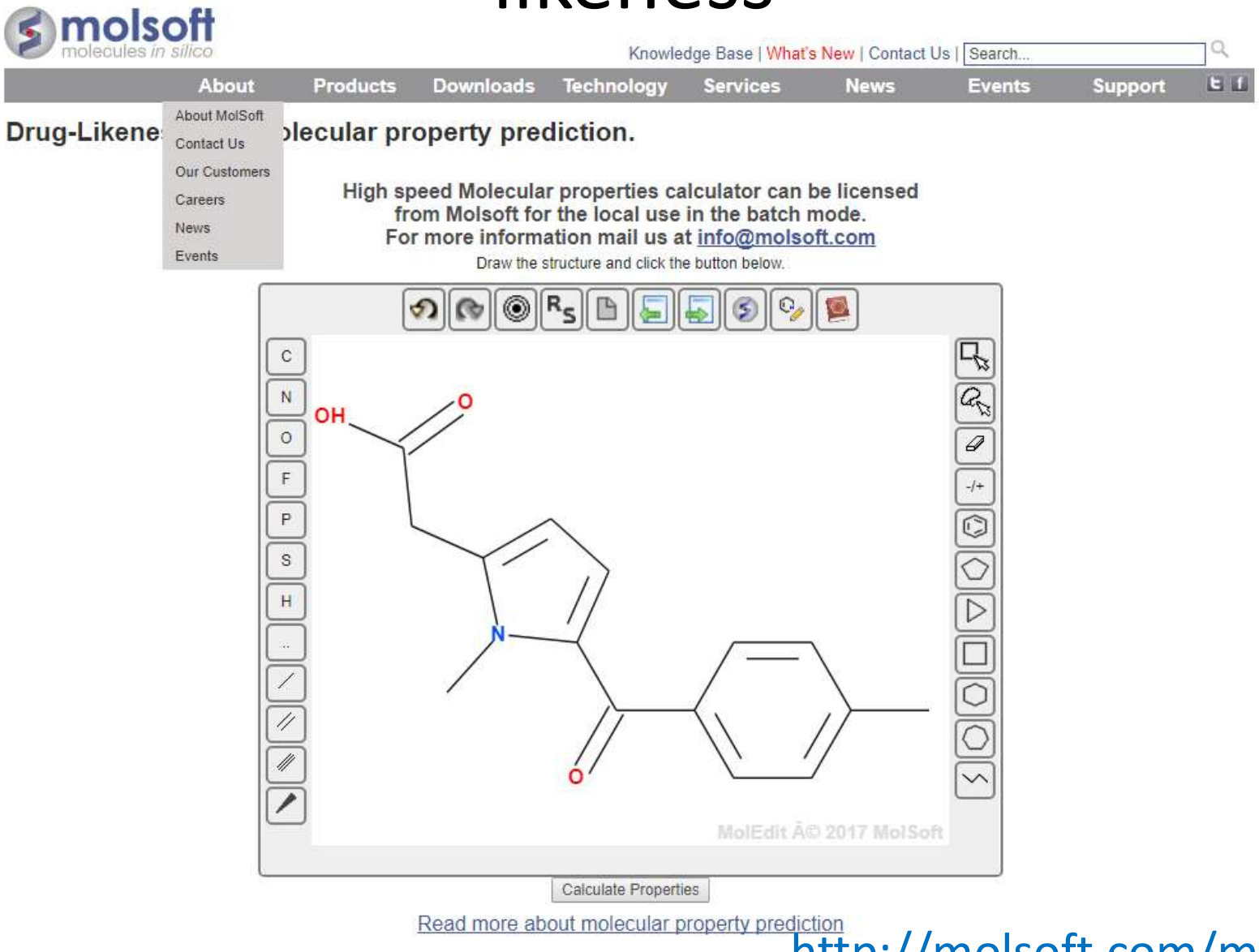
Typical drugs < 5.0



ClogP, XlogP – theoretical estimates of logP baseed on structure

Martin(2018) *J.Comp.Aided Mol.Des.*, 32:809

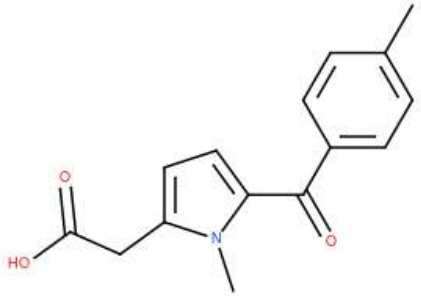
Computational prediction of drug likeness



http://molsoft.com/mprop

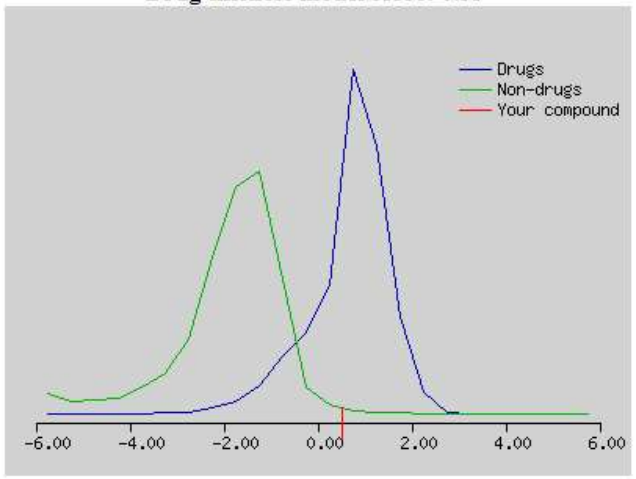
Computational prediction of drug likeness

Molecular Properties and Drug-likeness.



Molecular formula: C15 H15 N O3
Molecular weight: 257.11
Number of HBA: 3
Number of HBD: 1
MolLogP : 1.93
MolLogS : -3.09 (in Log(moles/L)) 210.05 (in mg/L)
MolPSA : 43.86 A^2
MolVol : 262.08 A^3
Number of stereo centers: 0

Drug-likeness model score: 0.53

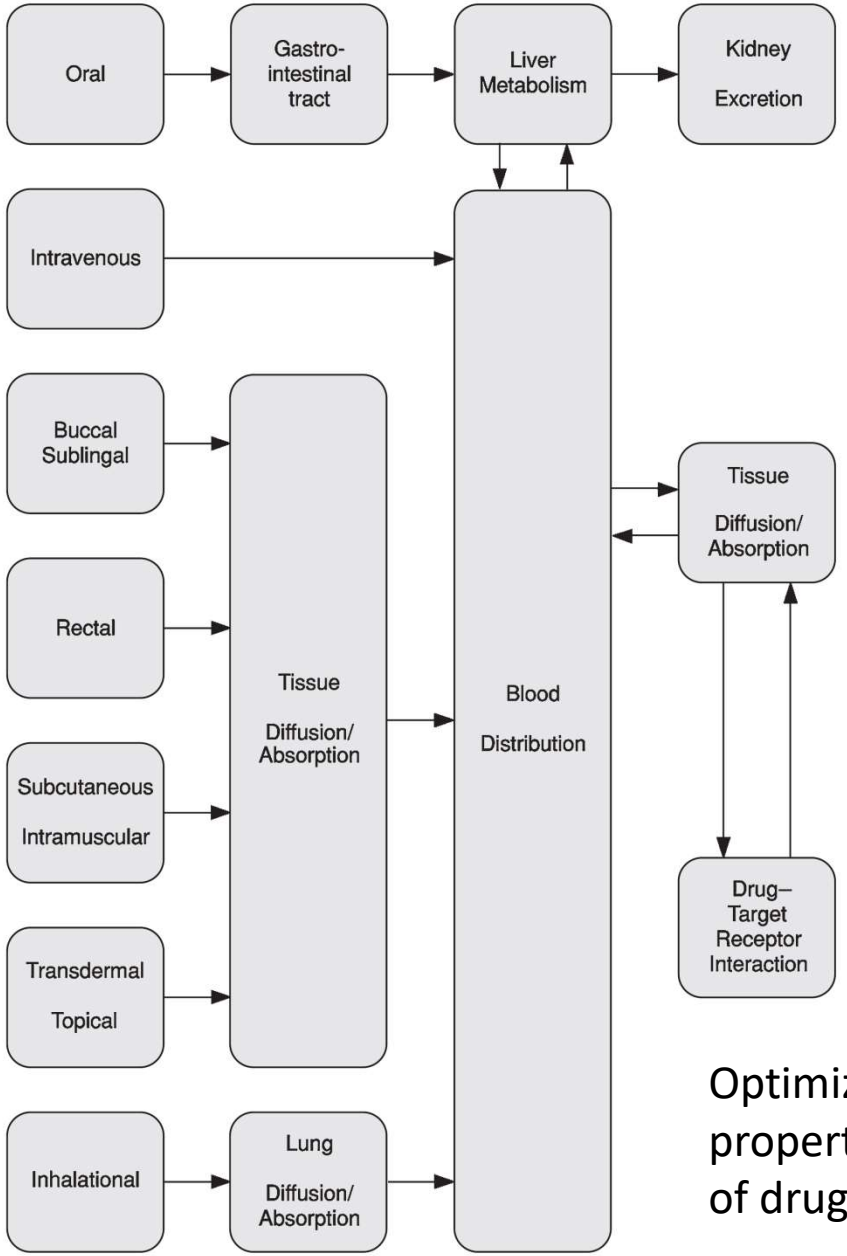


New molecule Modify molecule Search molecule

http://molsoft.com/mprop

ADMET

Absorption
Distribution
Metabolism
Excretion
Toxicity

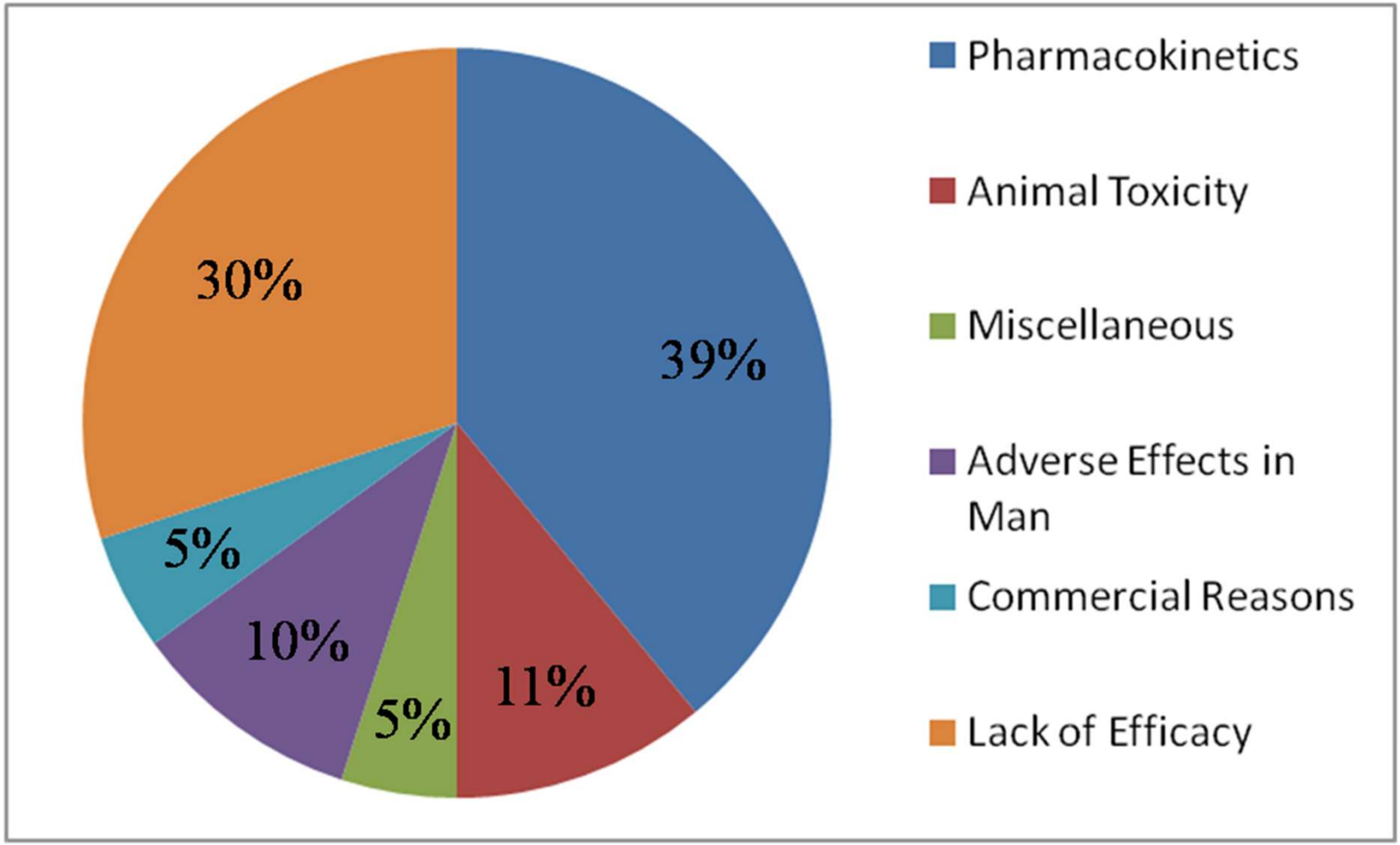


Optimization of ADMET properties is a crucial aspect of drug design!

Structure modification strategies for solubility improvement

Structure modification
Add ionizable group
Reduce Log P
Add hydrogen bonding
Add polar group
Reduce molecular weight
Out-of-plane substitution to reduce crystal packing
Construct a prodrug

Why drugs fail – the importance of pharmakocinetics



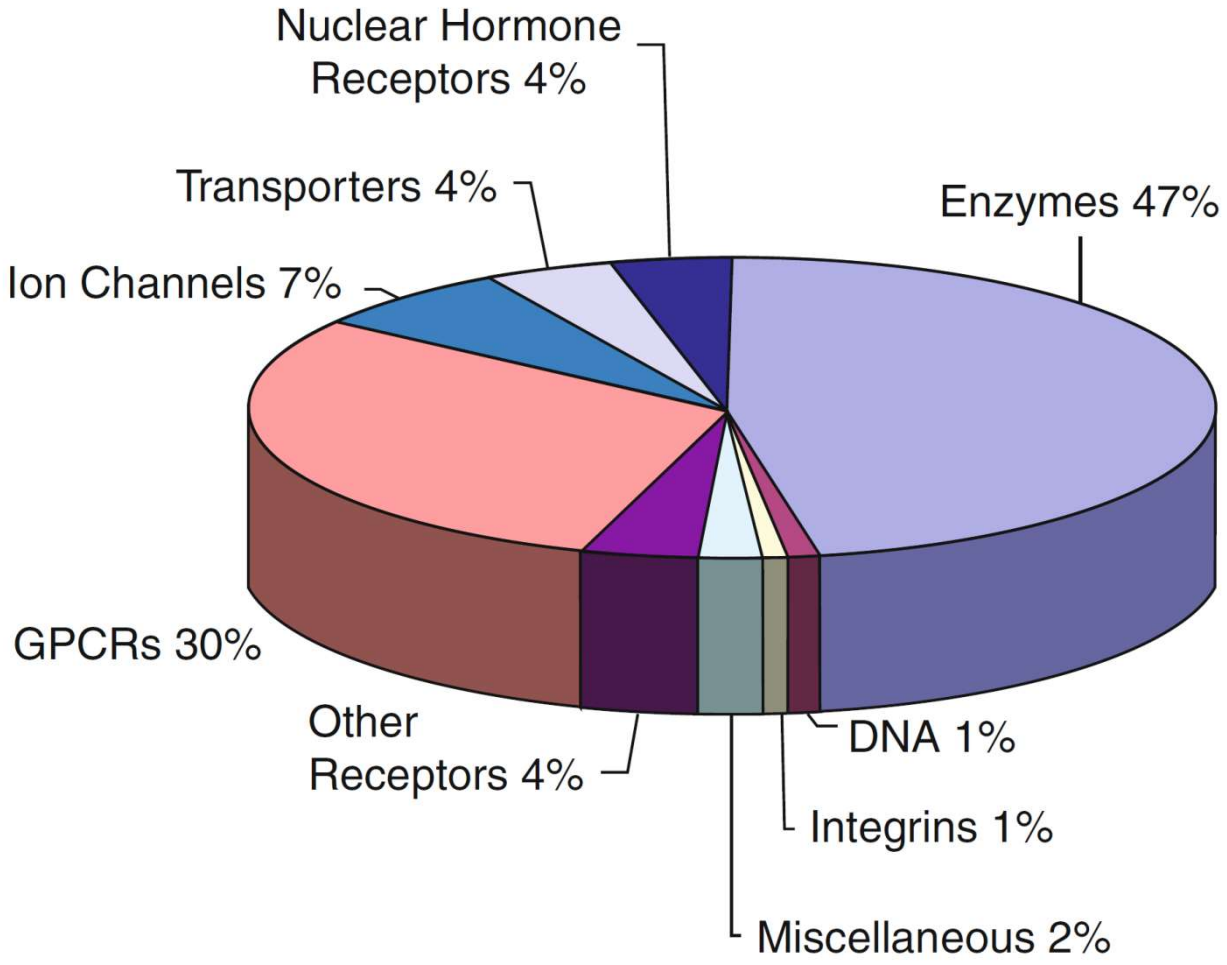
Pharmokinetics and Pharmcodynamics

- **Pharmacodynamics** or “what the drug does to the body” :
  - Mechanisms of drug action, interaction with target recepetor or enzyme, mode of inhibition, allostery. Concepts such as affinity, selectivity, agonist, antagonist,…
- **Pharmacokinetics** or“what the body does to the drug”:
  - Processes of drug absorption, transport and metabolization. Concepts such as half-life, solubility, permeability, therapeutic index…

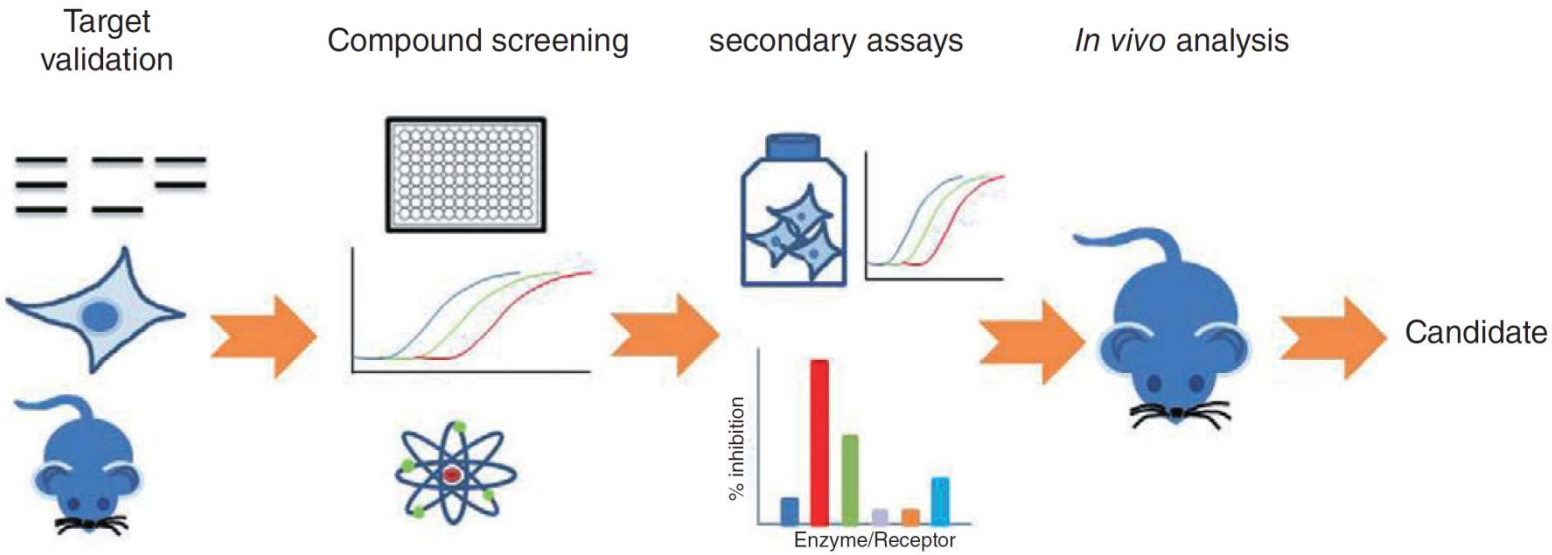
Drug targets

- **Enzymes:** There are many different types of enzymes in the human body. They are required for a variety of functions. Drugs can interact with enzymes to modulate their enzymatic activities.
- **Intracellular Receptors:** These receptors are in the cytoplasm or nucleus. Drugs or endogenous ligand molecules have to pass through the cell membrane (a lipid bilayer) to interact with these receptors. The molecules must be hydrophobic or coupled to a hydrophobic carrier to cross the cell membrane.
- **Cell Surface Receptors:** These receptors are on the cell surface and have an affinity for hydrophilic binding molecules. Signals are transduced from external stimuli to the cytoplasm and affect cellular pathways via the surface receptors. There are three main super families (groups) of cell surface receptors: G-protein coupled receptors, ion channel receptors, and catalytic receptors using enzymatic activities.
- **Nucleic Acids:** DNA and RNA support genetic information and its replicaion and translation. NA drugs can be groove binders, intercalators, chain terminators or alkylating agents.

The drug targets



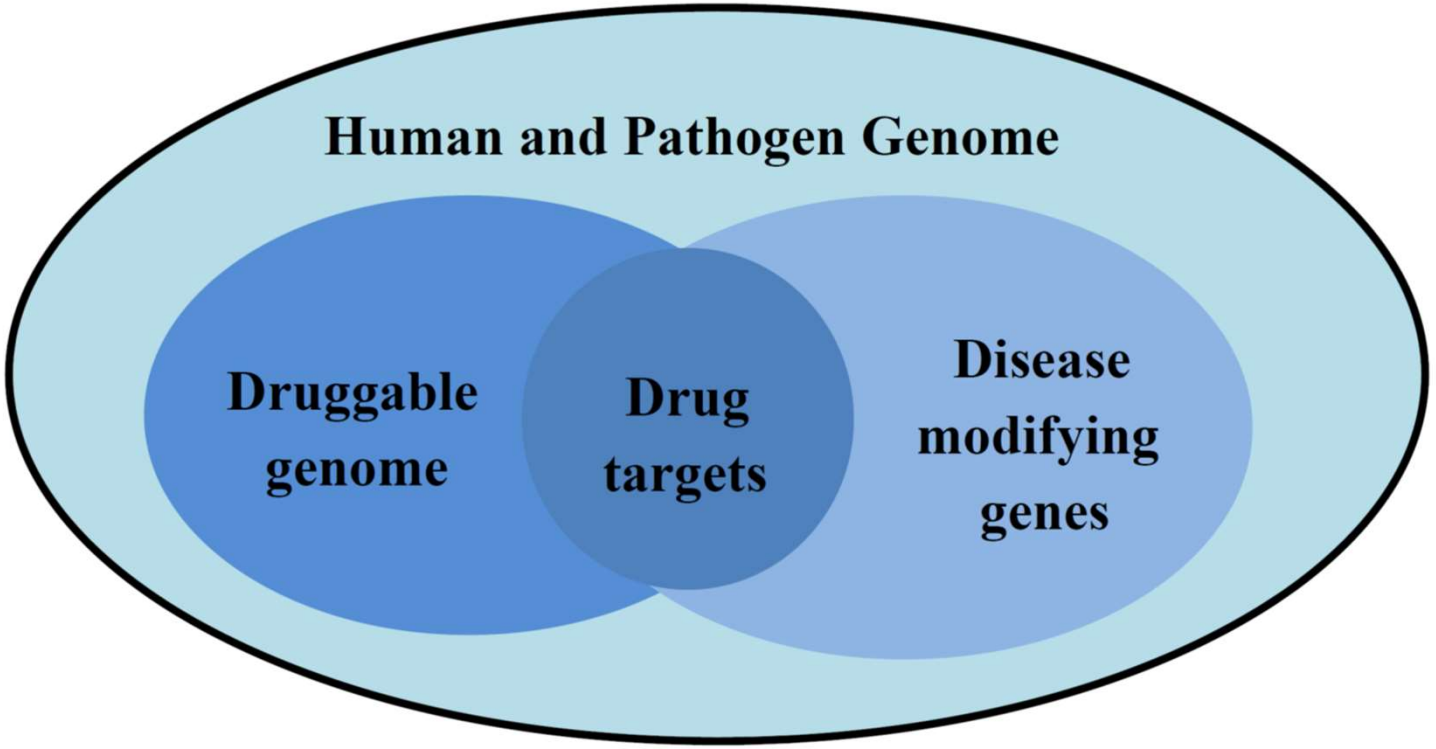
The early DD phase



- Genetic, cellular and *in vivo* experimental models to identify and validate target
- HTS & selective library screens; structure based design
- Reiterative directed compound synthesis to improve compound properties
- *in vitro* & *ex vivo* secondary assays (mechanistic)
- Selectivity & liability assays
- Compound pharmacology
- Disease efficacy models
- Early safety & toxicity studies
- Preclinical safety & toxicity package

Brit.J.Pharm. (2011) **162**:1239–1249

The “druggable” genome



~5000 druggable genes ?

Evaluating druggability

Druggability – the ability of a macromolecular target to bind small drug-like molecules

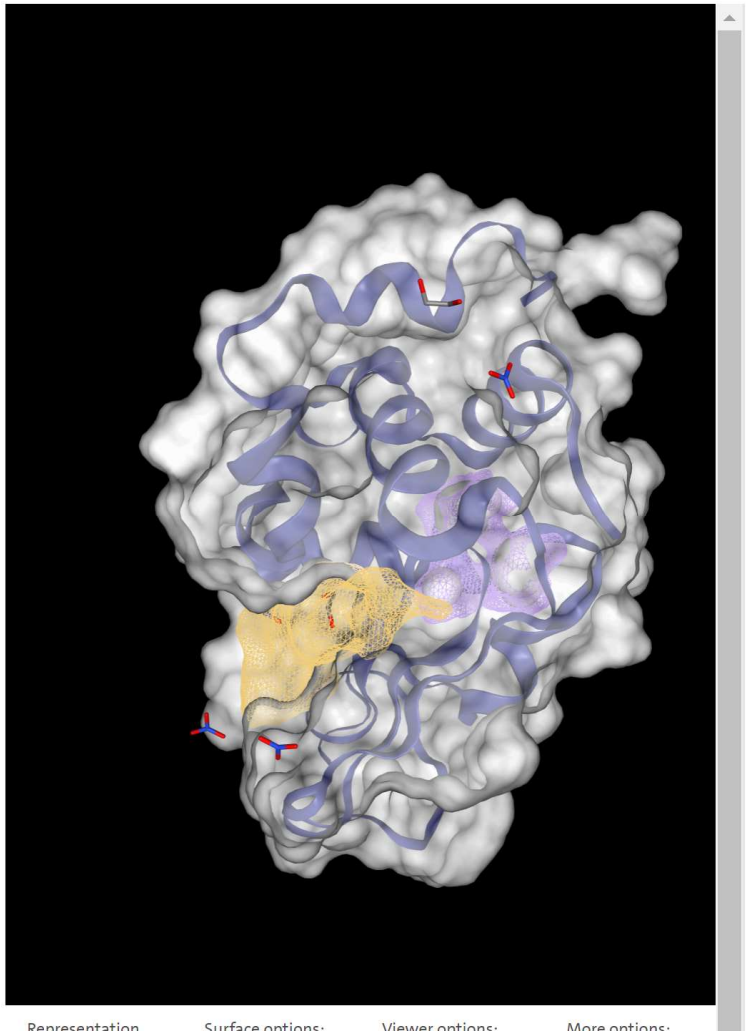
Ways to evaluate druggability:

- **Precedence –** target is a member of a family known to bind small moleculers
- **Structural analysis –** 3D structural analysis of the target aiming at the idenfication of structural features relevant to binding:
  - Identification of cavities or pockets in the structure
  - Calculation of physico-chemical properties of pockets
  - Assessing fitness of structural properties to a training set of druggable targets (machine-learning methods)
- **Feature based –** using other properties of the target, like those that can be derived from the aminoacid sequence,

"Out of the nearly 20,000 protein-coding genes in the human genome, approximately 3,000 are estimated to be part of the druggable genome, the subset of genes expressing proteins with the ability to bind drug-like molecules. Yet, less than ten percent of the druggable proteins are currently targeted by FDA-approved drugs" - NIH, Illuminating the Druggable Genome

DoGSiteScorer @ Protein+

(previsão de “pockets” e drugabilidade)



Ligands Pockets

Pockets

Cookies note

ProteinsPlus saves cookies in your browser. Cookies are used for temporary caching personal pocket data up to seven days.

Ok

Pocket: Empty_Pocket_1

Show pocket grid:

Origin: GeoMine

DoGSiteScorer

Back

DoGSiteScorer is a grid-based method which uses a Difference of Gaussian filter to detect potential binding pockets - solely based on the 3D structure of the protein - and splits them into subpockets. Global properties, describing the size, shape and chemical features of the predicted (sub)pockets are calculated. Per default, a simple druggability score is provided for each (sub)pocket, based on a linear combination of the three descriptors describing volume, hydrophobicity and enclosure. Furthermore, a subset of meaningful descriptors is incorporated in a support vector machine (libsvm) to predict the (sub)pocket druggability score (values are between zero and one). The higher the score the more druggable the pocket is estimated to be. [1]

1. A. Volkamer, D. Kuhn, T. Grombacher, F. Rippmann, M. Rarey. Combining global and local measures for structure-based druggability predictions. J. Chem. Inf. Model. 2012,52,360-372.

Result

Click on the plus to see your selected parameters:

Show 25 entries Search:

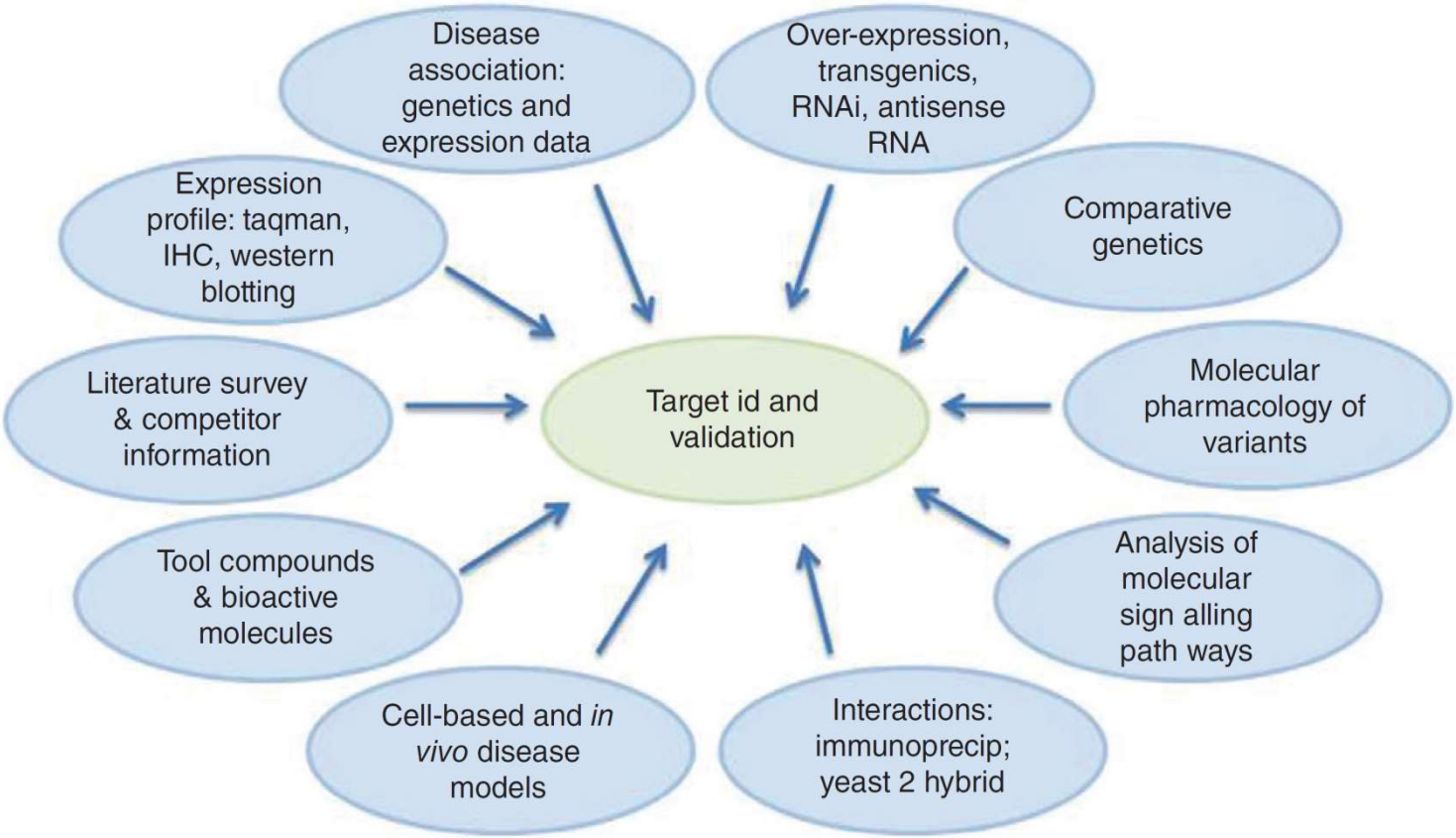
		Name	Volume Å³	Surface Å²	Drug Score	Simple Score	Additional information
		P_0	368.58	462.49	0.64	0.26	Click here to show/hide
		P_1	166.53	175.44	0.5	0.0	Click here to show/hide

Showing 1 to 2 of 2 entries Previous 1 Next

Download Start over Dogsite

https://proteins.plus/

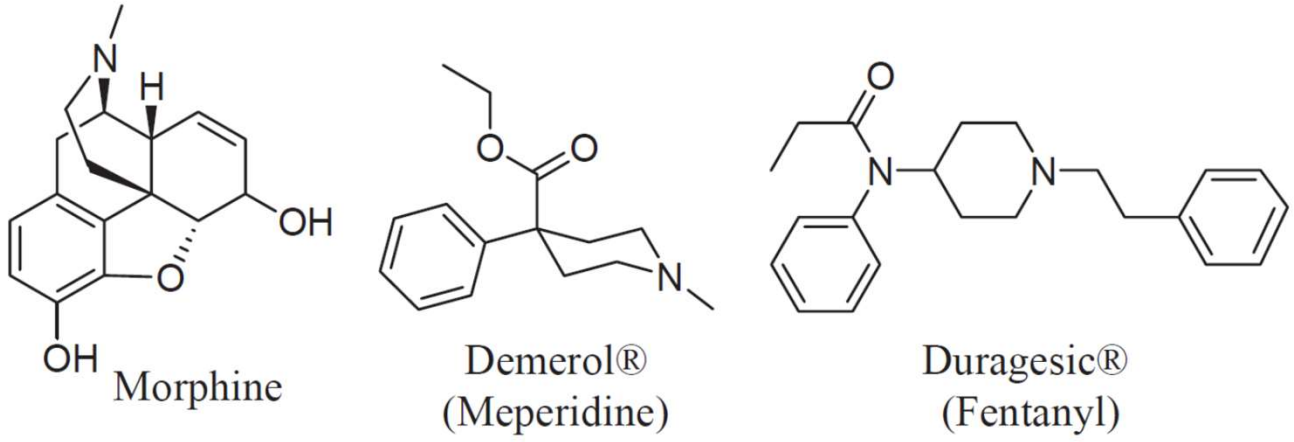
Target selection



Brit.J.Pharm. (2011) **162**:1239–1249

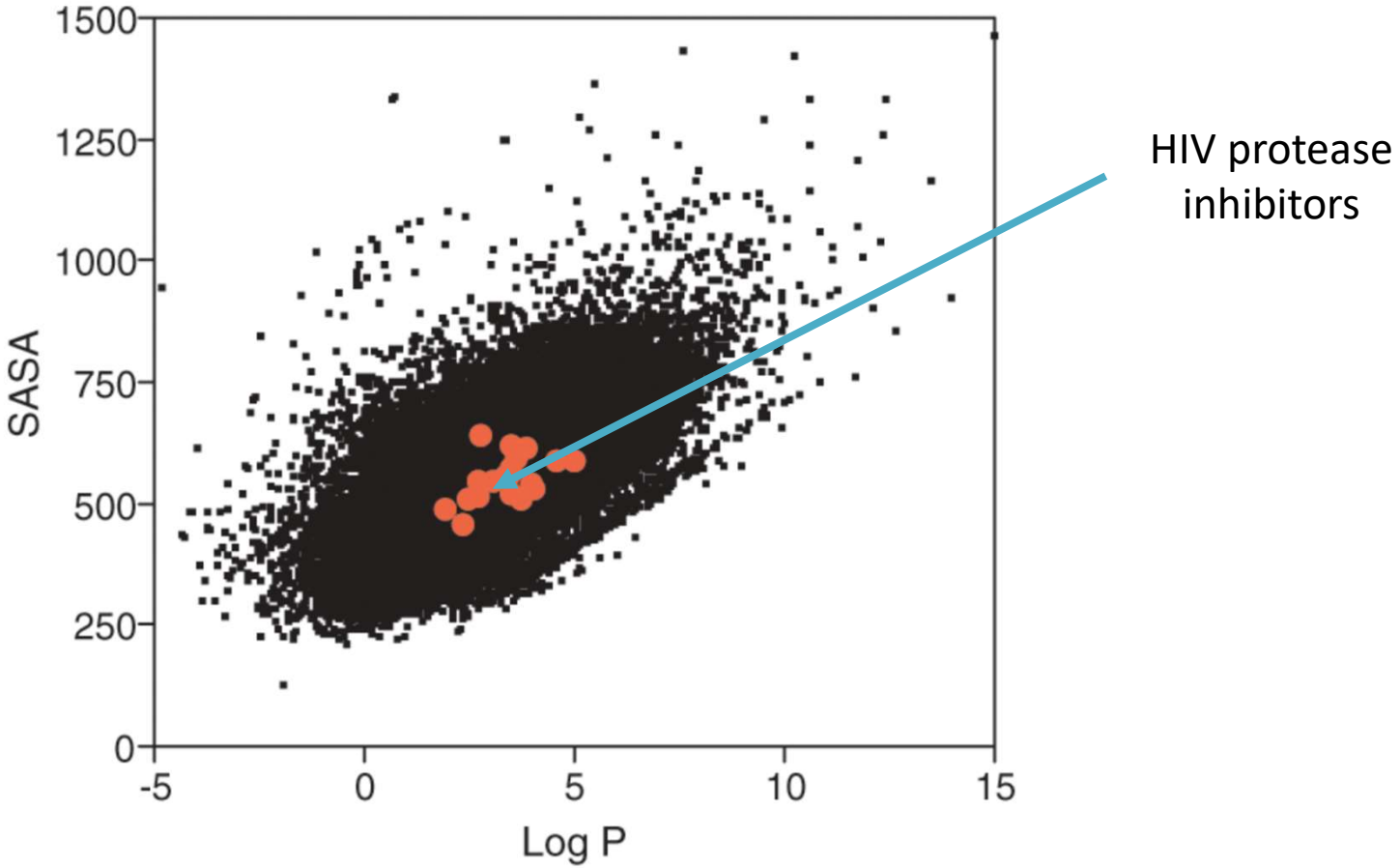
Choosing the test compounds

- There are over 70 million compounds registered in the Chemical Abstacts Service (CAS)
- Filtering this compounds for drug-likeness will still leave a big number
- Structural analogy is often not required for binding the same target
- First-in-class drugs are more profitable, but much harder to discover
- High Throughput Screening (HTS), real or virtual (*in sillico)* may be used to deal with a large subset of the chemical space



Structurally diverse μ-opiod receptor agonists

In Silico filtering by descriptor



The 70k compounds in the Maybridge catalog are presented as dots of **logP** *versus* **Solvent Accessible Surface Area** (SASA). Know HIV-1 protease inhibitors (red dots) cluster on a narrow region of SASA and logP.

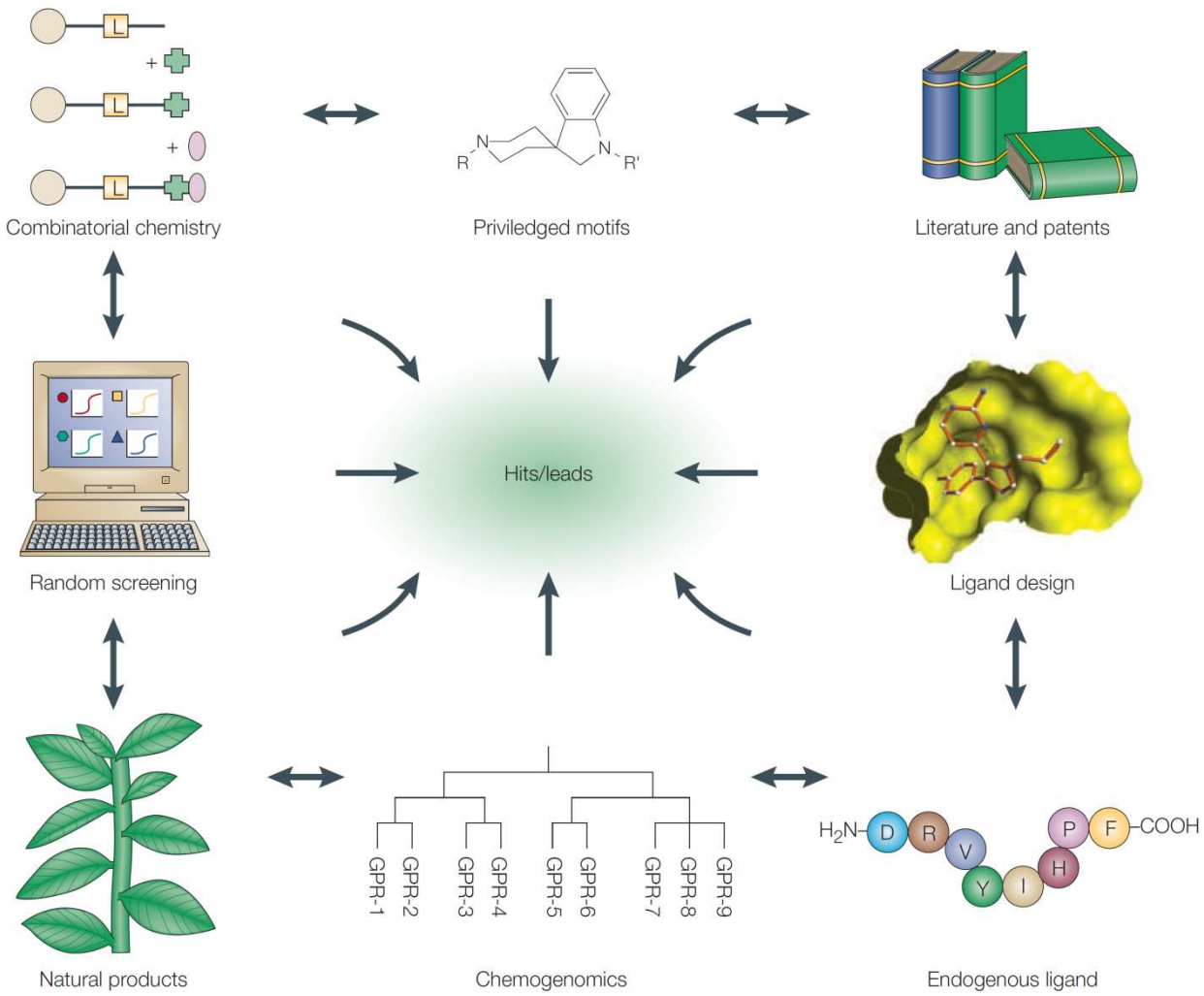
Jorgensen(2004) *Science* **303**:1813

Hits and leads

- Hit – compound which has the desired activity in a compound screen and whose activity is confirmed upon retesting

- Lead – a hit compound with sufficient potency, selectivity, drug-likeness, bioavailability, and *in vivo* effect to be selected as drug candidate

Generation of Hits/Leads

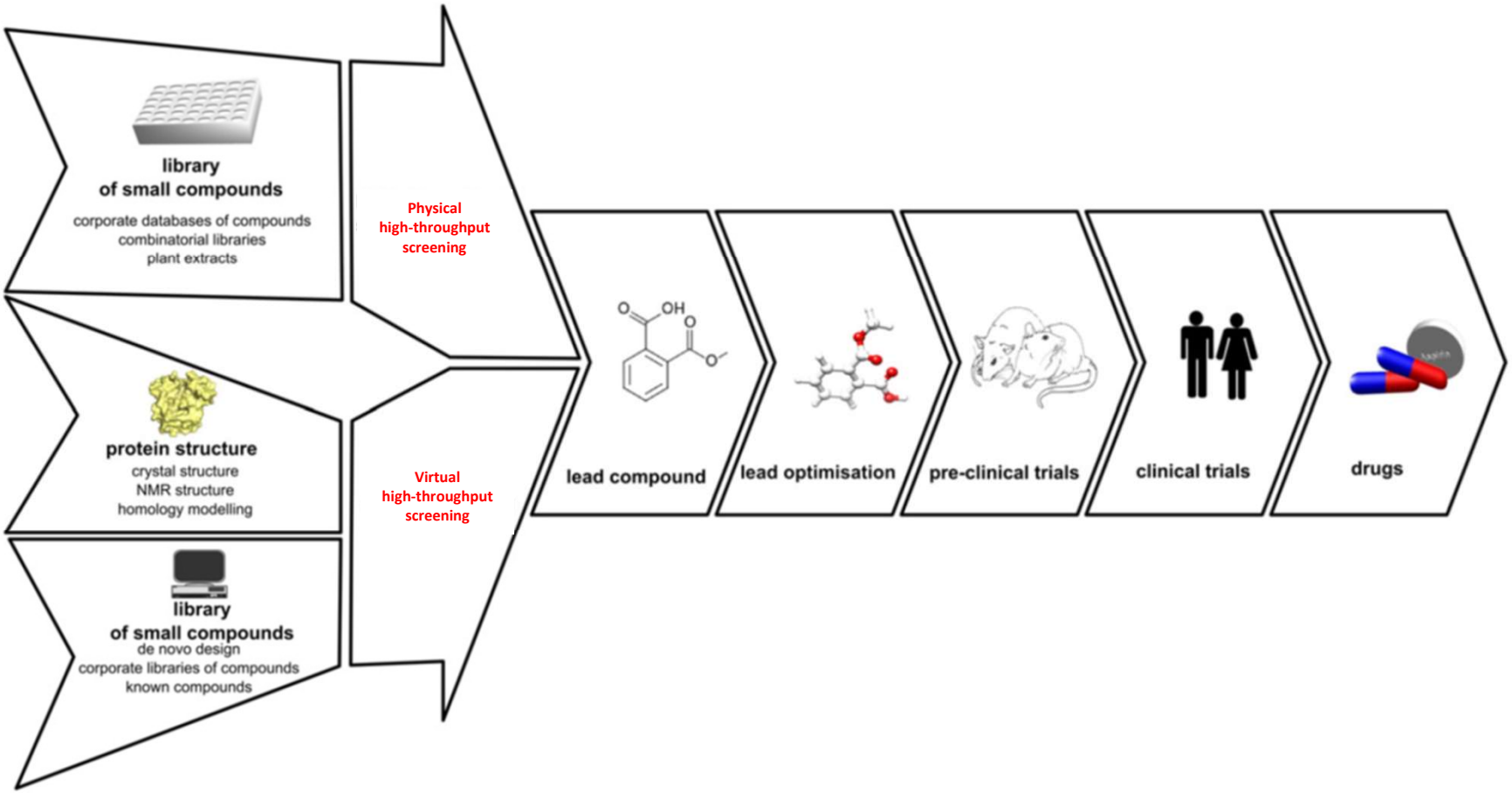


Screening strategies

Screen	Description	Comments
High throughput	Large numbers of compounds analysed in a assay generally designed to run in plates of 384 wells and above	Large compound collections often run by big pharma but smaller compound banks can also be run in either pharma or academia which can help reduce costs. Companies also now trying to provide coverage across a wide chemical space using computer assisted analysis to reduce the numbers of compounds screened.
Focused screen	Compounds previously identified as hitting specific classes of targets (e.g. kinases) and compounds with similar structures	Can provide a cheaper avenue to finding a hit molecule but completely novel structures may not be discovered and there may be difficulties obtaining a patent position in a well-covered IP area
Fragment screen	Soak small compounds into crystals to obtain compounds with low mM activity which can then be used as building blocks for larger molecules	Can join selected fragments together to fit into the chemical space to increase potency. Requires a crystal structure to be available
Structural aided drug design	Use of crystal structures to help design molecules	Often used as an adjunct to other screening strategies within big pharma. In this case usually have docked a compound into the crystal and use this to help predict where modifications could be added to provide increased potency or selectivity
Virtual screen	Docking models: interogation of a virtual compound library with the X-ray structure of the protein or, if have a known ligand, as a base to develop further compounds on	Can provide the starting structures for a focused screen without the need to use expensive large library screens. Can also be used to look for novel patent space around existing compound structures
Physiological screen	A tissue-based approach for determination of the effects of a drug at the tissue rather than the cellular or subcellular level, for example, muscle contractility	Bespoke screens of lower throughput. Aim to more closely mimic the complexity of tissue rather than just looking at single readouts. May appeal to academic experts in disease area to screen smaller number of compounds to give a more disease relevant readout
NMR screen	Screen small compounds (fragments) by soaking into protein targets of known crystal or NMR structure to look for hits with low mM activity which can then be used as building blocks for larger molecules	Use of NMR as a structure determining tool

Brit.J.Pharm. (2011) **162**:1239–1249

Finding Hits with HTS



Finding Hits with Physical HTS

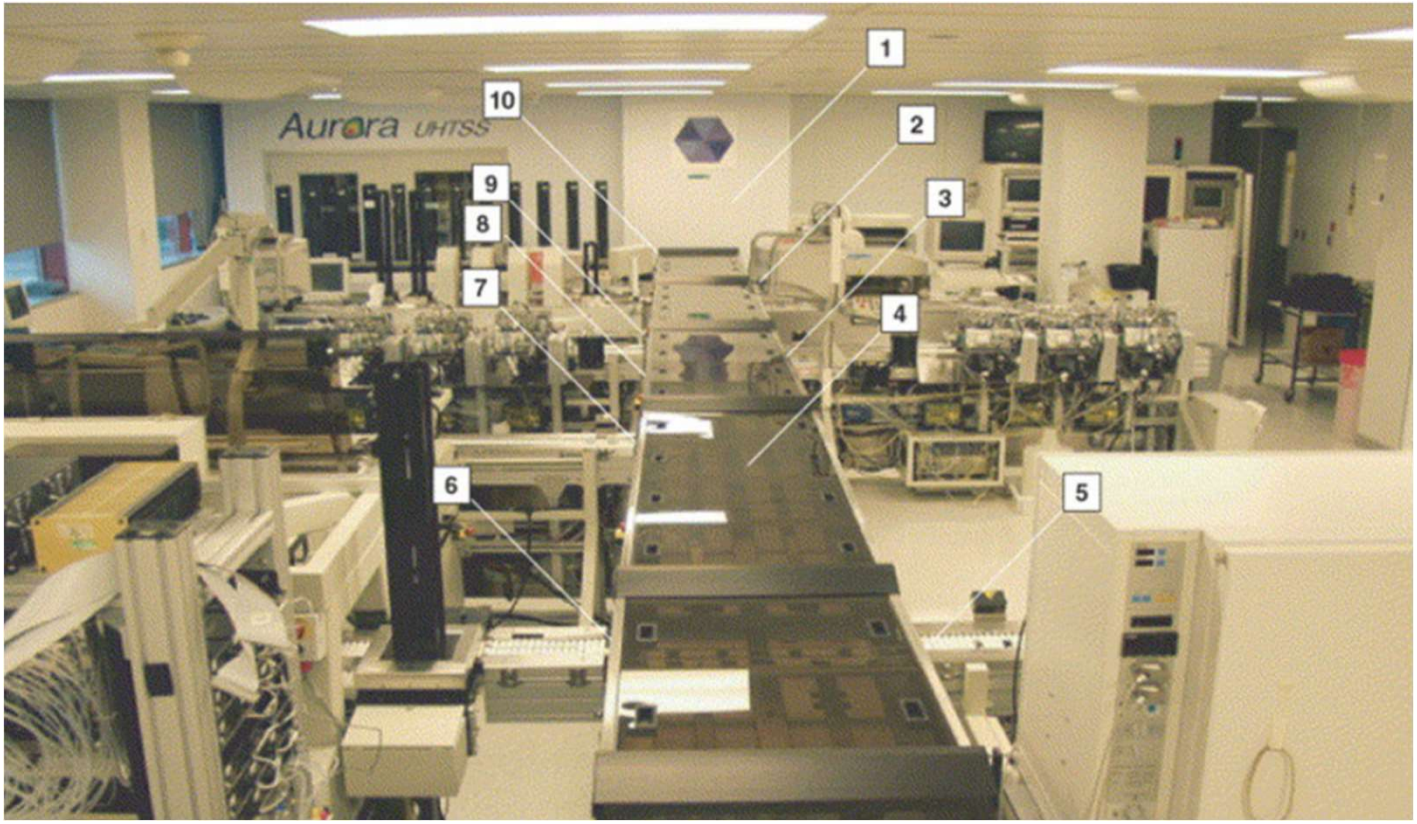
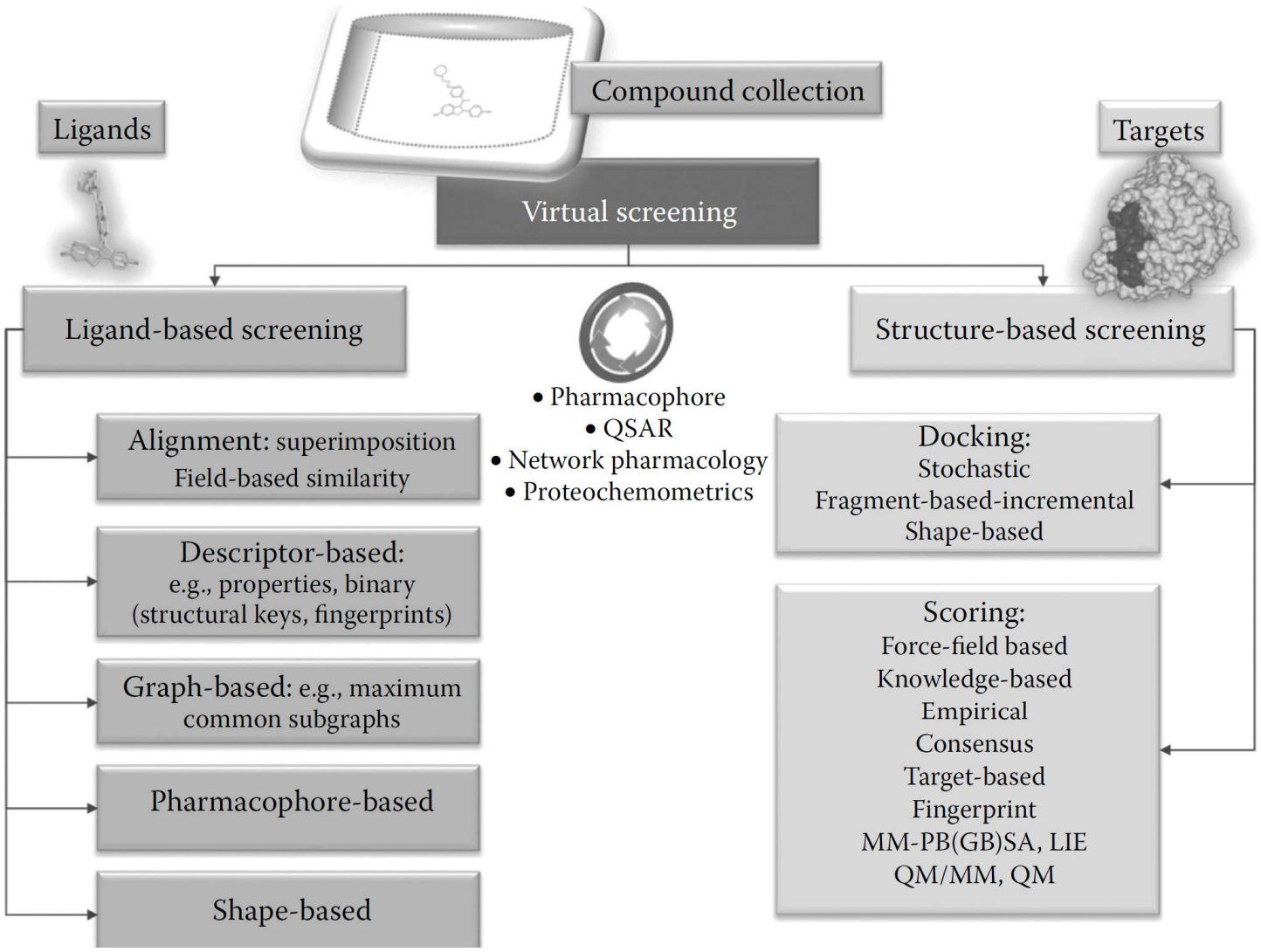
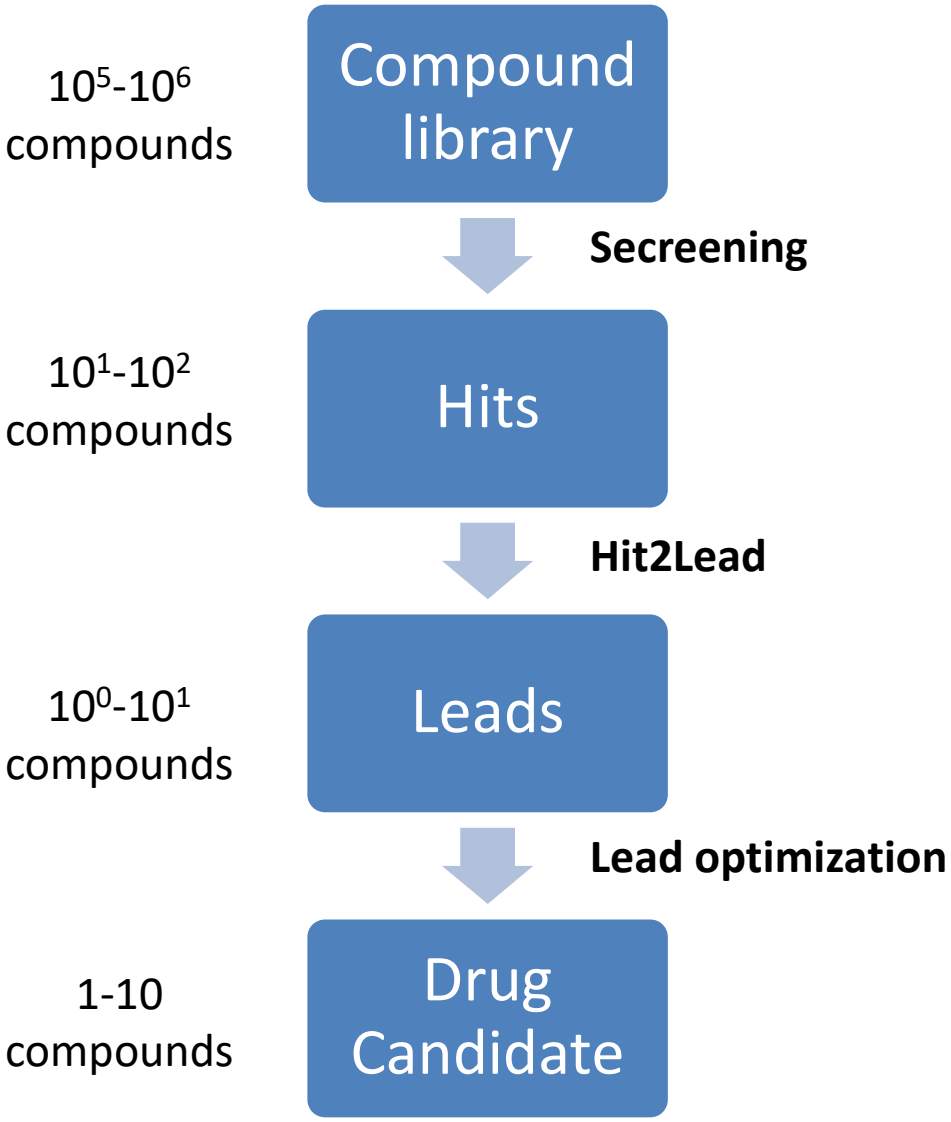


FIGURE 2.17 The automated uHTS system at Bristol-Myers Squibb. Integral components and subsystems are shown; (1) Compound store, (2) Hit-picking robot, (3) 3456 reagent dispensing robot, (4) Transport, (5) Incubators, (6) Piezo-electric distribution robot, (7) Topology compensating plate reader, (8) 1536 reagent dispensing robot, (9) Automated plate replicating system, (10) High-capacity stacking system. *Source: Reprinted from Cacace, A.; Banks, M.; Spicer, T.; Civoli, F.; Watson, J. An ultra-HTS process for the identification of small molecule modulators of orphan G-protein-coupled receptors. Drug Discovery Today, 8 (17), 785–792, copyright 2003, with permission from Elsevier.*

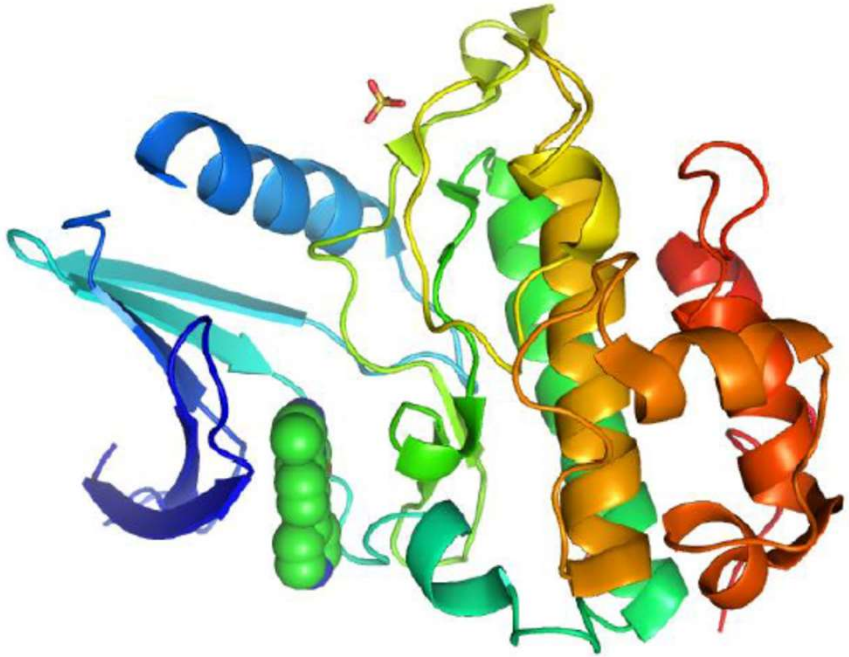
Finding Hits with Virtual HTS




10^5-10^6 compounds
Compound library
Secreening
10^1-10^2 compounds
Hits
Hit2Lead
10^0-10^1 compounds
Leads
Lead optimization
1-10 compounds
Drug Candidate

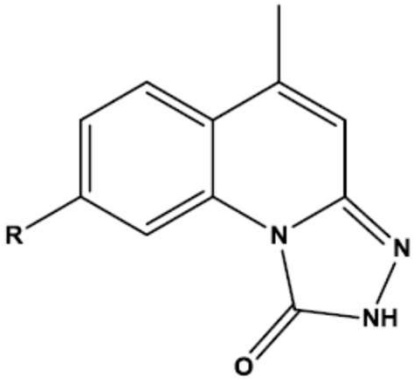
Hiy2Lead by Example: Checkpoint kinase inhibitor

Checkpoint kinase (ChK) is protein kinase that is activated in response to DNA damage and is involved in cell cycle arrest. This is a highly prospective target for the treatment of cancer (particularly breast cancer, Li-Fraumeni syndrome, but also other types of cancer).



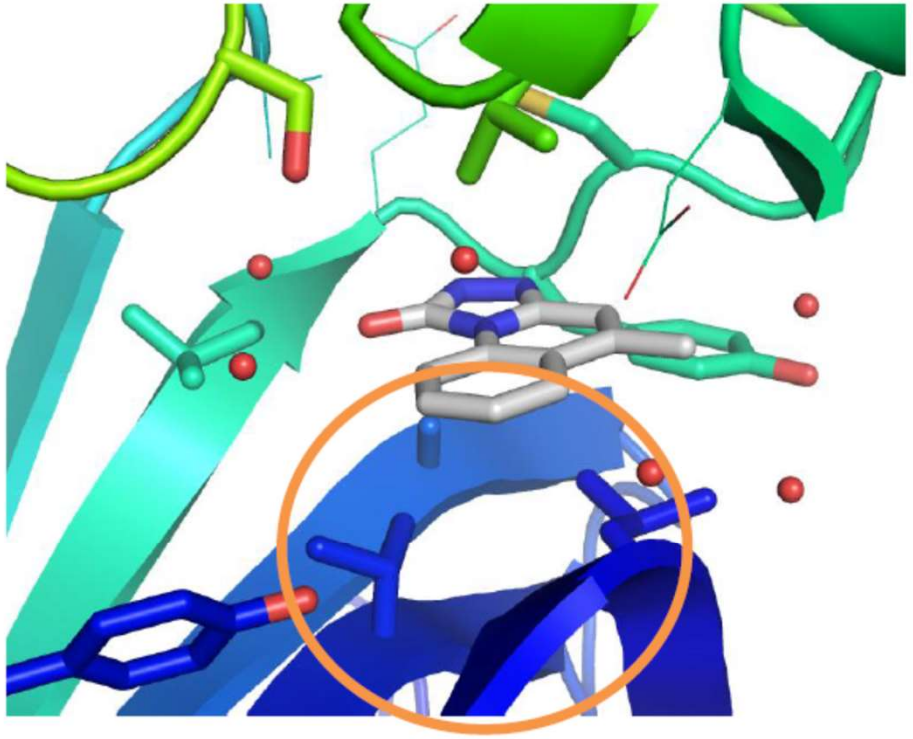
Discovery of a novel class of triazolones as Checkpoint Kinase inhibitors—Hit to lead exploration (AstraZeneca). DOI: 10.1016/j.bmcl.2010.07.015. PDB: 2BXD, 2BXI, 2BXE.

Hit2lead by example: Checkpoint kinase inhibitor



Hit, R = H

The hit on the left was found to be selective for the checkpoint kinase (Chk) among a collection of compounds tested with High-Troughput Screening (HTS).



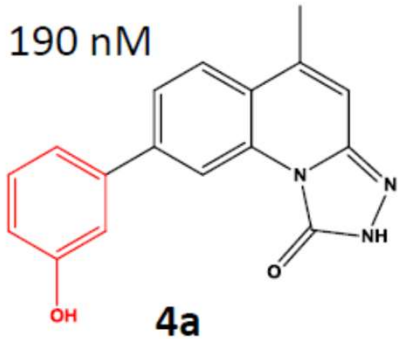
Synthesis of the derivates was made to fill the so-called sugar pocket.

PDB: 2BXD

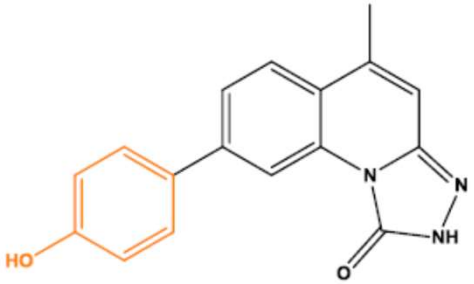
Discovery of a novel class of triazolones as Checkpoint Kinase inhibitors—Hit to lead exploration (AstraZeneca). DOI: 10.1016/j.bmcl.2010.07.015. PDB: 2BXD, 2BXI, 2BXE.

Hit2lead by example: Checkpoint kinase inhibitor

190 nM

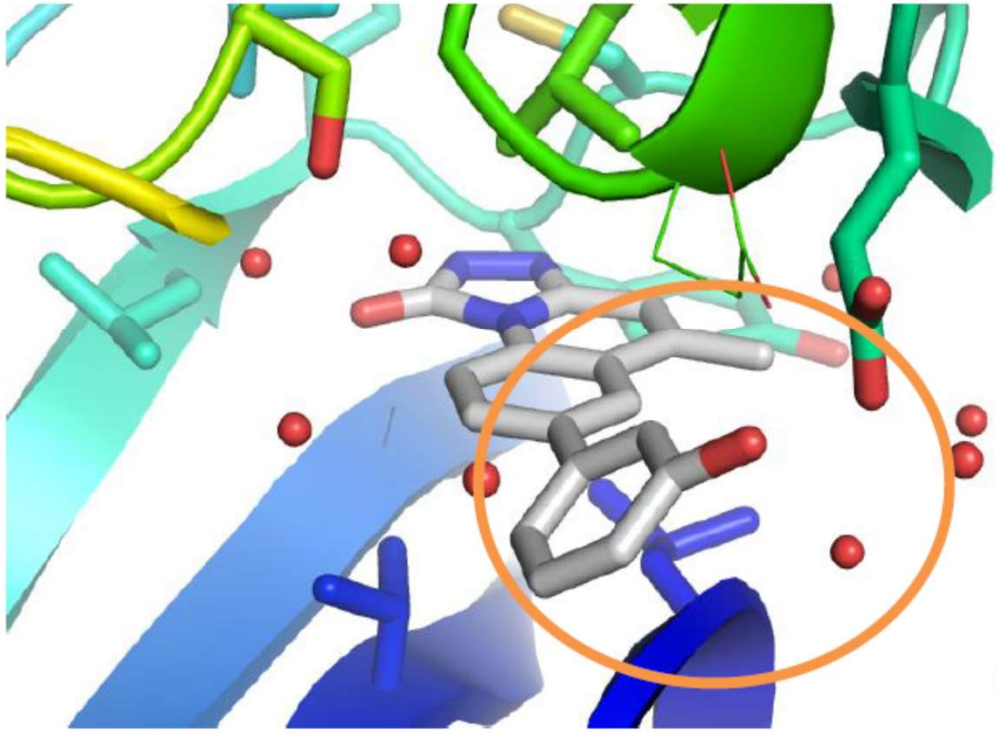


4a



4b

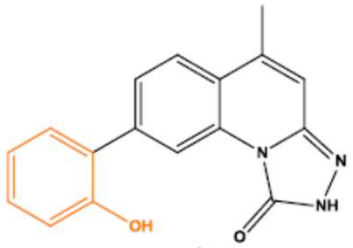
10 nM



4a and **4b** analogues filled the sugar pocket.

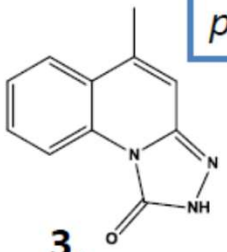
PDB: 2BXI

Hit2lead by example: Checkpoint kinase inhibitor

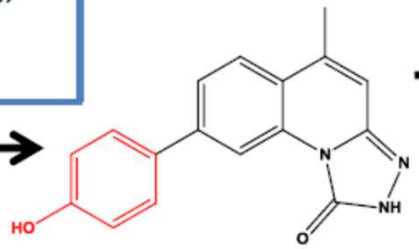


4a, 190 nM

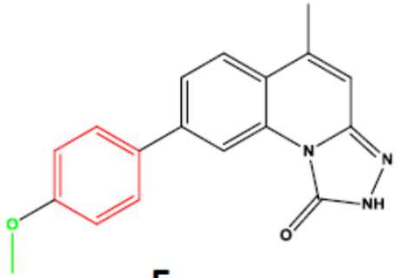
Filling the "sugar" pocket



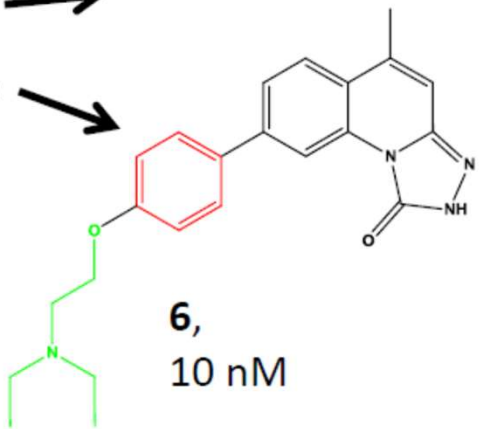
3, 800 nM



4b, 10 nM



5, 520 nM

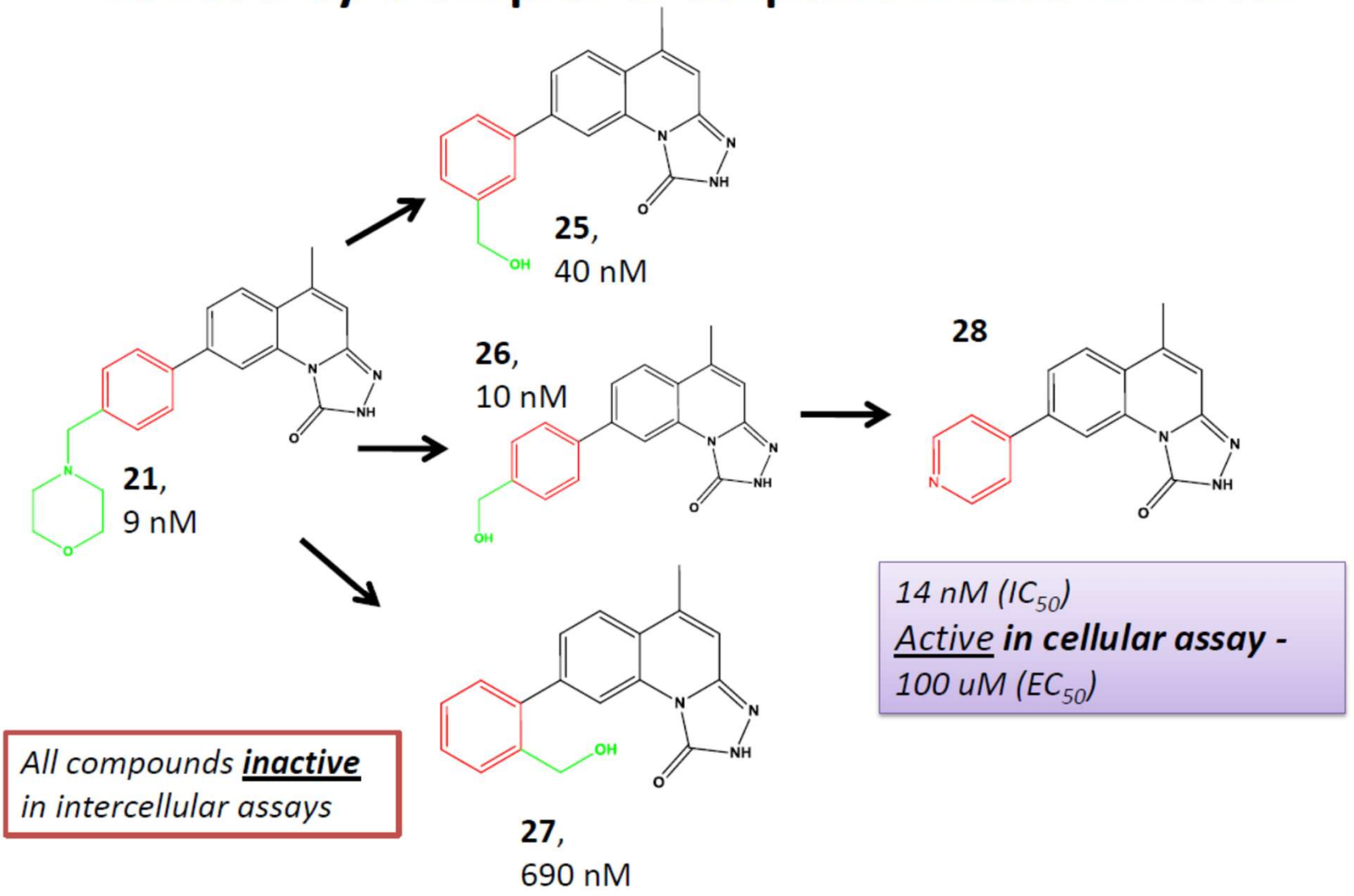


6, 10 nM

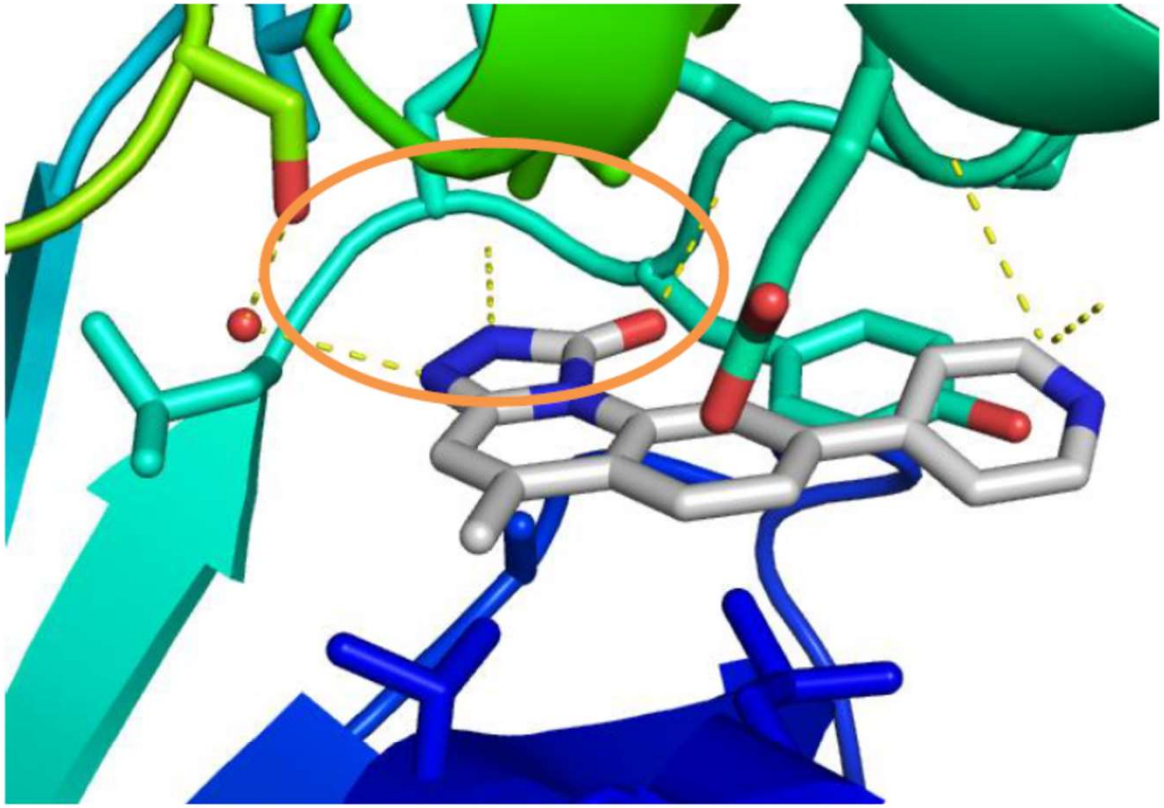
Further modifications - search for the more active compounds

*All compounds **inactive** in intercellular assays*

Hit2lead by example: Checkpoint kinase inhibitor

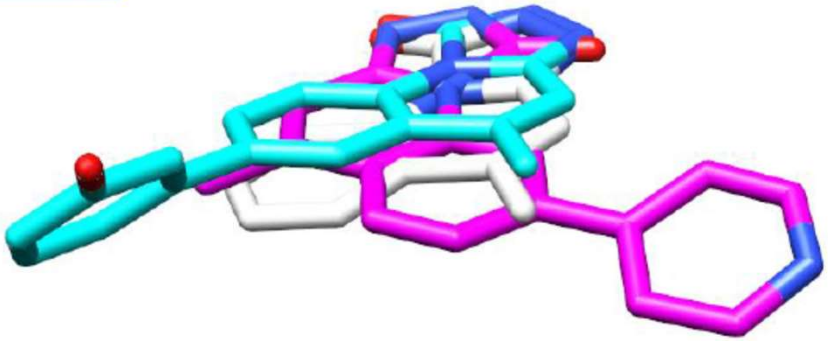


Hit2lead by example: Checkpoint kinase inhibitor



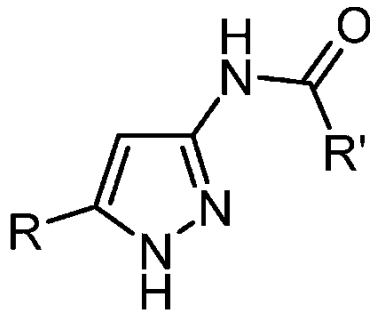
PDB: 2BXE

Superposition of the ligands from the three PDB structures. The resultant derivate binds in different way but forms almost the same pattern of hydrogen bonds.



Lead Optimizartion Example: CDK2 inhibitors

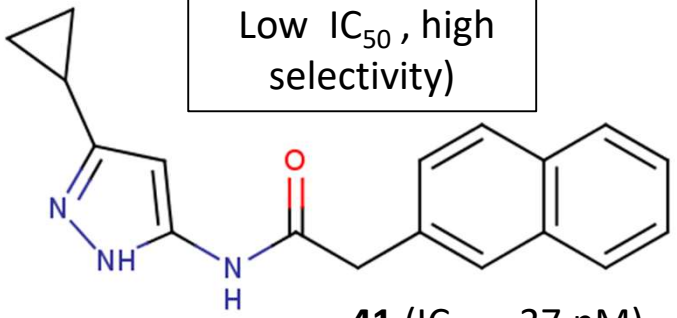
42 hit compounds



1-42

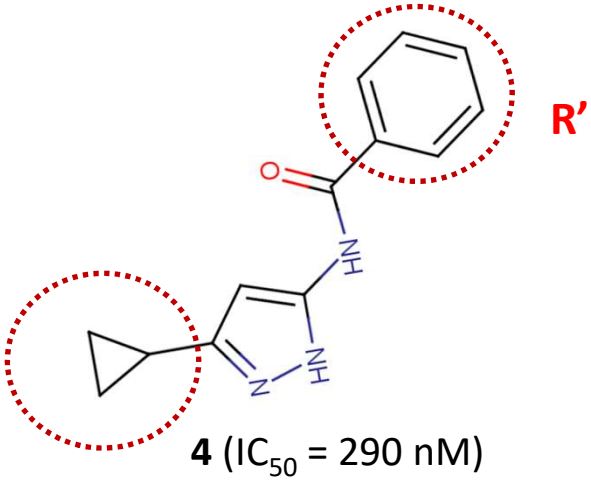
Select lead

Low IC_{50} , high selectivity)



41 (IC_{50} = 37 nM)

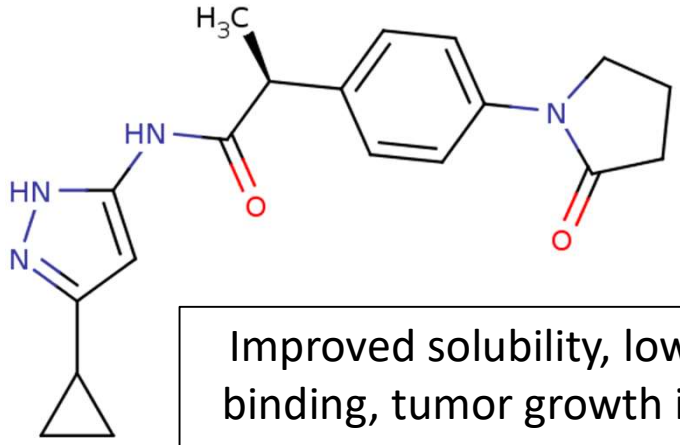
Optimize lead



R

R'

4 (IC_{50} = 290 nM)



Preclinical stage

Improved solubility, lower plasma protein binding, tumor growth inhibition in *in vivo* mouse model

J.Med.Chem (2005) **148**:2944

J. Med. Chem. **2005,** *48,* 2944−2956

3-Aminopyrazole Inhibitors of CDK2/Cyclin A as Antitumor Agents. 2. Lead Optimization

Paolo Pevarello,*[,†] Maria Gabriella Brasca,[†] Paolo Orsini,[†] Gabriella Traquandi,[†] Antonio Longo,[†] Marcella Nesi,[†] Fabrizio Orzi,[†] Claudia Piutti,[†] Pietro Sansonna,[†] Mario Varasi,[†] Alexander Cameron,[†] Anna Vulpetti,[†] Fulvia Roletto,[‡] Rachele Alzani,[‡] Marina Ciomei,[‡] Clara Albanese,[‡] Wilma Pastori,[‡] Aurelio Marsiglio,[‡] Enrico Pesenti,[‡] Francesco Fiorentini,[⊥] Jim R. Bischoff,[‡,§] and Ciro Mercurio[‡]

https://github.com/pjmartel/teaching/raw/gh-pages/mmdf/tutorials/pymol_tutorial.pdf

2

THE EVOLUTION OF CLASSICAL DRUG DESIGN

Early beginings

- Eber Papyrus, an Egyptian document which is one of the oldest known medical texts (1550 B.C.)
- Covers subjects like the heart, respiratory disease, cancer, mental diseases, prediction and prevention of pregnancy, intestinal disease, abscesses, skin problems, etc.…
- Practical recipes mingle with enchantments and rites to exorcise demons ….
- Example: inhalation of the smoke of heated plants for the treatment of asthma

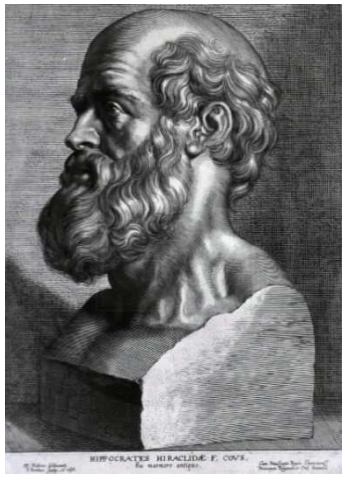
Fragmento do Papiro de Ebers

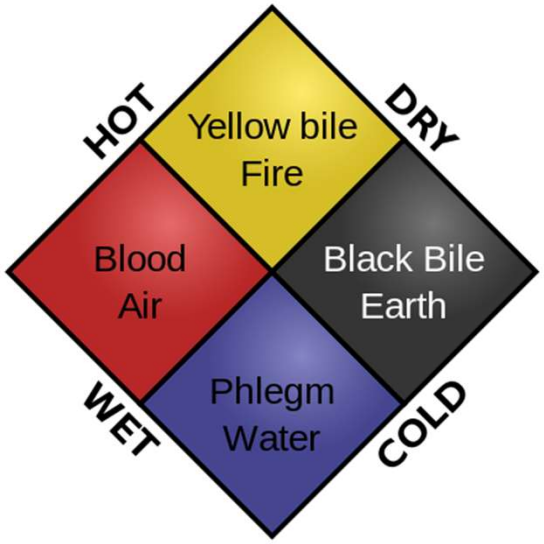
The Eber papyrus

- The Eber Papyrus includes around 800 medical “recipes”.
- Recipes for appeasing the gods…
- Empiric knowledge and common sense lead to the discovery of many plant extracts with medicinal properties which are still in use today…
- Opiate alkaloids, ephedrine, cannabis, etc.…

Greek Medicin

- Non-theistic interpretation
- Fundamentally theoretical
- Theory of the 4 humors
- Very little emphasis on medicinal plants





Traditional medicines

- Chinese
- Indian
- Arabic

Still practiced to this day. They have provided many compounds and active principles of pharmacological interest. Mix of practical knowledge with theories of disease and organism “equilibrium” based on scientifically unsound concepts like “energies”, “fluxes”, “chakras”, etc…

Paracelso

"Just as women can be recognized and appraised on the basis of their shape, drugs can easily be identified by appearance. God has created all diseases, and he has also created an agent or drug for every disease. They can be found everywhere in nature, because nature is the natural pharmacy.."
-- "Doctrine of Signatures", Paracelsus

Paracelso (1493-1541)

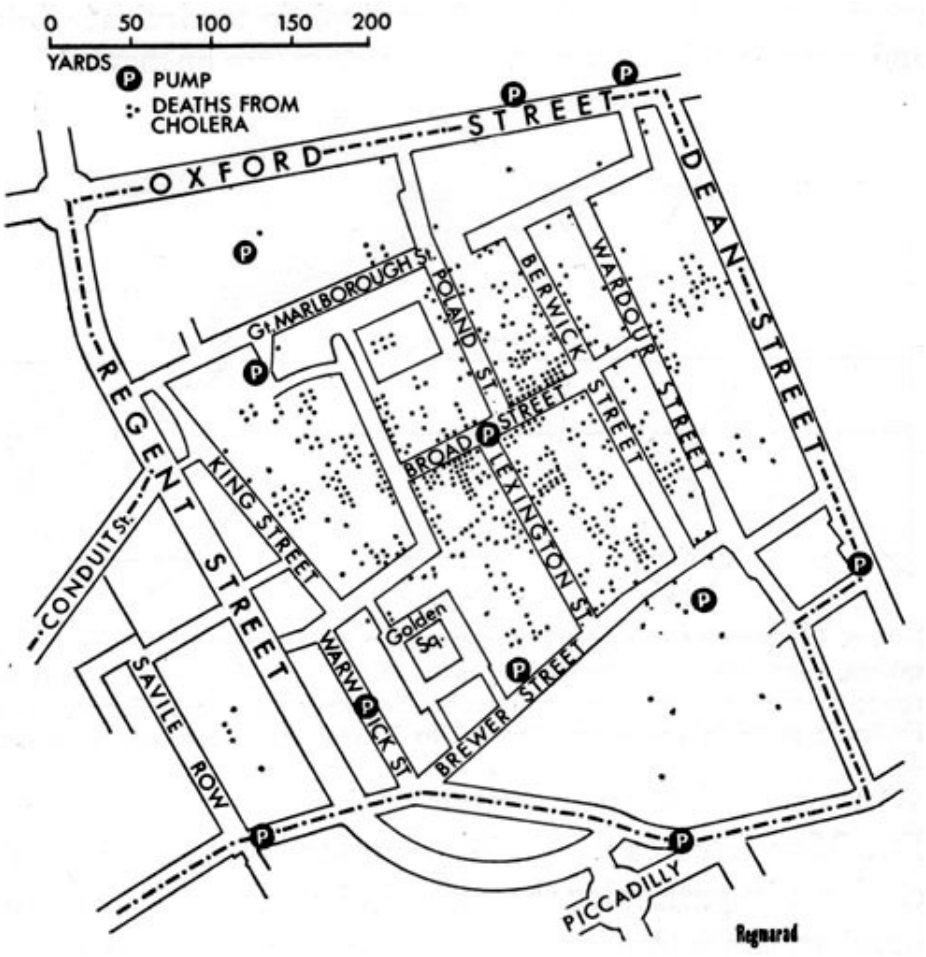
- He recognizes the existence of active principles in medical preparations
- Hypothesizes on the possibility of a match between active principle and disease
- Predicts the advent of rational drug design!

Success cases in disease prevention

- Vaccination (Jenner 1798, Pasteur 1864)
- Scurvy (Lind, 1763)
- Anti-septics (Lister, 1867)
- Control of the 1854 cholera outbreak 1854 (John Snow, 1854)

In spite of these achievements, therapeutic medicine is practically non-existent until late XIX century.

Cholera outbreak, 1854



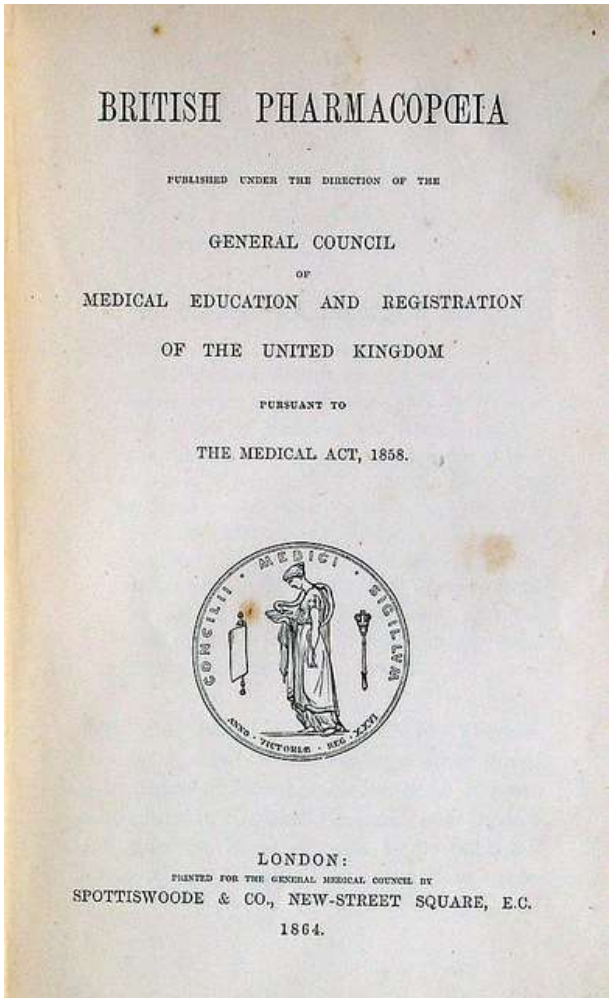
London Cholera Outbreak, 1854

Oliver Wendell Holmes (1809-1894)

“I firmly believe that if the whole Materia Medica, as now used, could be sunk to the bottom of the sea, it would be all the better for mankind – and the worse for the fishes”

-- Oliver Wendell Holmes, 1860

The status of pharmacology in the XIX century



BRITISH PHARMACOPŒIA

PUBLISHED UNDER THE DIRECTION OF THE

GENERAL COUNCIL

OF

MEDICAL EDUCATION AND REGISTRATION

OF THE UNITED KINGDOM

PURSUANT TO

THE MEDICAL ACT, 1858.

LONDON:

PRINTED FOR THE GENERAL MEDICAL COUNCIL BY

SPOTTISWOODE & CO., NEW-STREET SQUARE, E.C.

1864.

- The first edition of *British Pharmacopeia* (1864), lists 311 preparations:
  - 187 extracts plant, of only 9 are pure substances, and almost none of them with pharmacological action
  - 103 inorganic chemical substances: iron, iron sulfur, sodium bicarbonate and many toxic salts of arsenic, lead and mercury
  - Some synthetic compounds, like diethyl ether and chloroform
  - Some animal products

Dawning of the pharmaceutical industry (end of XIX century)

- Biomedicine & Pharmacology
- Development of Synthetic Chemistry
- European Chemical Industry
- Trade of medical supplies

All of these gave a decisive boost to the search for new synthetic compounds.

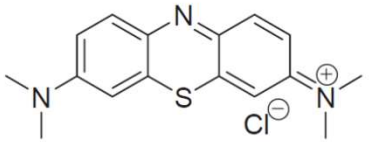
Pharmacology & Biomedicine

- Cell Theory (Virchow, 1858)
- Dorpat Pharmacological Institute (Buccheim, 1847)
- Physiology (Claude Bernard)
- Microbial theory of disease (Pasteur, 1878)
- Direct observation of pathogens (Koch)
- Beginning of chemotherapy (Paul Erlich)

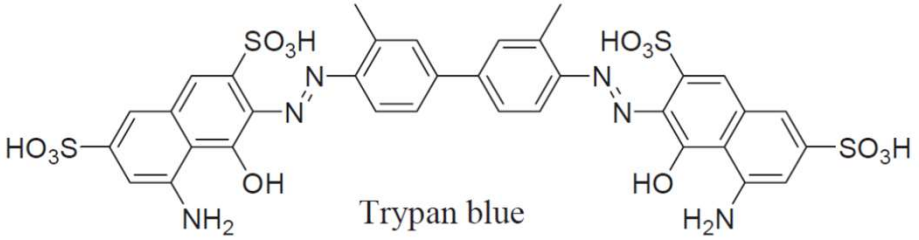
Ehrlich and the dawn of rational drug design

- Study of the selective affinity of dyes for the different cellular structures
- Searching for chemical compounds with therapeutic activity
- Concept of "receptor” e “magical bullet”
- Diphtheria anti-toxin
- Anti-syphilitic drugs (Salvarsan and Neosalvarsan)
- Theory of antibody action
- The first organized effort to modify the activity of a lead compound through systematic chemical modifications
- Ehrlich coined the word *chemotherapy*

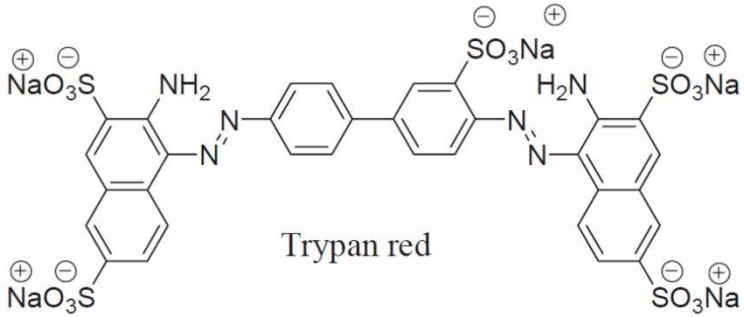
Paul Ehrlich
(1854-1915)



Methylene blue

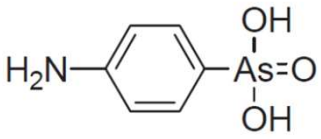


Trypan blue



Trypan red

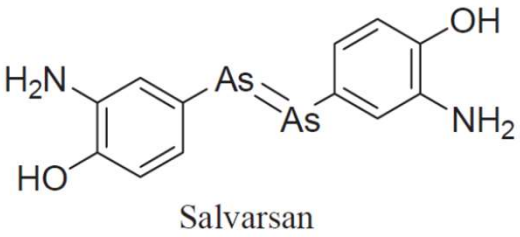
Dyes studied by Erlich



Atoxyl

African trypanossomiasis

Rabbit animal model of syphilis

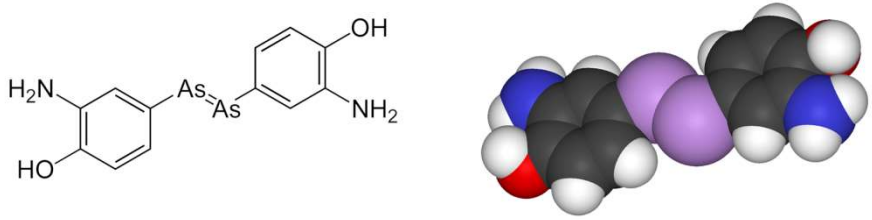


Salvarsan

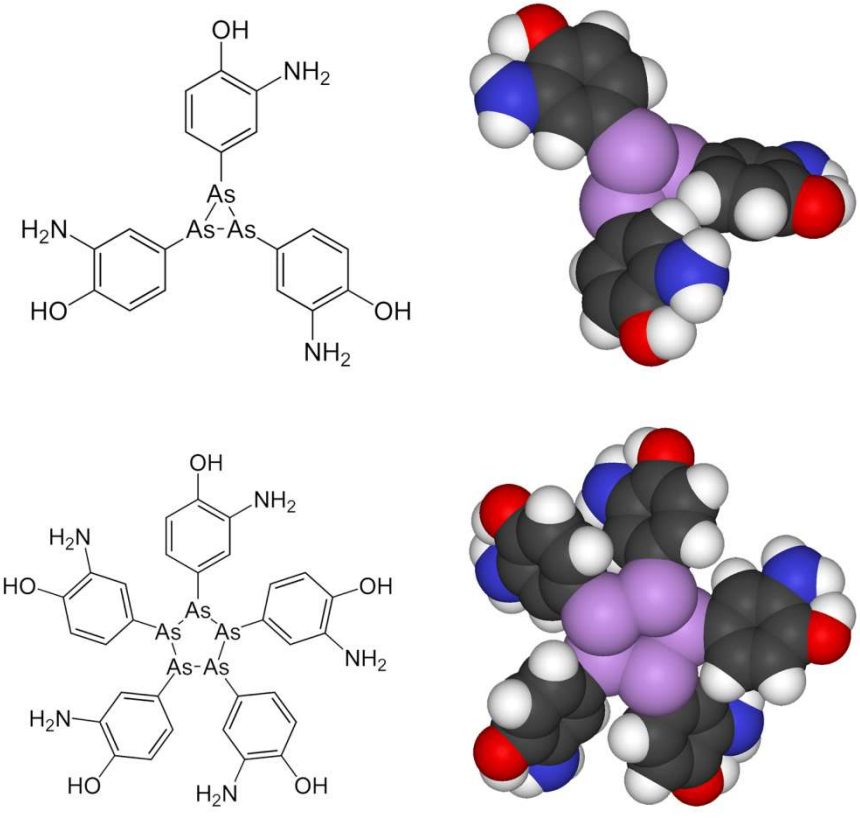
Syphilis

Arsphenamine (Salvarsan)

Before 2005



Structures resolved in 2005 using Mass Spectrometry

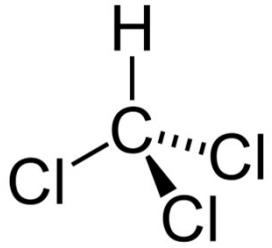


Lloyd NC, Morgan HW, Nicholson BK, Ronimus RS (2005). ".*Angew. Chem. Int. Ed. Engl.* **44** (6): 941–4

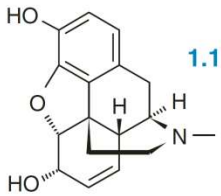
Synthetic chemistry and the development of new drugs

- The first synthetic compounds finding medical use were anesthetics rater than therapeutic agents
- Diethyl ether synthesis in 1540

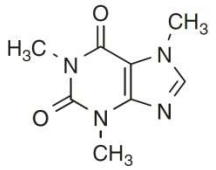
- Humphrey Davy synthesizes nitrous oxide (N_2O) in 1799
- These compounds where used as anesthetics starting from 1840, as well as chloroform



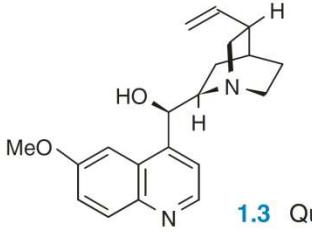
- The chemical industry of dyes gave a decisive boots to synthetic organic chemistry
- Valence theory and benzene structure (von Kekulé, 1865)



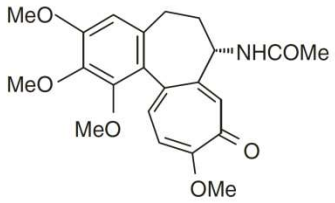
1.1 Morphine



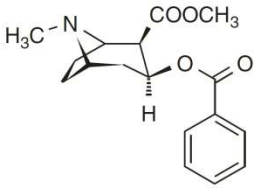
1.2 Caffeine



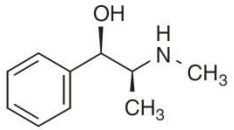
1.3 Quinine



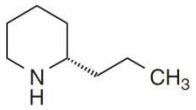
1.4 Colchicine



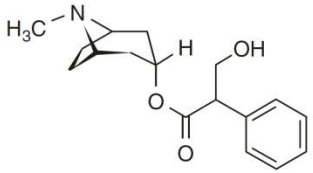
1.5 Cocaine



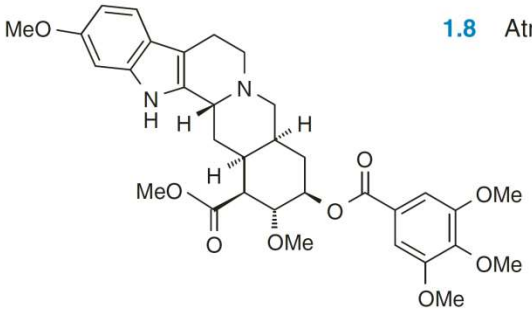
1.6 Ephedrine



1.7 Coniine



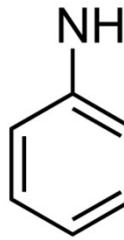
1.8 Atropine (racemate)



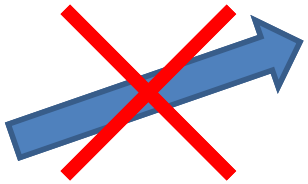
1.9 Reserpine

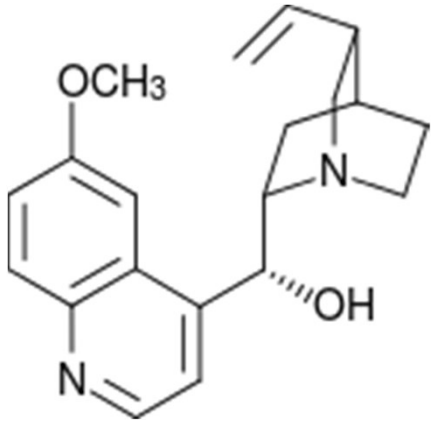
Serendipity: the discovery of *mauvein*

Perkin 1856

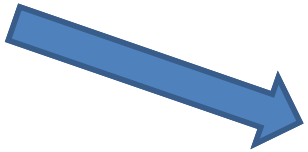


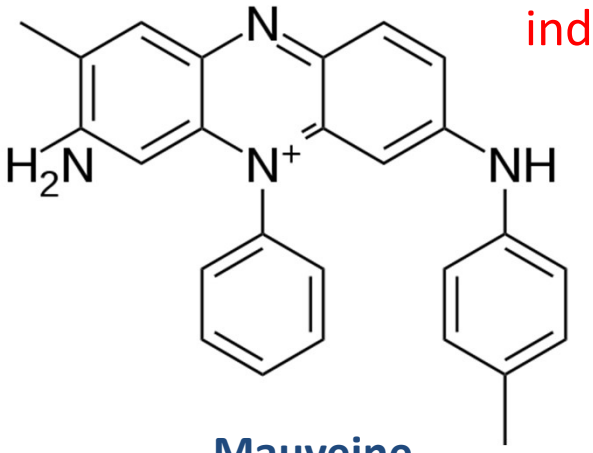
Anilin





Quinine



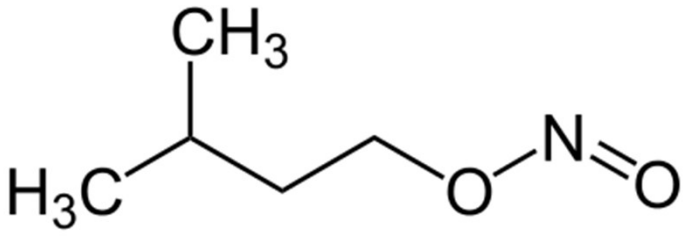


Mauveine

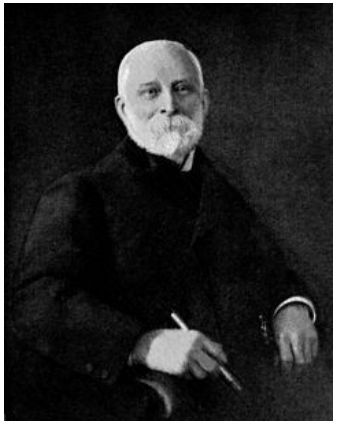
A failed attempt to synthesize quinine from aniline lead to the discovery of the first synthetic dye, mauveine!

Strong purple dye, its discovery kick started the synthetic dye industrv.

The first synthetic drug: amyl nitrite



Frederick Guthrie
(1833–1886)



Lauder Brunton
(1844-1916)

- Synthesis by Guthrie in 1859
- Very powerful vasodilator
- Used by Brunton in 1864 for treating angina pectoris
- 40 years would pass before another synthetic drug was created

Pharmacology at the turn of XX century

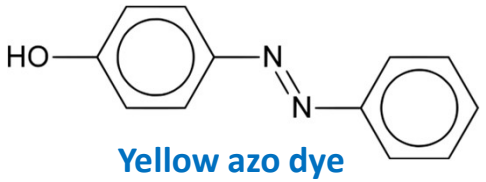
- Several convergent approaches:
- Animal models
- Target identification
- Advances in synthetic chemistry
- Molecular structure and bonding theories
- Birth of quantitative Enzymology
- First attempts at racional drug design

Synthetic chemistry dominates drug discovery, but the ideas of Fischer and Erlich raise interest in the analysis of the *targets* of pharmacologically active substances.

At this time, mechanism is almost always overlooked, with the main focus on the optimization of therapeutic effect, in what it is mostly a trial and error approach (irrational drug design).

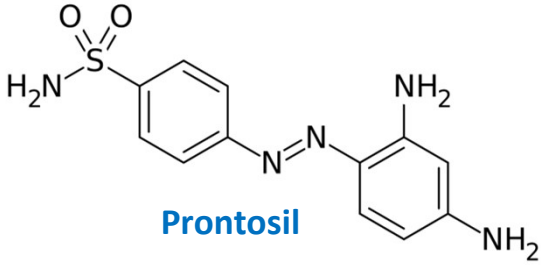
An example of “classic” drug design: sulfa drugs

- Gerhard Domagk from IGFarben researches some azo dyes with antibacterial properties and low toxicity in humans.



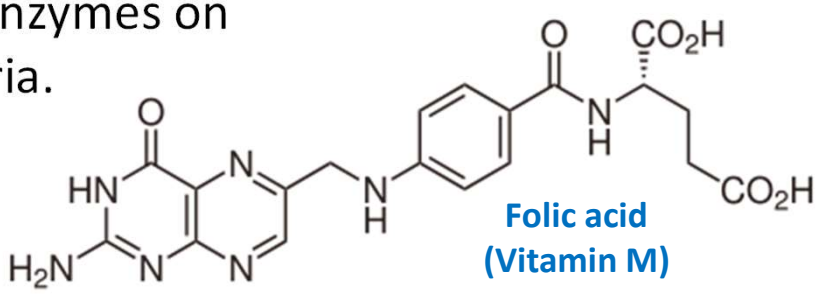
Yellow azo dye

- Sulfonamidochrysoidine is marketed in 1935 under the trade name *Prontosil* by Bayer, the first commercially available antibacterial drug and starting point for the family of sulfonamide compounds produced in the following years.



Prontosil

- In 1940, D.D.Woods discovers that sulfonamides are competitive inhibitors of DHPS, one of the enzymes on the folic acid biosynthesis pathway in bacteria.

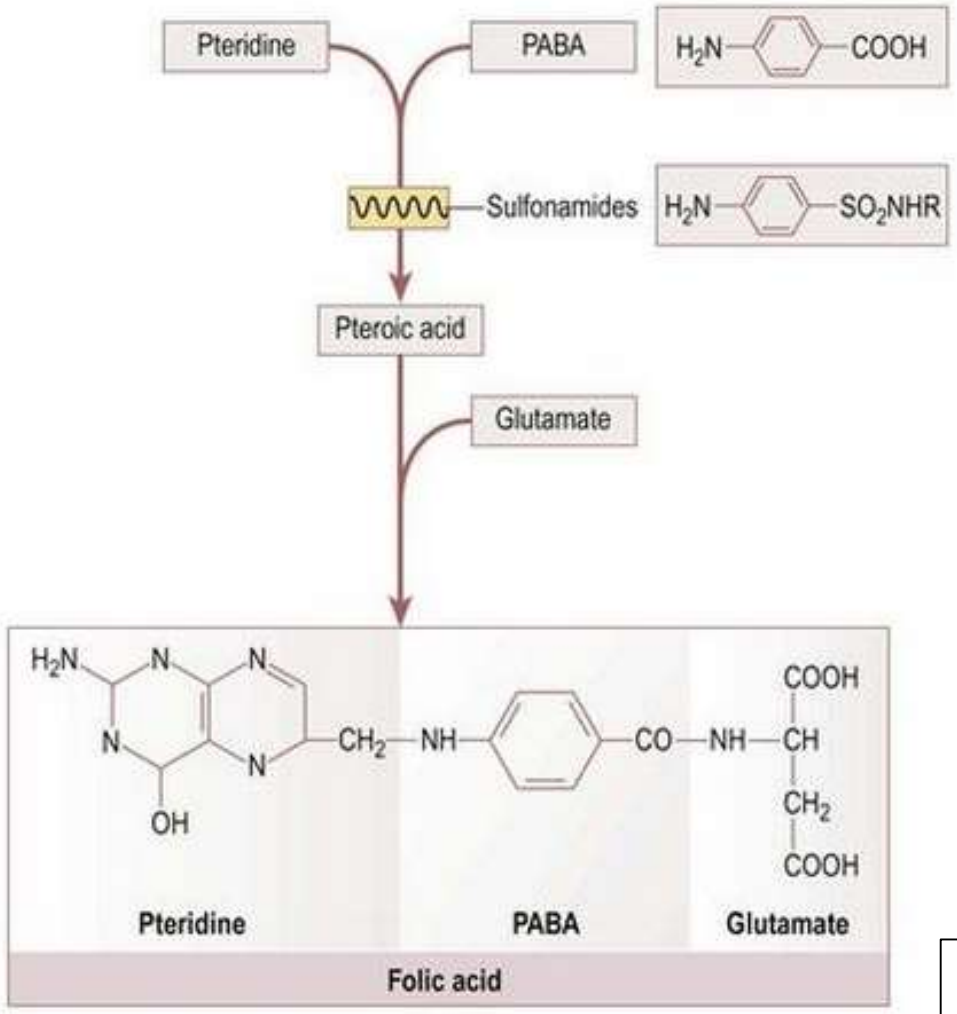


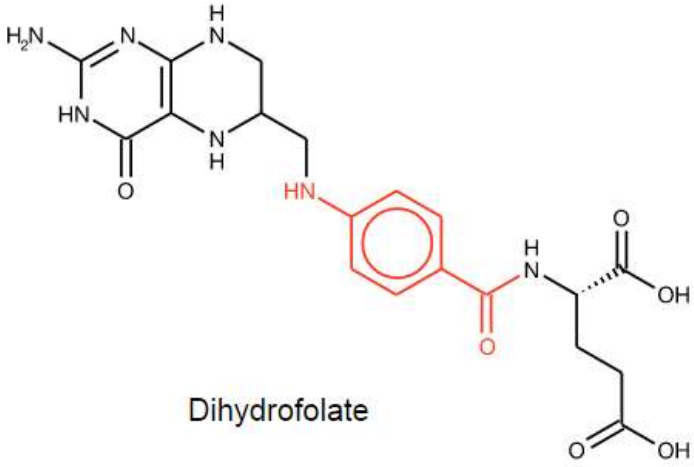
Folic acid
(Vitamin M)

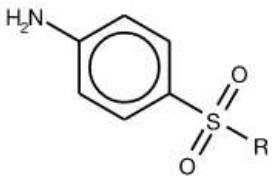
DHPS - Dihydropteroate synthase

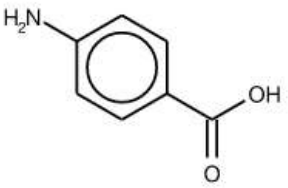
IGFarben – Bayer+BASF+Hoechst+AGFA

Sufanilamides are structural analogues of *p*-aminobenzoic acid









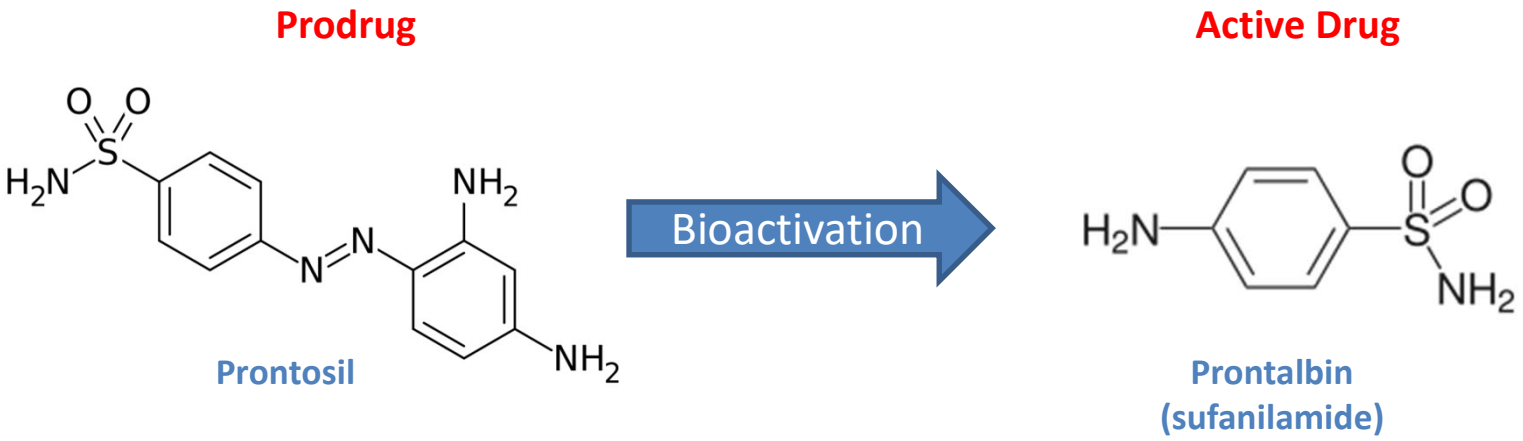
Sufanilamides are structural analogues of PABA (competitive inhibition)

PABA – *p*-aminobenzoic acid

Prontosil is a prodrug

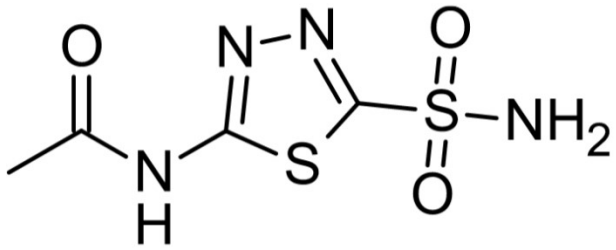
- In late 1935, working at the Pasteur Institute in Paris in the laboratory of Dr. Ernest Fourneau, Jacques and Thérèse Tréfouël, Dr. Daniel Bovet and Federico Nitti discovered that Prontosil is metabolized to sulfanilamide, a much simpler, colorless molecule, reclassifying Prontosil as a prodrug
- Sulfanilamide was market by Bayer under the trade name *Prontalbin*

- These findings help establish the concept of **bioactivation**, the process by which a **prodrug** is metabolized in the body to an active drug.



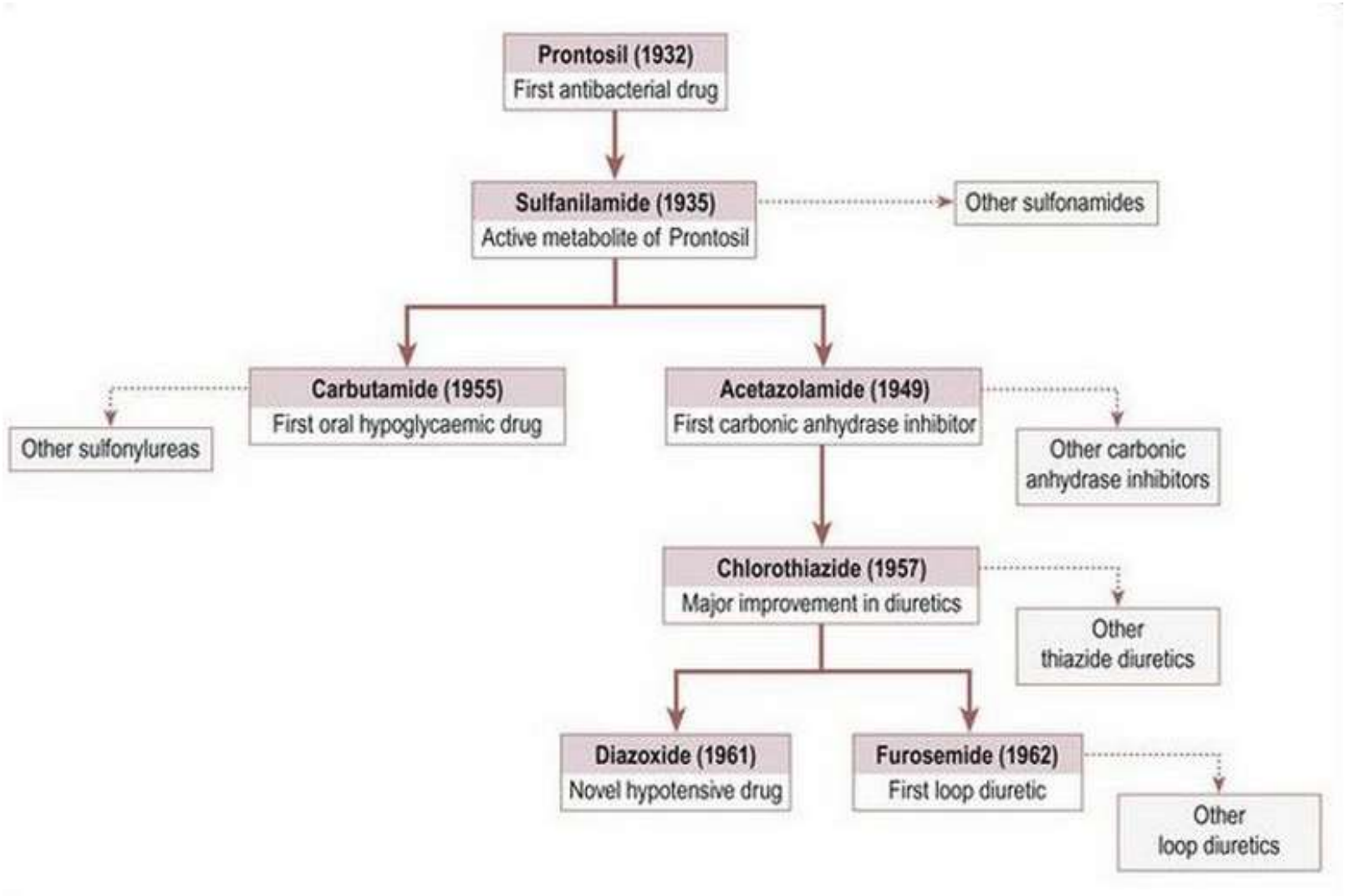
Serendipitous side effect: carbonic anhydrase inhibition

- Some sulfonamides were found to be diuretic (unexpected side effect)
- The discovery of carbonic anhydrase in 1940, and its role in bicarbonate secretion, lead to the experimental demonstration of the inhibitory effect of some sulfonamides on this enzyme.
- Modification of the structure of diuretic sulfonamides led to the making of the first commercially available carbonic anhydrase inhibitor, *acetazolamide*, marketed as a diuretic drug under the trade name *Diamox* (1952).



Acetazolamide

Sulfa family tree



Lessons from the sulfa story

Transition from the *synthetic chemistry* paradigm to *therapeutic target* paradigm (target-derived drug design).

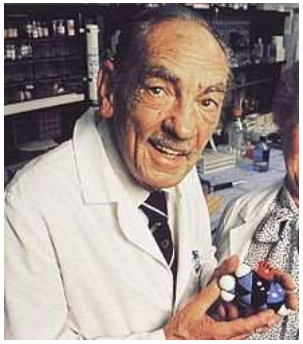
The active drug may be a metabolic product of a prodrug.

Serendipity in the discovery of new drugs: the diuretic action was an unsought side effect of sulfa drugs, but the researchers were able to recognize its utility.

"Chance favors only the prepared mind."
-- Louis Pasteur

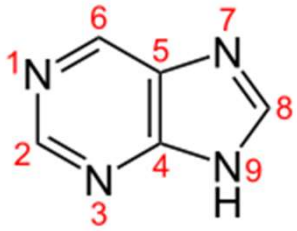
The “anti-metabolic principle”

- George Hitchings and Gertrude Elion worked together a the Wellcome Research Labs (1944). Development of inhibitors of folic acid biosynthesis
- Search for potential anti-metabolites for the purine and pyrimidine biosynthetic pathways
- Discovery of the enzyme DHFR (dihydrofolate reductase)
- Discovery of DHFR inhibitors with specificity towards particular microbial species.
- Development of several drugs with anti-bacterial, anti-cancer and immunosuppressive action
- Development of allopurinol, a Xanthine Oxydase inhibitor effective in gout treatment.
- They received the 1998 Nobel Prize in Phys. & Med.

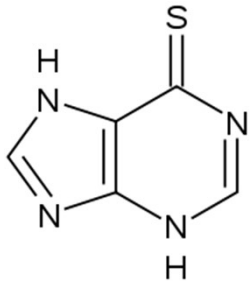


George Hitchings
(1905-1998)

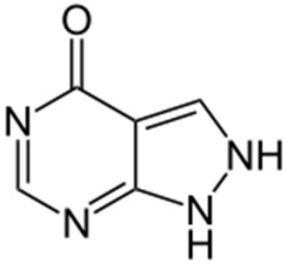
Gertrude Elion
(1918-1999)



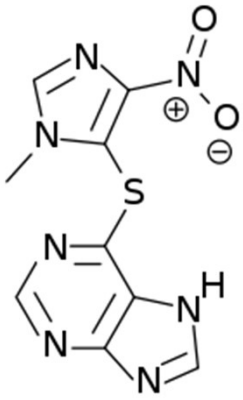
Purine



Mercaptopurine



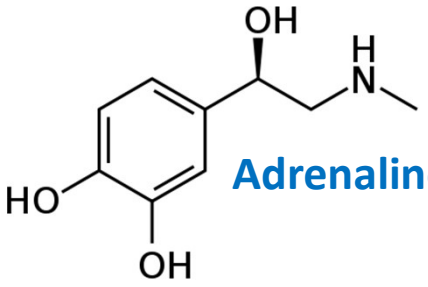
Allopurinol



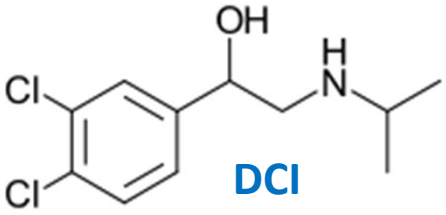
Azathioprine

Receptor pharmacology

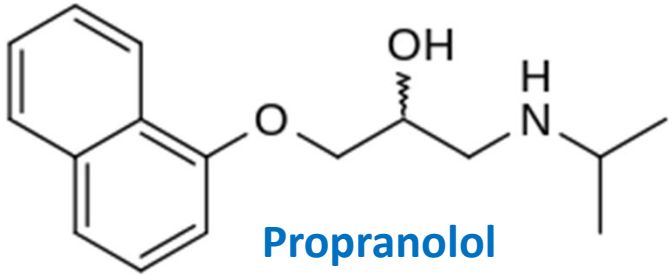
- James Black develops the first beta-blocker in 1960, pronethalol
- Pronethalol was found to be carcinogenic in mice, and was quickly replaced by *propranolol*
- This was the first a drug designed based on a previous specification of its target (the *β-adrenergic receptor*).
- Propranolol was marketed in 1964 under the name *Inderal*
- Propranol is an non-selective *antagonist* of the β-adrenergic receptors that revolutionized management of angina pectoris and later became the world's best selling drug



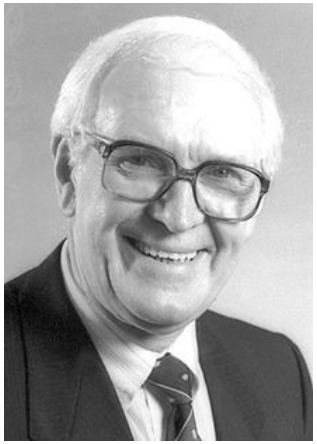
Adrenaline



DCI

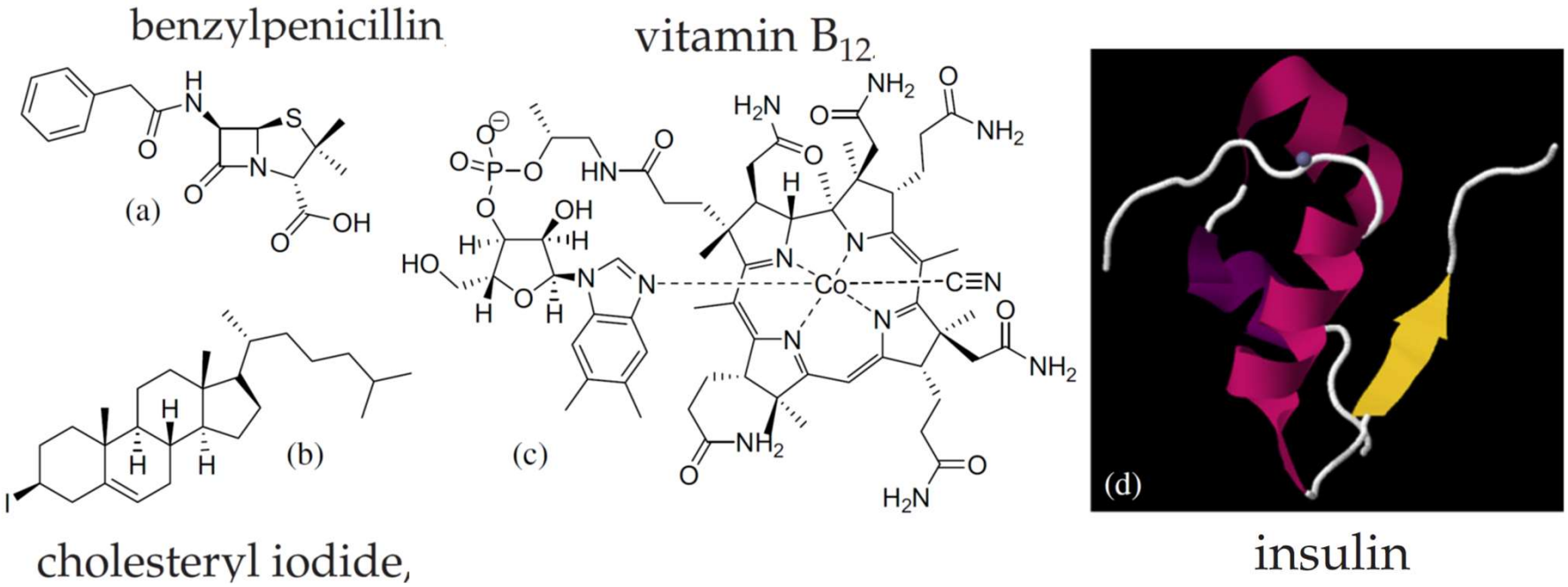


Propranolol



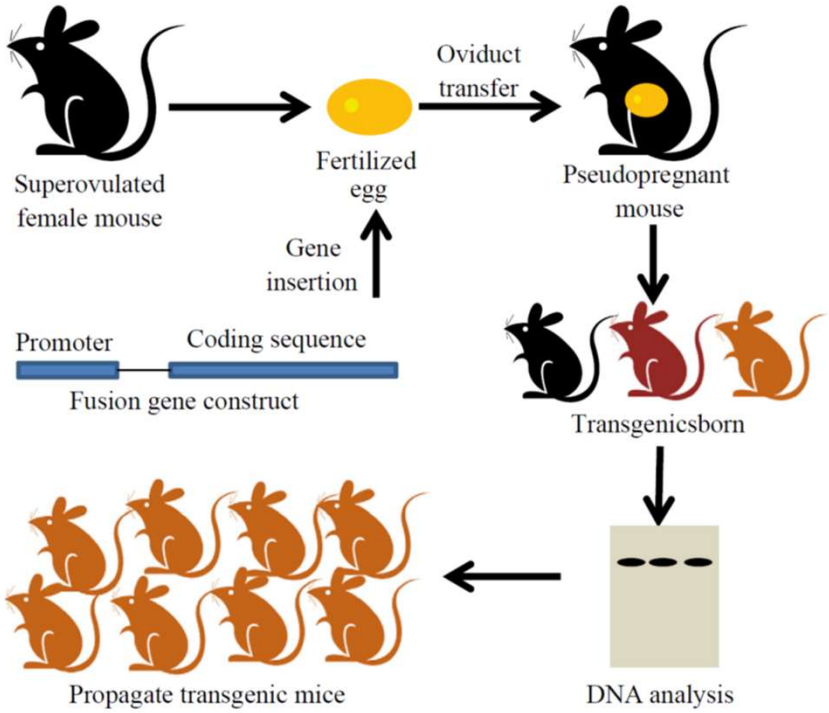
James Black
(1924-2010)

Structural chemistry

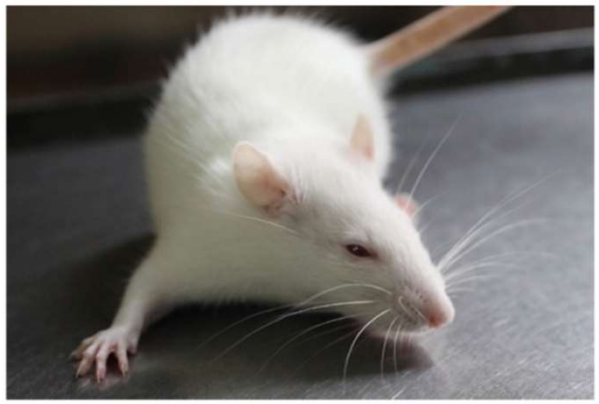


- The development of crstllographic methods in the early XX century permitted the discovery of many chemical structures, from simple to complex
- Quantum mechanics provide the theoretical framework to understand chemical bonding and reactivity

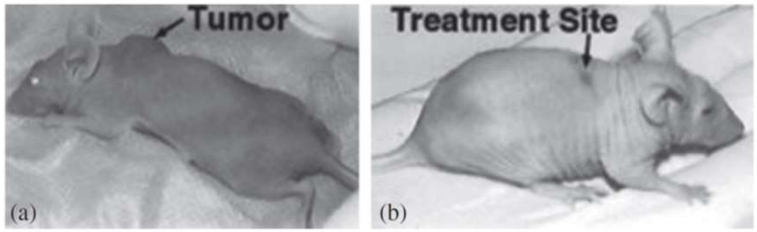
Animal models



Transgenic animal models

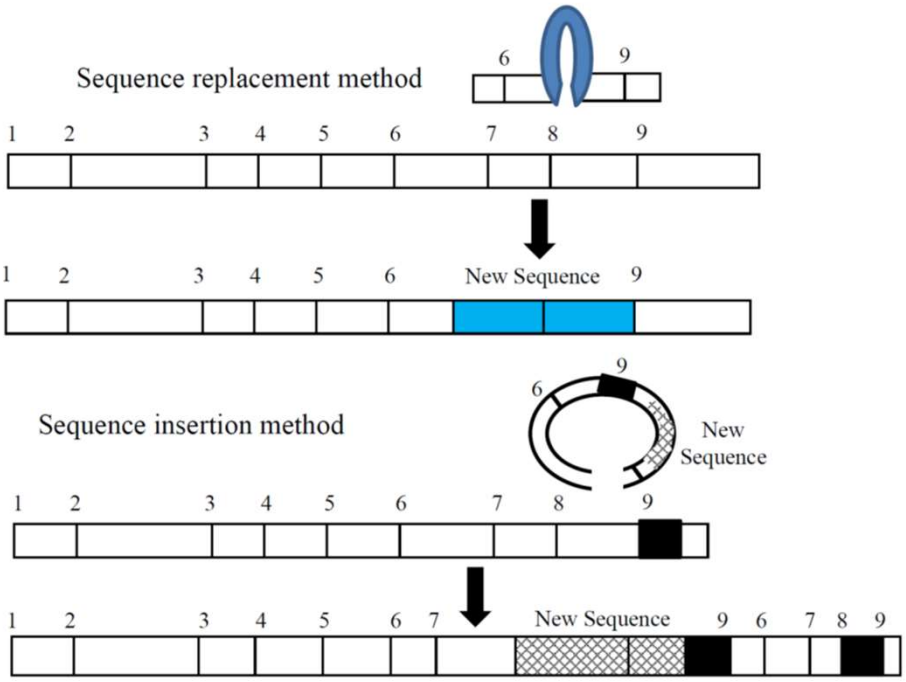


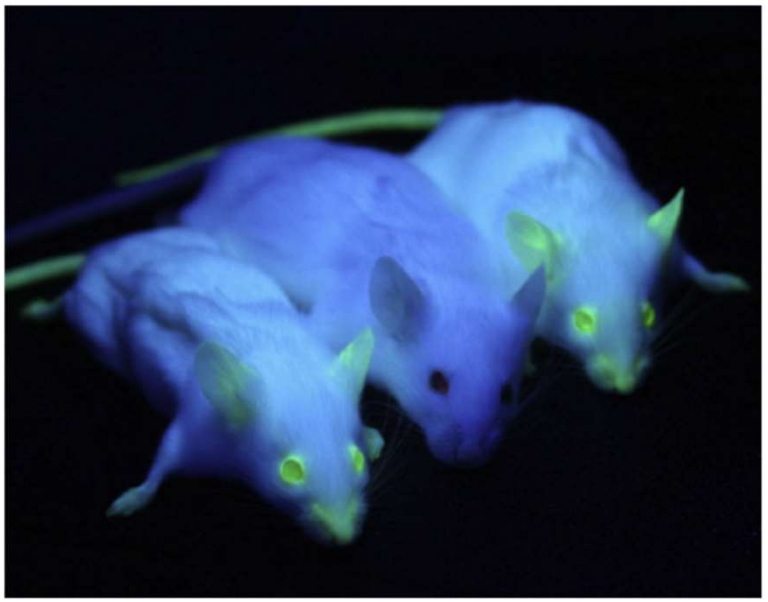
Wistar rat



Nude mice

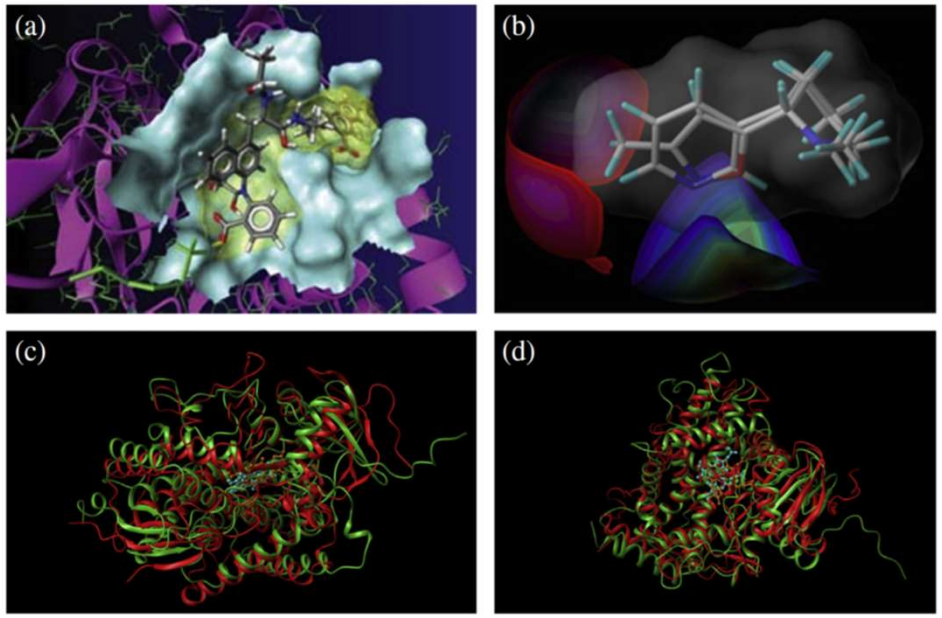
Molecular Genetics





Transgenic insertion of GFP

Computational methods



IBM 709 (1958)

XX Century

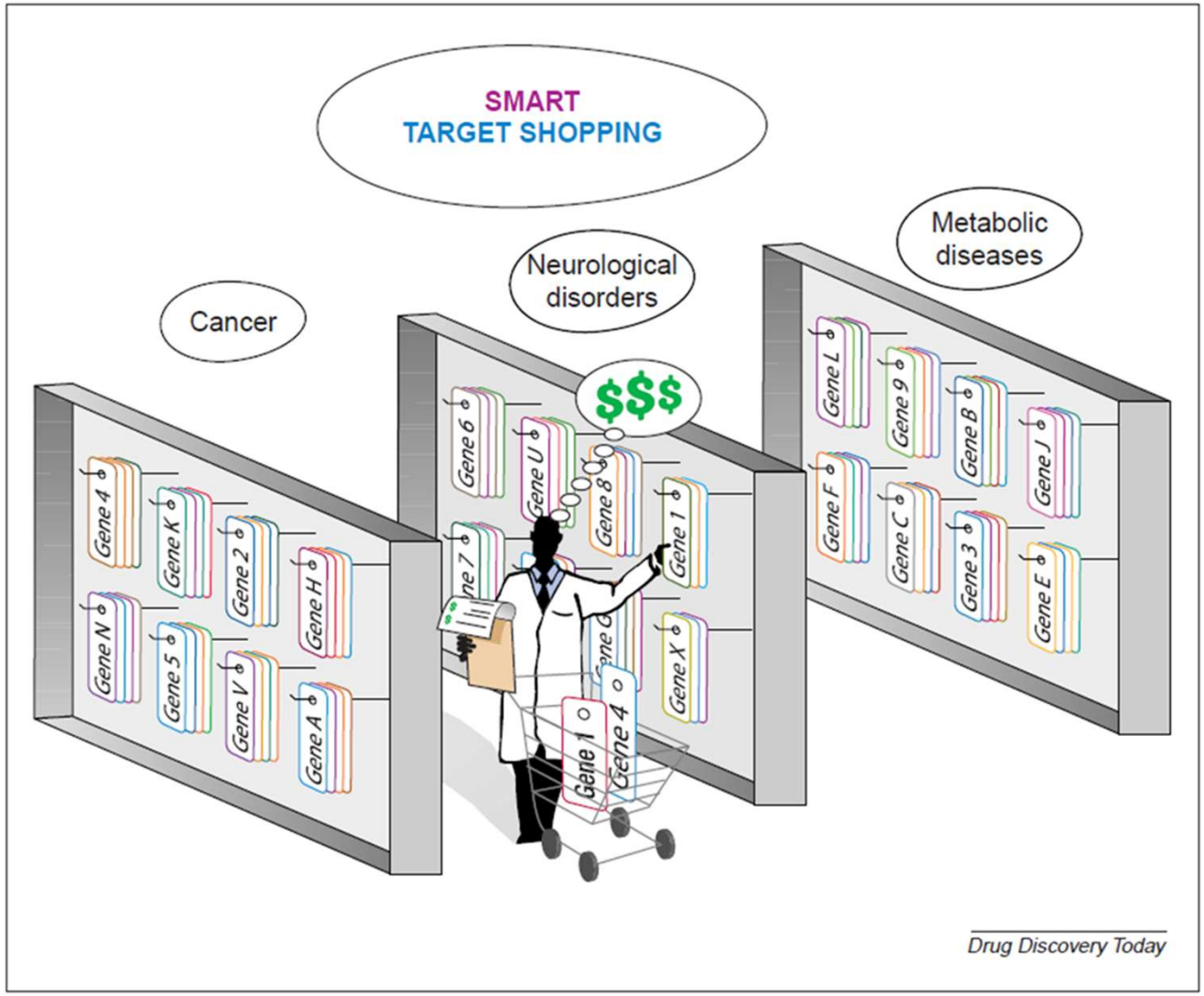
- Racional Drug Design
- Molecular Genetics
- Genomics (and other “omics”)
- High throughput screening
- Structural methods (NMR, X-ray, etc)
- Molecular Modelling
- Systems Biology

A new paradigm: Structure-based drug design

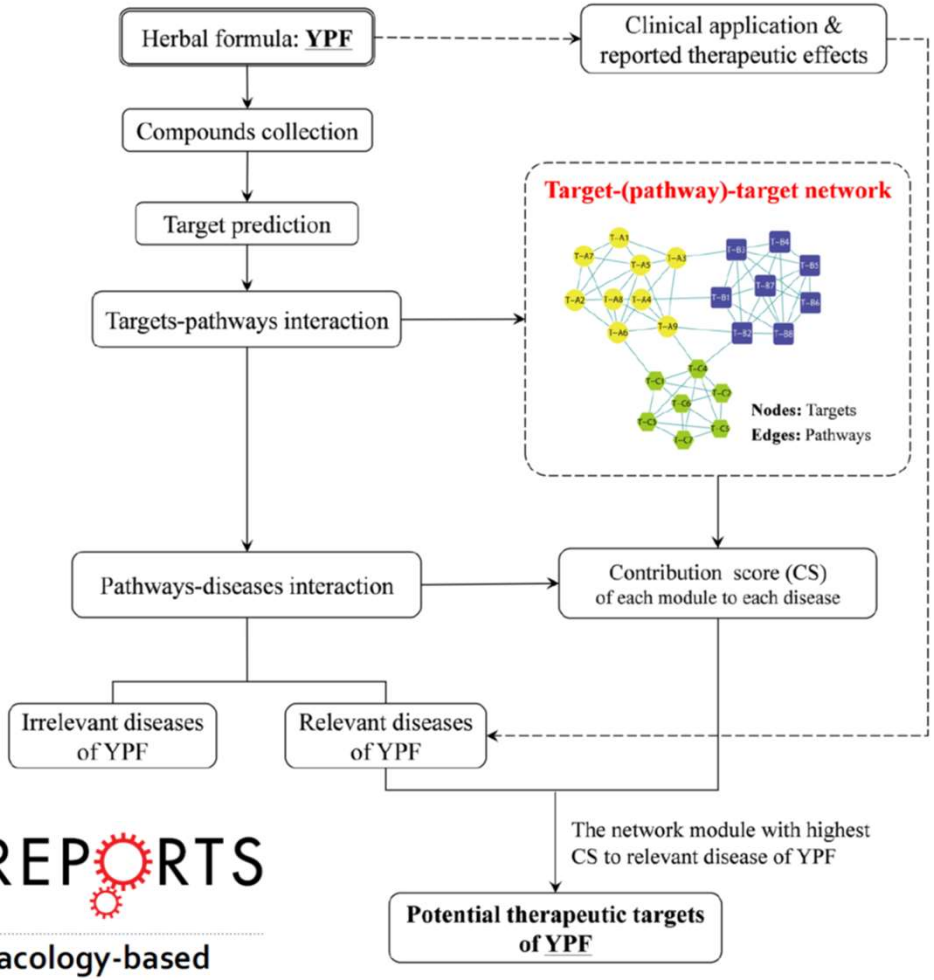
XX century's last quarter has witnessed multiples advances in several areas of crucial impact in drug development:

- Elucidation of DNA structure and protein synthesis mechanism, leading to molecular genetic techniques.
- Big advances in the methods for the determination of the 3D structure of proteins and other macromolecules.
- Complete draft of the Human Genome and development of new bioinformatics tools to analyze it.
- High-throughput screening methods for the discovery of new lead compounds
- Advances in both hardware, methods and algorithms for the computational modeling of proteins, ligands and their interactions (docking, virtual screening, molecular dynamics, computational chemistry/QM).
- Collection, organization and generation of very large databases of small molecule compounds, macromolecules, genomes, pathways, interactions and other biological data.
- Real and virtual fragment libraries, fragment-based design, click chemistry, cheminformatics methods.

Systems Biology and Smart Target Finding



Network Pharmacology



SCIENTIFIC REPORTS

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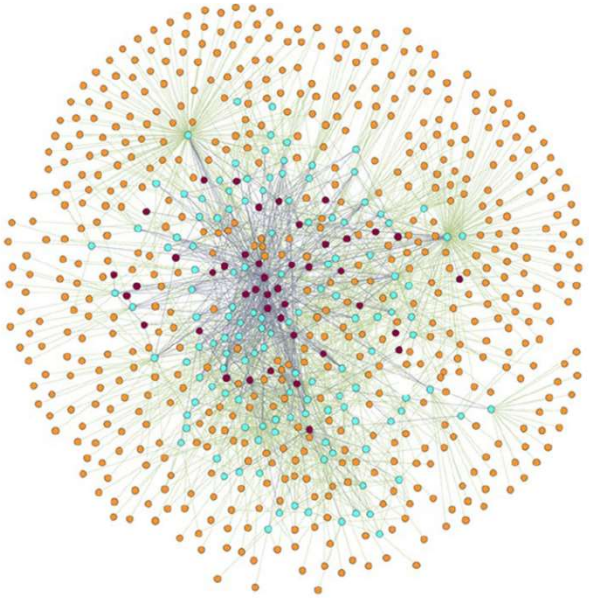
A network pharmacology-based approach to analyse potential targets of traditional herbal formulas: An example of Yu Ping Feng decoction

d: 17 January 2018
d: 3 July 2018
d online: 30 July 2018

Huali Zuo[1], Qianru Zhang[1,2], Shibing Su[3], Qilong Chen[3], Fengqing Yang[3]* & Yuanjia Hu[1]

Zuo (2018) *Scientific Reports* ***8***:*11418*

Network Pharmacology



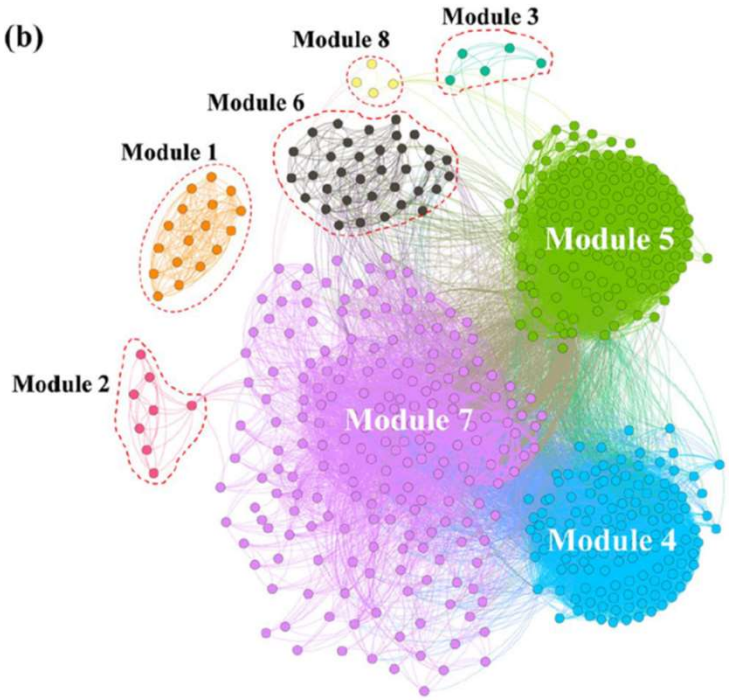
target-pathway-disease network

250 Compounds

549 Targets

132 Pathways

43 Diseases



target-pathway-target network

Zuo (2018) *Scientific Reports* ***8****:11418*