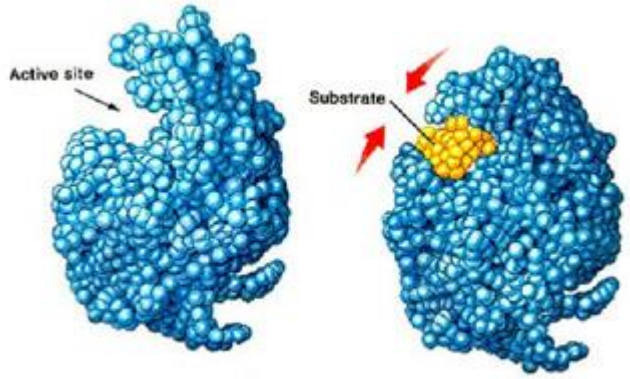
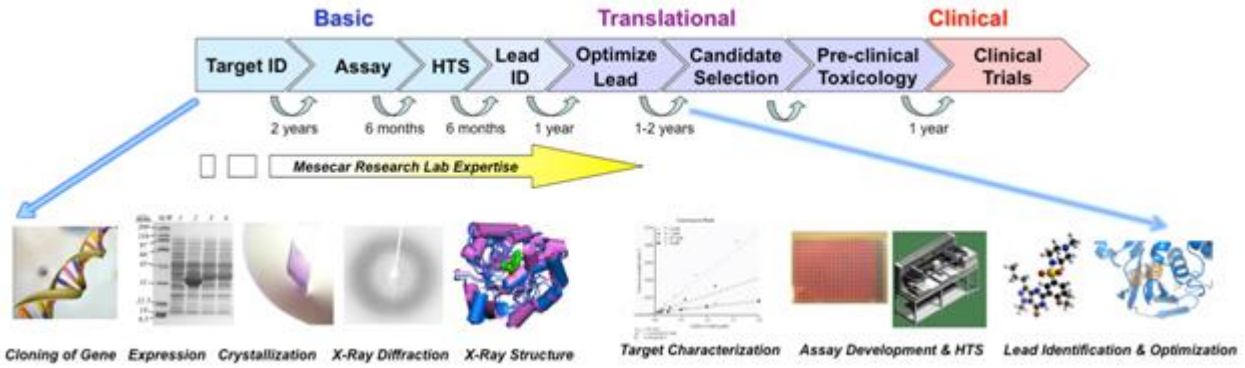
Enzimas como alvos de drogas





Interações químicas das drogas e das enzimas

Hydrophobic interactions plays an important role in stabilizing the conformation of proteins and in the association of hydrophobic structure between the drug and its target.

Hydrogen bonding is strongly directional and has considerable importance both in the maintaining the secondary and tertiary structure of the target itself and in the target-drug interaction.

Charge transfer complexes formed between electron- rich donor molecules and electron-deficient acceptors are also often involved in drug-target interaction.

Ionic bonds are of importance in the actions of ionizable drugs since they act across long distances; ionic bonds result from the electrostatic attraction that occurs between oppositely charged ions; most targets have a number of ionizable groups (COO-, —O —, NH3+) at physiological pH that are available for the binding with charged drugs.

Covalent bonds resulting in the formation of a long- lasting complex are less important in drug-target interaction. Although most drug-target interactions are readily reversible, some drugs, such as anticancer nitrogen mustards and alkylating compounds form reactive cationic intermediates that can react with electron donor groups on the target.

Características do sítio ativo:

-Cavidade hidrofóbica

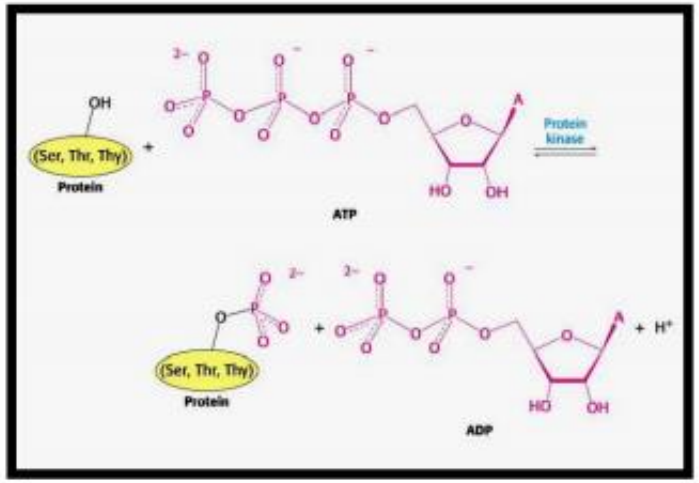
-Liga cofatores e substrates

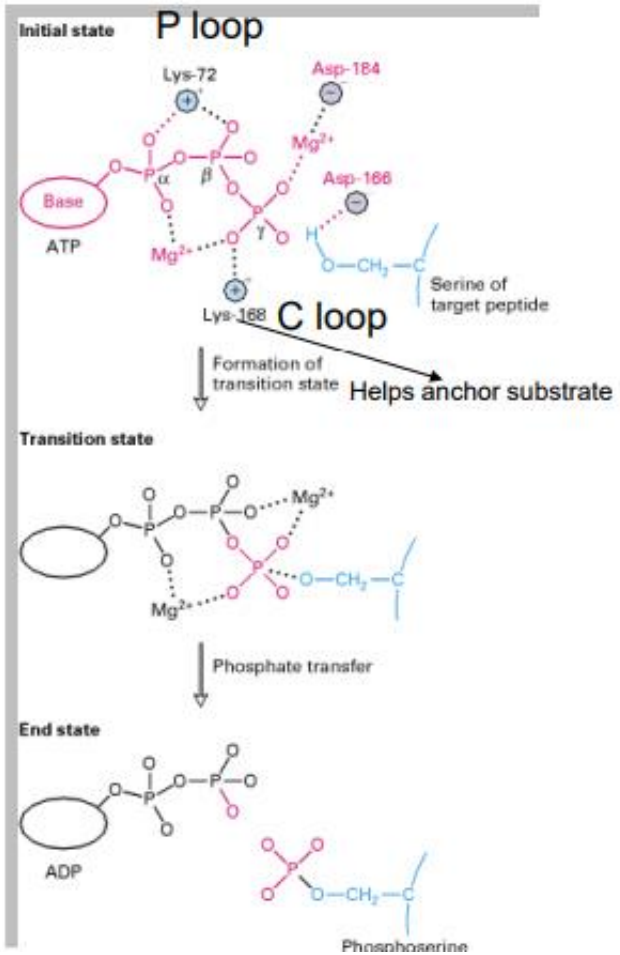
-Contém amino ácidos que:

Interagem com substrates e cofatores

Catalizam as reações

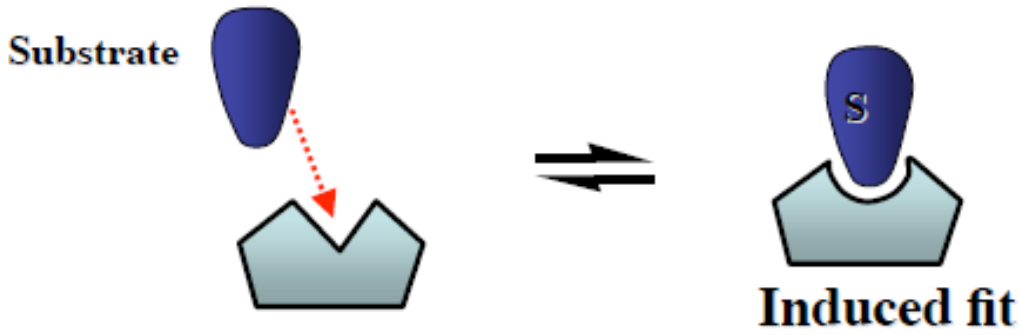
Características do sítio ativo:





"Induced fit"

- O sítio ativo está próximo da conformação para se ligar ao substrato
- Ligação do substrato altera o "formato" da enzima
- Ligações entre a enzima e o substrato
- Ligações intramoleculares (no substrato e na enzima).

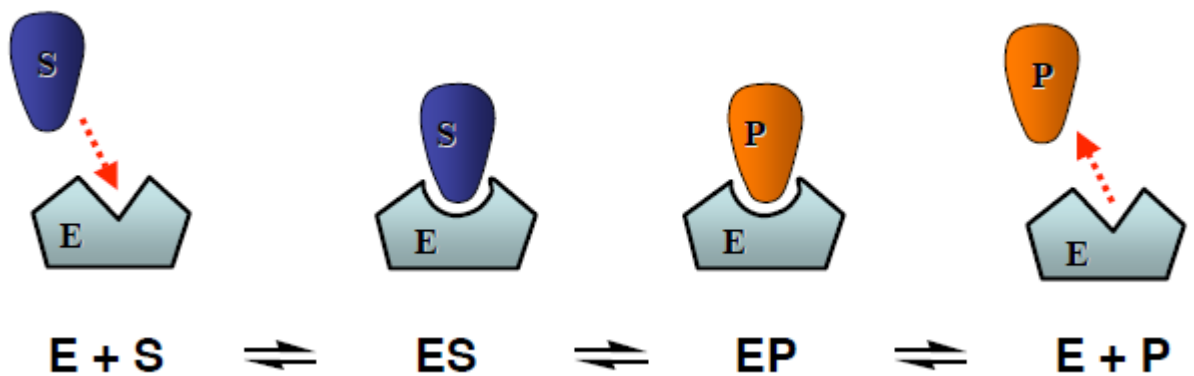


"Catálise"

- A interação do substrato com a enzima deve:
  - Ser forte o suficiente para manter o substrato ligado permitindo que a catálise ocorra.
  - Fraca o suficiente para perimitr que o produto se desligue da enzima.

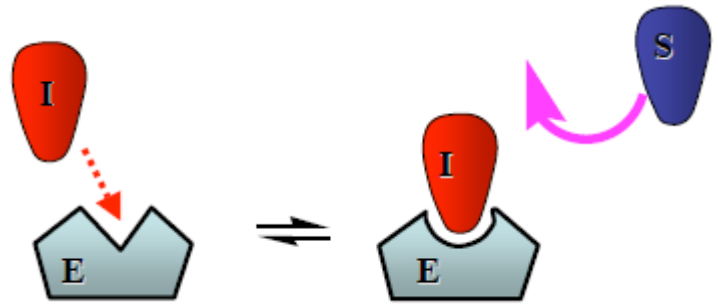
Drogas:

-Moléculas com interações mais fortes resultam em inibidores que bloqueiam o sítio ativo.



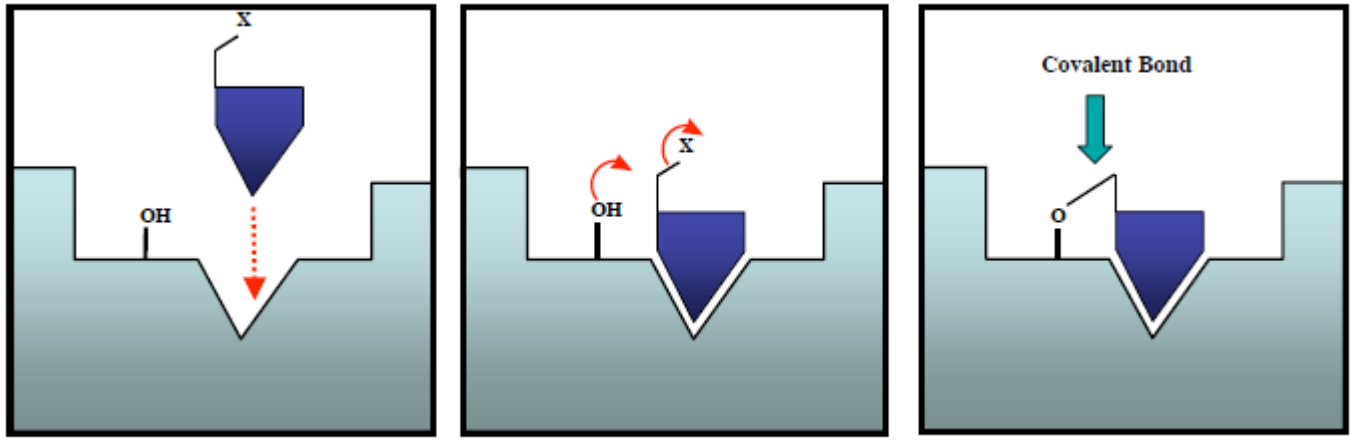
Inibidor competitivo (reversível)

- O inibidor se liga de forma reversível ao sítio ativo da enzima.
- Ligações intermoleculares entre a enzima e o inibidor
- A reação está inibida na presença do inibidor
- A inibição depende da força de ligação do inibidor e da sua concentração.
- A entrada do substrato fica bloqueada do sítio ativo.
- Aumentando a concentração do substrato reverte a inibição.
- O inibidor é provavelmente parecido com o substrato.



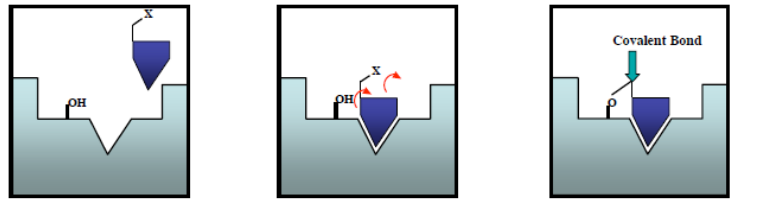
Inibidor não competitivo (irreversível)

- O inibidor se liga de forma irreversível ao sítio ativo.
- Uma ligação covalente se forma entre a droga e a enzima.
- O substrato está bloqueando o sítio ativo.
- Um aumento na concentração do substrato não reverte a inibição.
- O inibidor provavelmente tem uma estrutura semelhante ao do substrato.



Irreversible inhibition

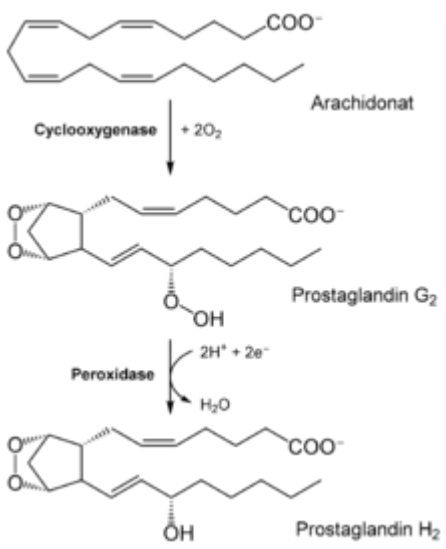
Inibidor não competitivo (irreversível)

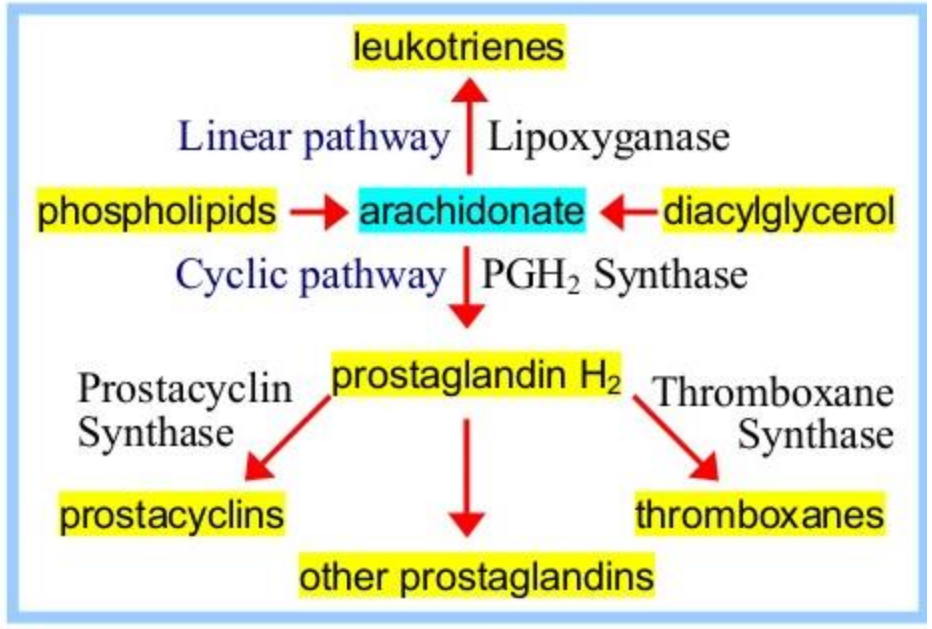


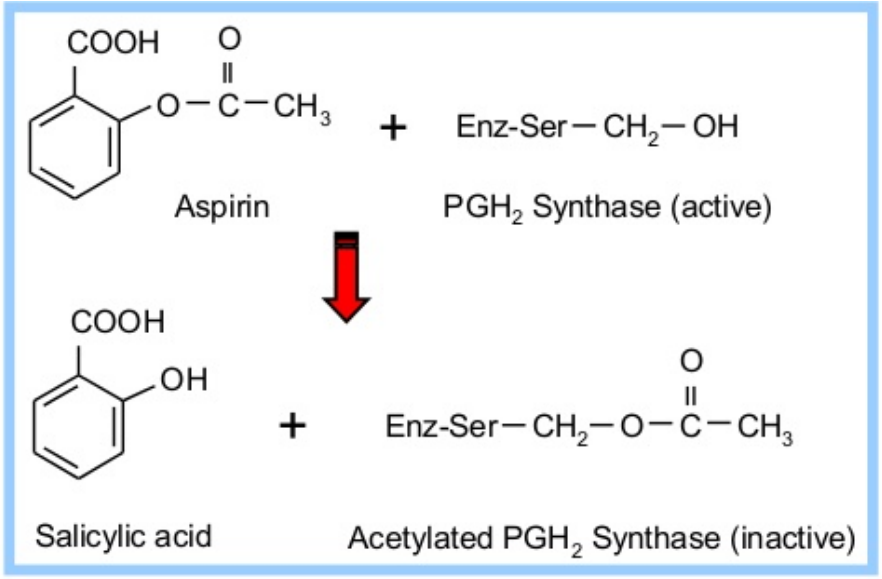
Example: aspirin for COX (PGH_2 synthase)

Prostaglandin-endoperoxide synthase 2, cyclooxygenase-2 ou COX-2

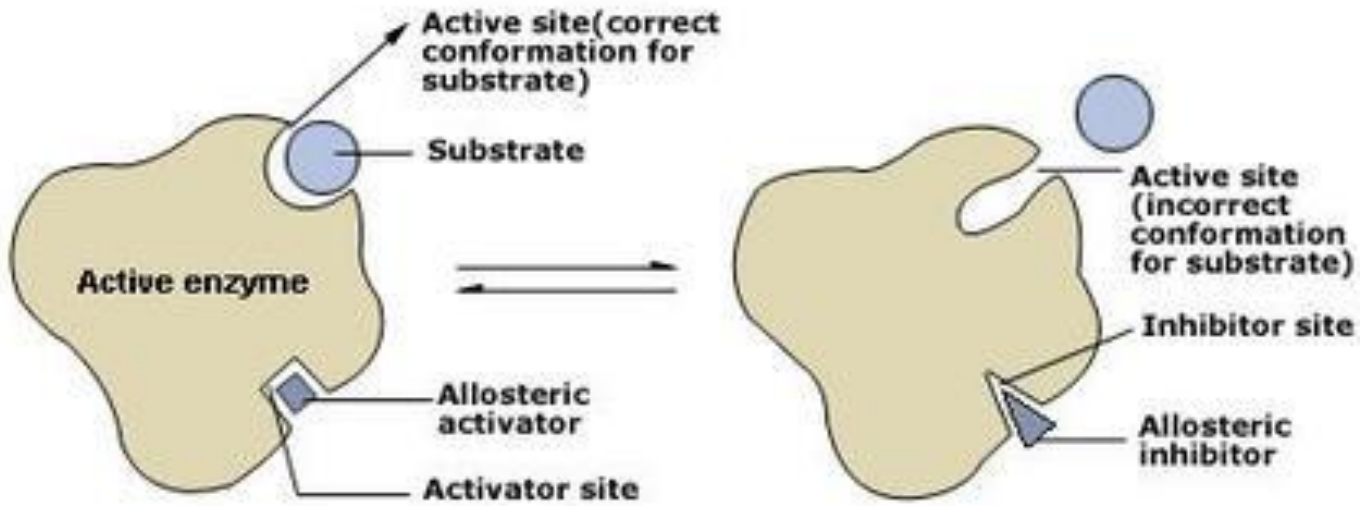
Prostaglandinas são vasodilatodores e inibidores da agregação plaquetária atuam na reação inflamatória.







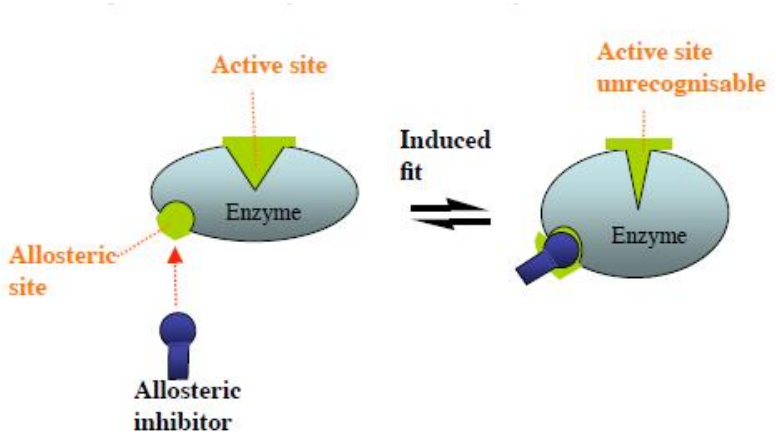
Sítio alostérico



Schematic representation of allosteric enzyme activity

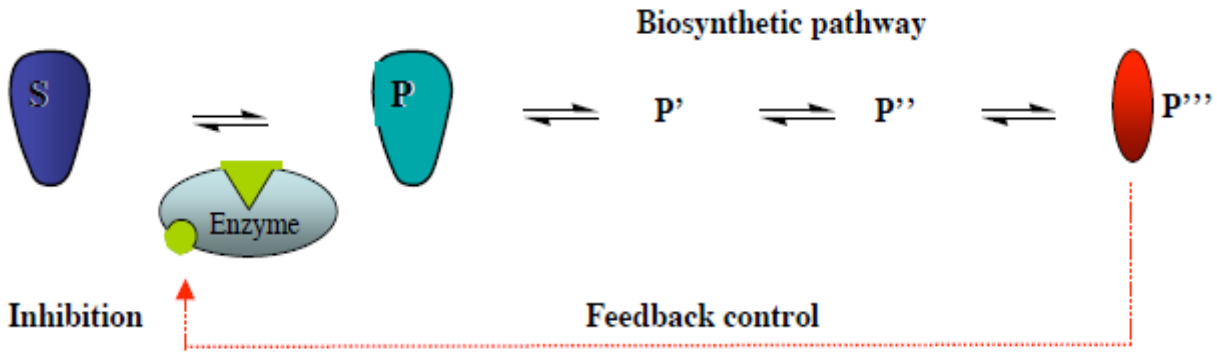
Inibidor não competitivo (reversível) alostérico

- O inibidor se liga reversivelmente ao sítio alostérico.
- Ligações intermoleculares se formam.
- Ligação do inibidor altera a *forma* da enzima.
- O sítio ativo está distorcido e não reconhece mais o substrato.
- Aumentando a concentração do substrato não reverte a inibição.
- O inibidor não tem uma estrutura semelhante ao do substrato.



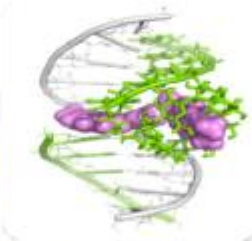
Inibidor não competitivo (reversível) alostérico

- Enzimas com sítios alostéricos geralmente estão no início das vias metabólicas.
- A enzima é controlada pelo produto final da via.
- O produto final se liga ao sítio alostérico e *desliga* a enzima.
- O inibidor pode ter uma estrutura semelhante ao do produto final da via.



O processo de descoberta de novas drogas

Stages of drug discovery

1	2	3	4	5	6
					
Disease identification	Therapeutic Target	Lead Identification -High throughput Screening -*In vitro* and *in vivo* pharmacology ...	Preclinical trials -Animal test -HTS	Clinical Trials	FDA Approval

High-throughput screening (HTS):
Lead identification and Preclinical toxicology

Investigational New Drug application

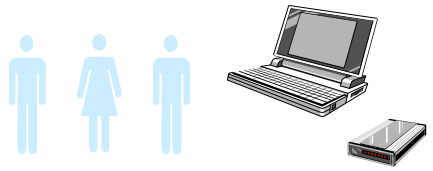
IND

Clinical Trials

Phase I

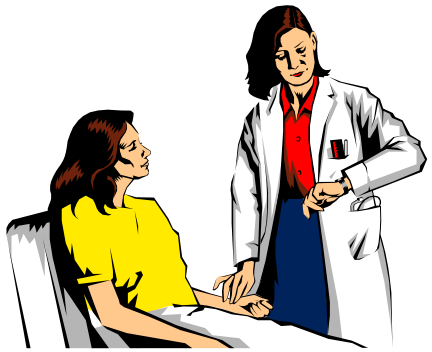
Product Profile → Marketing SOI

20 - 100 healthy volunteers take drug for about one month



Remote data entry

Information Learned
1. Absorption and metabolism
2. Effects on organs and tissue
3. Side effects as dosage is increased



Phase II

Several hundred health-impaired patients

Treatment Group Control Group

Information Learned
1. Effectiveness in treating disease
2. Short-term side effects in health -impaired patients
3. Dose range

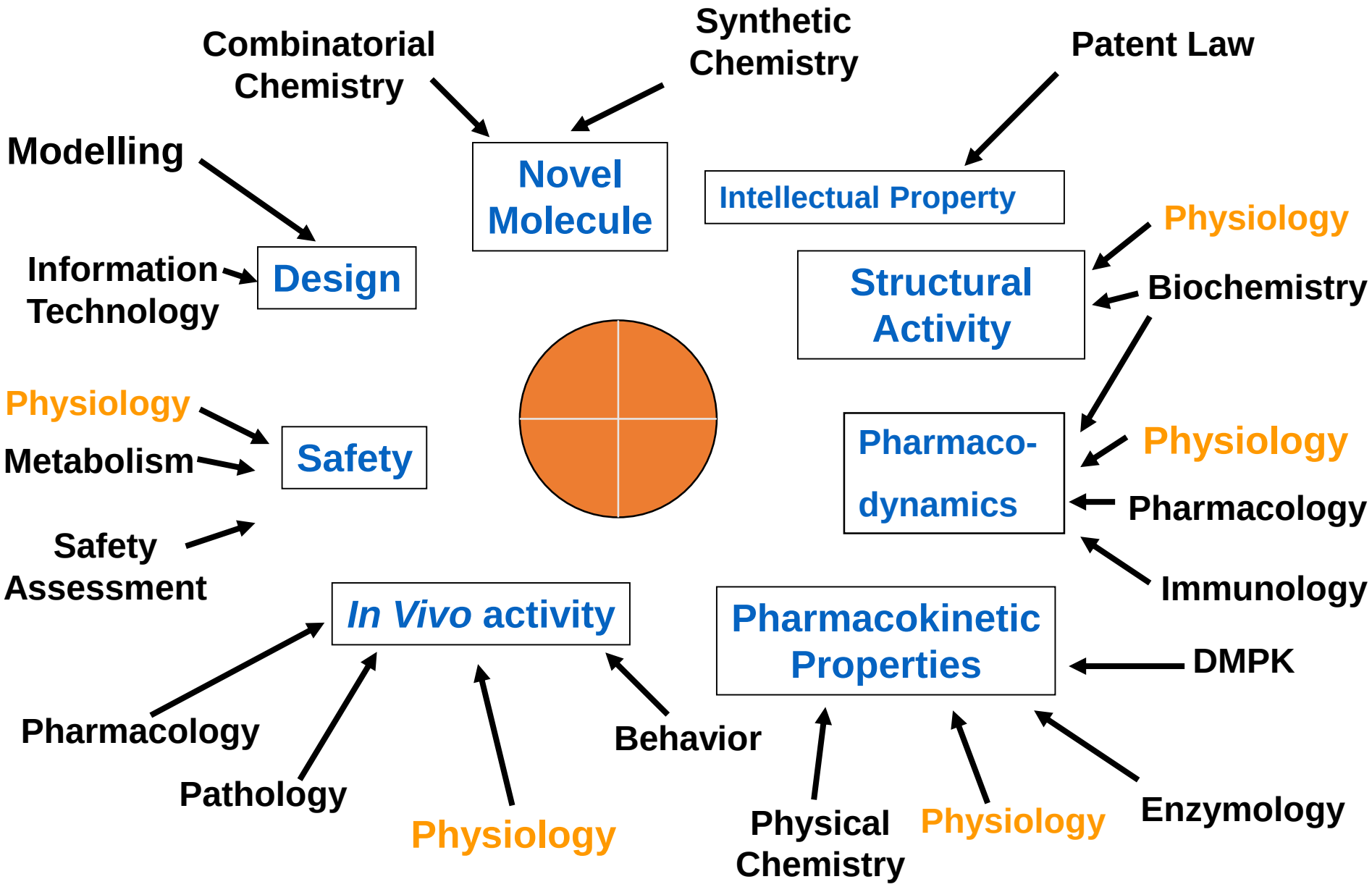
Phase III

Hundreds or thousands of health-impaired patients

Information Learned
1. Benefit/risk relationship of drug
2. Less common and longer term side effects
3. Labeling information

Compassionate Use

Drug Discovery—Convergence of Disciplines



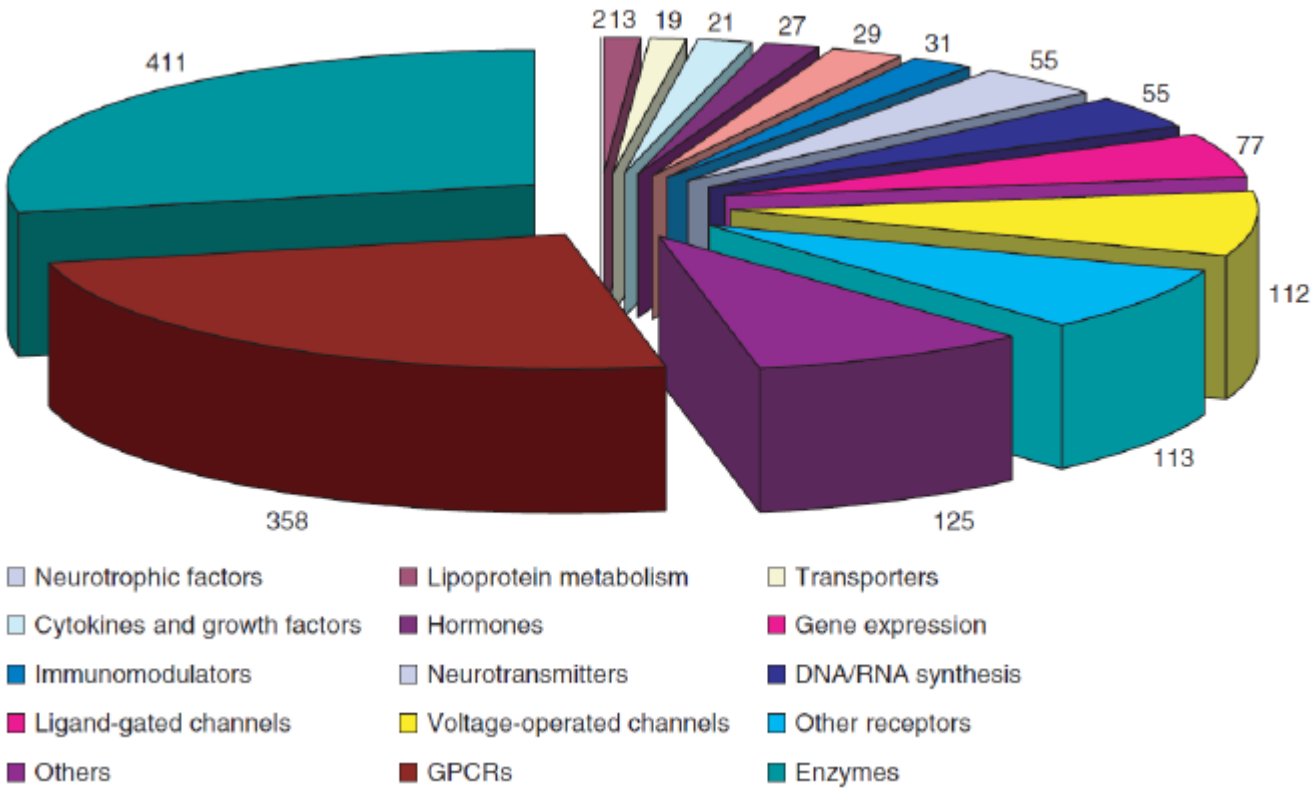
Alvos de drogas

- Interagem com as drogas seletivamente administradas para tratarem ou diagnosticar uma doença.

- Proteínas do genoma humano ou de organismos patogênicos. (exÇ enzima hiperativa em uma doença).

- Desconhecem-se os alvos de algumas drogas.

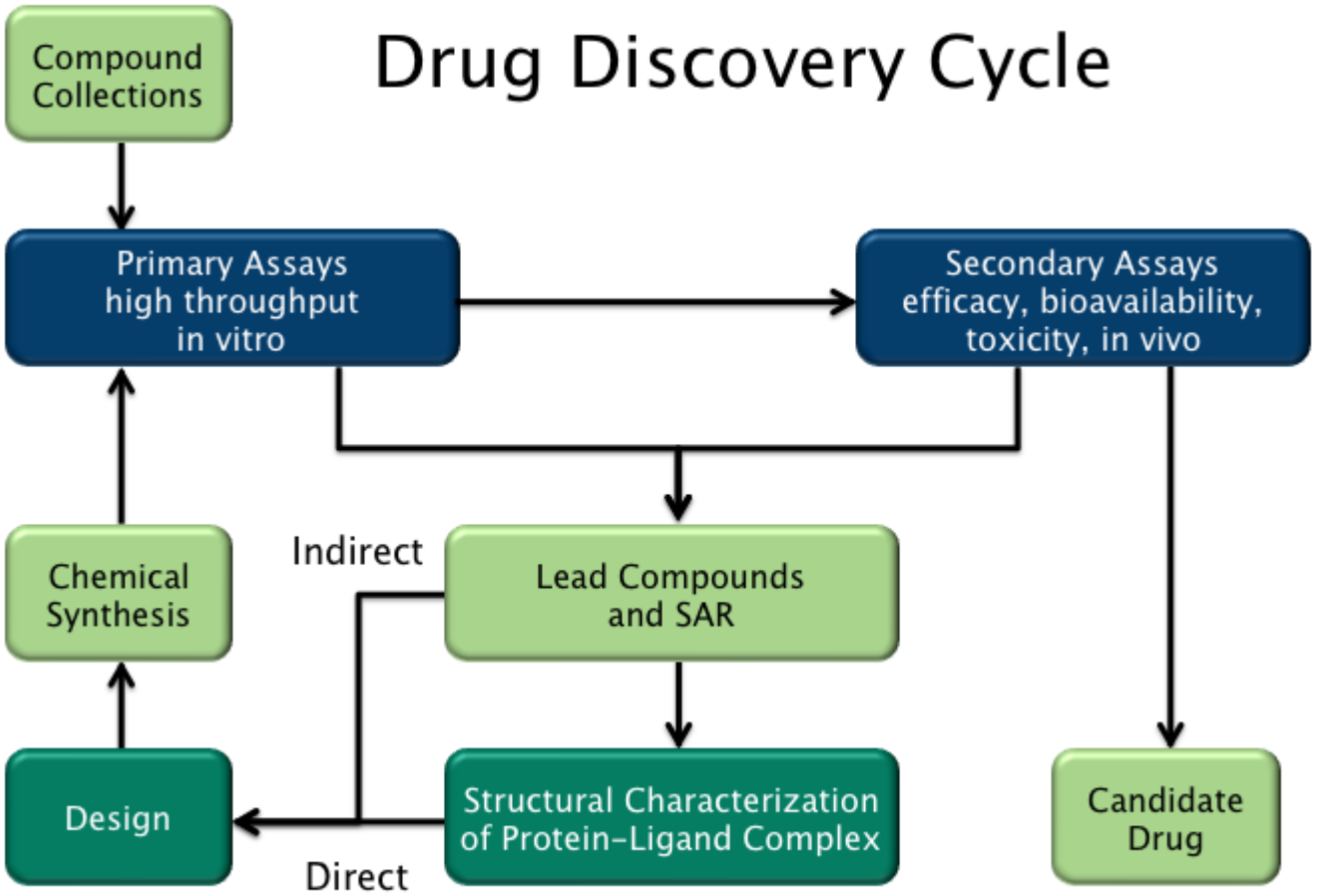
Alvos de drogas

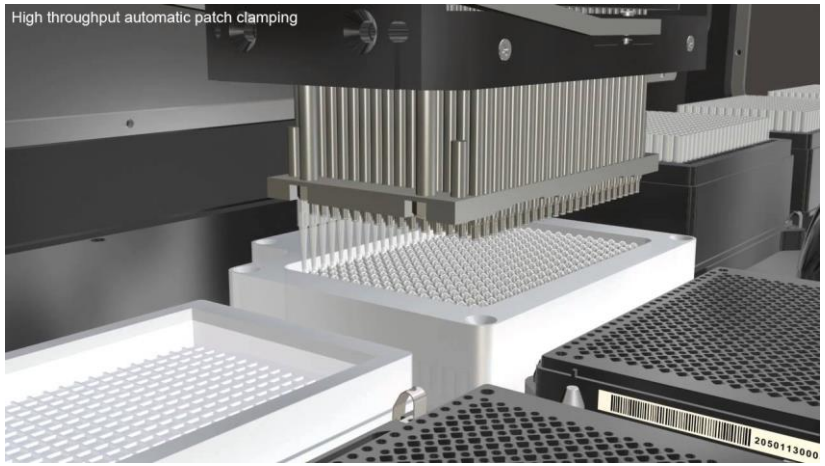


Alvos de drogas

Drug	Target enzyme	Field of therapy
Aspirin	Cyclooxygenase	Anti-inflammatory
Captopril and enalapril	Angiotensin converting enzyme (ACE)	Antihypertension
Simvastatin	HMG-CoA reductase	Lowering of cholesterol levels
Desipramine	Monoamine oxidase	Antidepressant
Clorgiline	Monoamine oxidase-A	Antidepressant
Selegiline	Monoamine oxidase-B	Treatment of Parkinson's disease
Methotrexate	Dihydrofolate reductase	Anticancer
5-Fluorouracil	Thymidylate synthase	Anticancer
Gefitinib and imatinib	Tyrosine kinases	Anticancer
Sildenafil (Viagra)	Phosphodiesterase enzyme (PDE5)	Treatment of male erectile dysfunction
Allopurinol	Xanthine oxidase	Treatment of gout
Zidovudine	HIV reverse transcriptase	AIDS therapy
Saquinavir	HIV protease	AIDS therapy
Aciclovir	Viral DNA polymerase	Treatment of herpes
Penicillins and cephalosporins	Bacterial transpeptidase	Antibacterial
Clavulanic acid	Bacterial p-lactamases	Antibacterial
Sulfonamides	Dihydropteroate synthetase	Antibacterial
Fluoroquinolones	Bacterial topoisomerases	Antibacterial
Ro41-0960	Catechol-O-methyltransferase	Treatment of Parkinson's disease
Omeprazole	H+/K+ ATPase proton pump	Ulcer therapy
Organophosphates	Acetylcholinesterase	Treatment of myasthenia gravis, glaucoma, and Alzheimer's disease
Acetazol amide	Carbonic anhydrase	Diuretic
Zileutin	5-Lipoxygenase	Anti-asthmatic

Rastreamento de drogas





High Throughput Screening

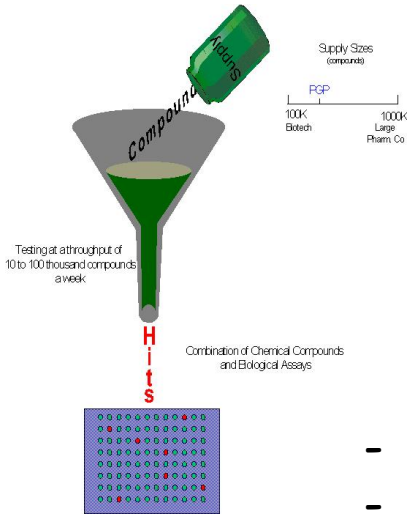
Commercial automation systems

- Biochemicals-based High Throughput Screening - microchips
- Cell-based High Throughput Screening – microtiter plates, microfluidic chips

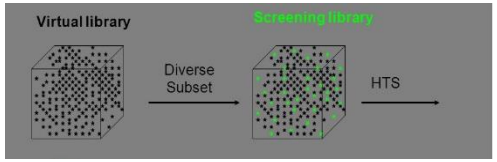
High throughput screening platform, Tecan

GNF systems for HTS

Escolha da biblioteca de compostos para o rastreamento

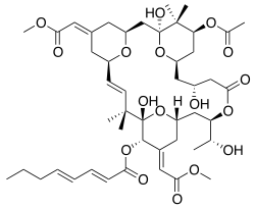


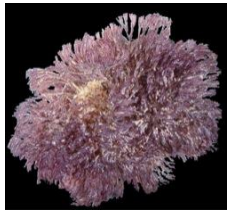
- Escolha da biblioteca análise virtual quando a estrutura do alvo é conhecida
- - Bibliotecas sintéticas



- Bibliotecas de produtos naturais

Bryostatins macrolide lactones isolated from the marine organism, *Bugula neritina*. Modulators of protein kinase C. They have been studied in clinical trials as anti-cancer agents, as anti-AIDS/HIV agents and in people with Alzheimer's disease.

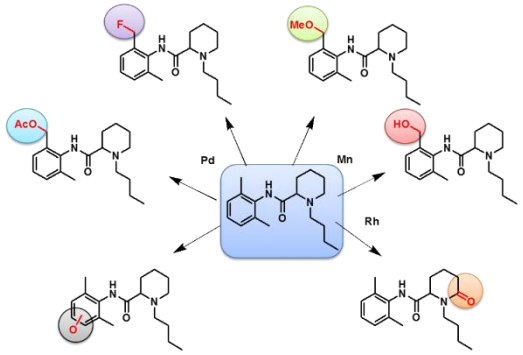




- Drogas existentes

lead compound (i.e. a "leading" compound)

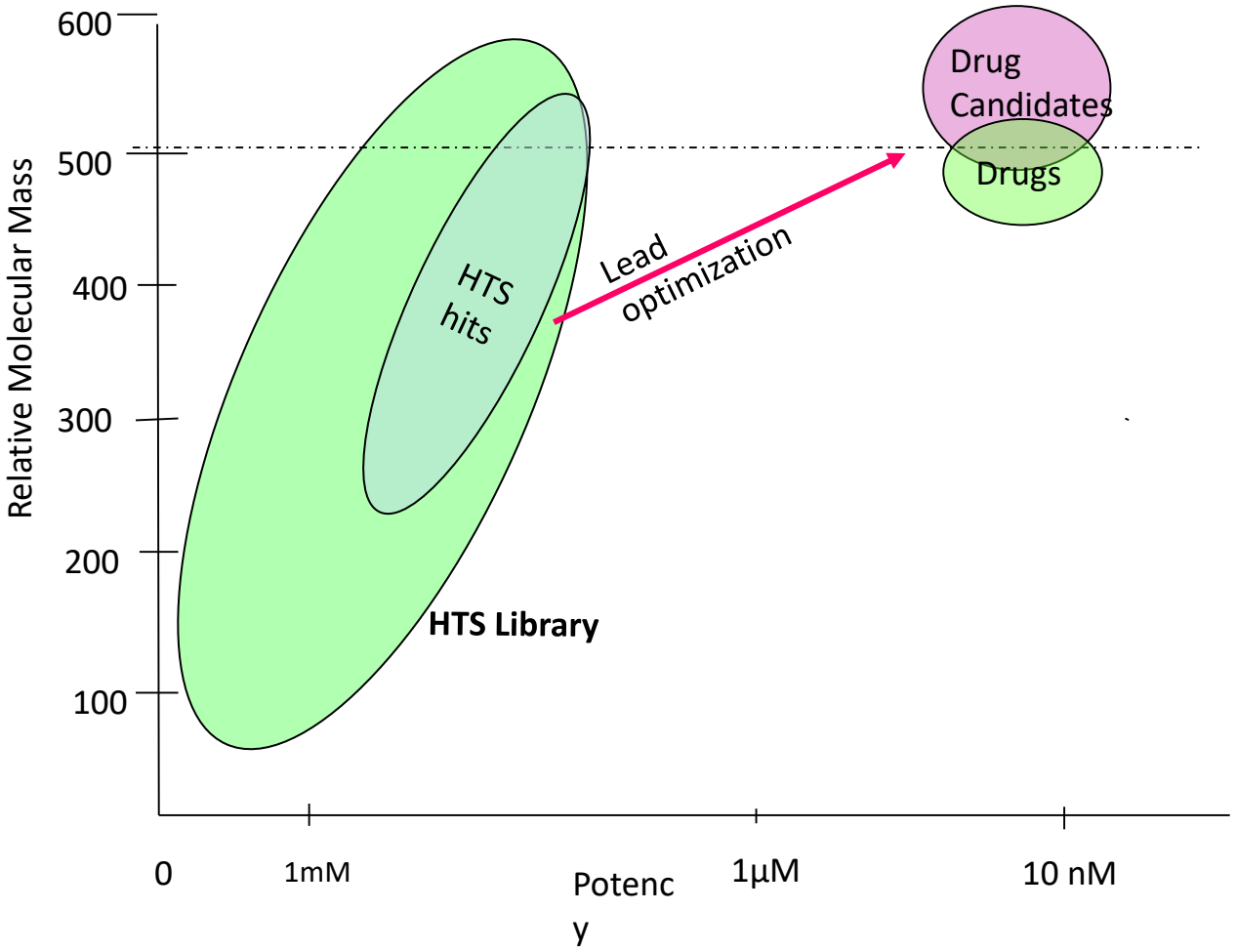
chemical compound that has pharmacological or biological activity likely to be therapeutically useful, but may still have suboptimal structure that requires modification to fit better to the target.



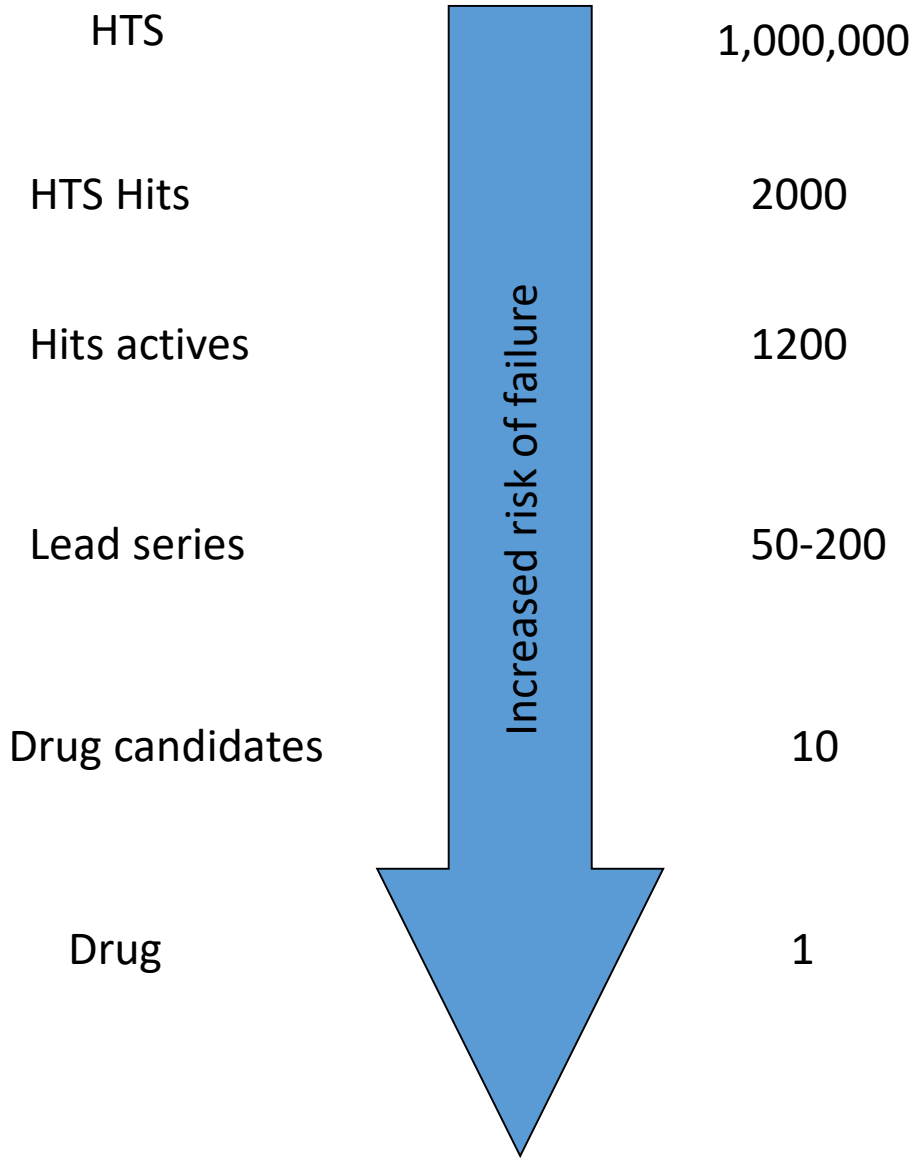
Lead drugs are followed by back-up compounds. Its chemical structure is used as a starting point for chemical modifications in order to improve potency, selectivity, or pharmacokinetic parameters.

Furthermore, newly invented pharmacologically active moieties may have poor druglikeness and may require chemical modification to become drug-like enough to be tested biologically or clinically.

From lead compound to drug



A Typical Drug Discovery Cascade



Opera ,T.I. *J Comput. Aided Mol Des* **2002**, 16, 323-334

From lead compound to drug
Lipinski's Rules (Pfizer)

HTS Drug like (Rule of 5)

Molecular weight ≤ 500

Hydrogen Bond acceptors ≤ 10

Sum of N and O

Hydrogen Bond Donors ≤ 5

Sum of NH and OH

Lipophilicity ClogP<5

Drug like behavior

Lead-likeness

Molecular weight ~300

Fewer Hydrogen Bond Acceptors

Sum of N and O

Lipophilicity ClogP<3

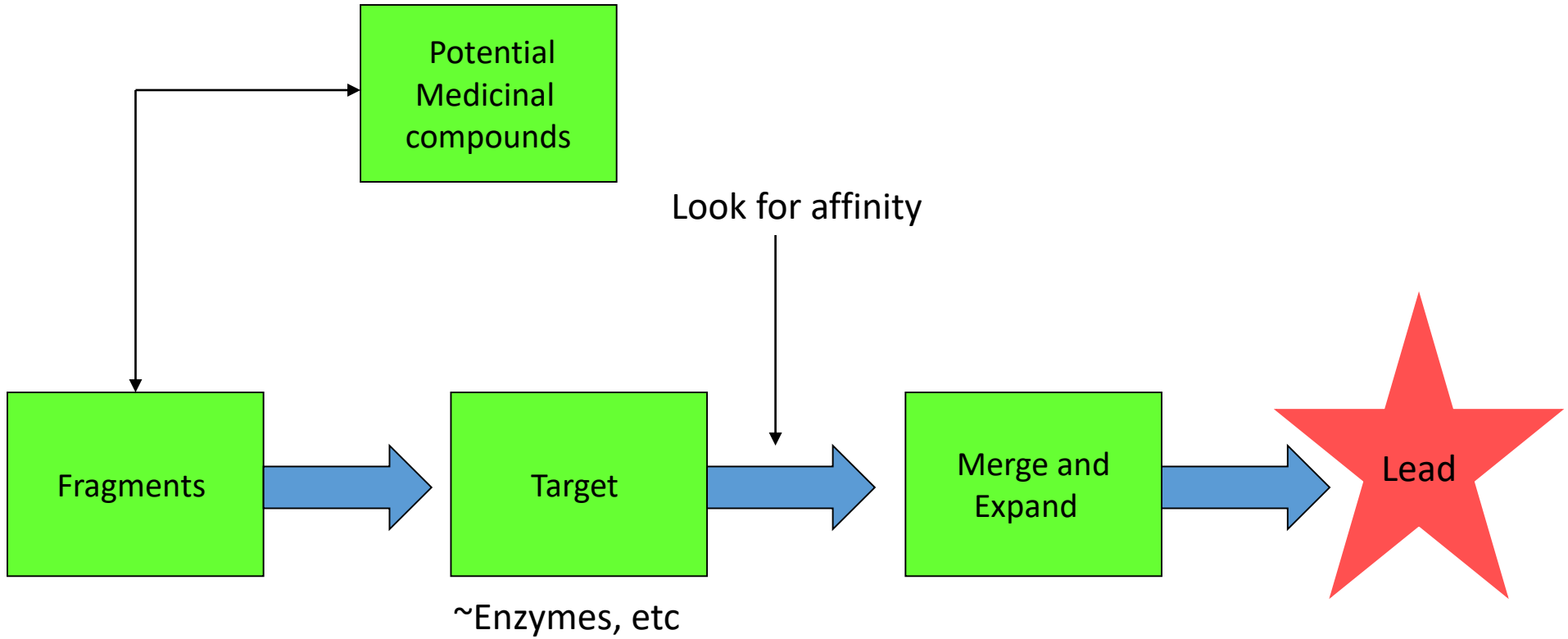
Low to high affinity for the target receptor

Lead like behavior

Congreve, M. et al. Drug Disco. Today.**2003**,8, 876-877
Lipinski, C.A. et al. Adv.Drug Deli.Rev.**1997**,23,3-25.

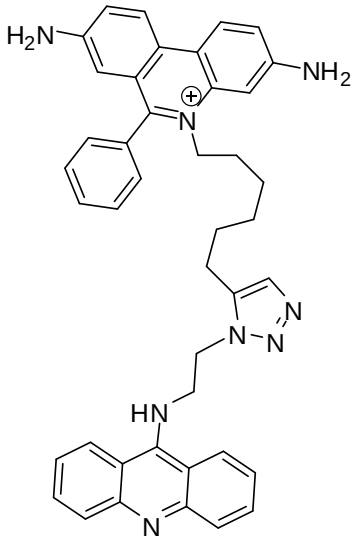
Fragnomics: Fragment Based Drug Design

- An approach that uses small and relatively simple molecules to make lead compounds

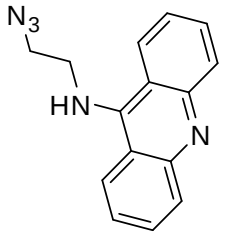


Fragnomics: Fragment Based Drug Design

Conventional HTS approach



Fragment based drug design



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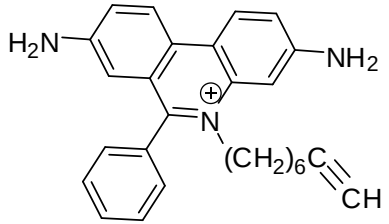


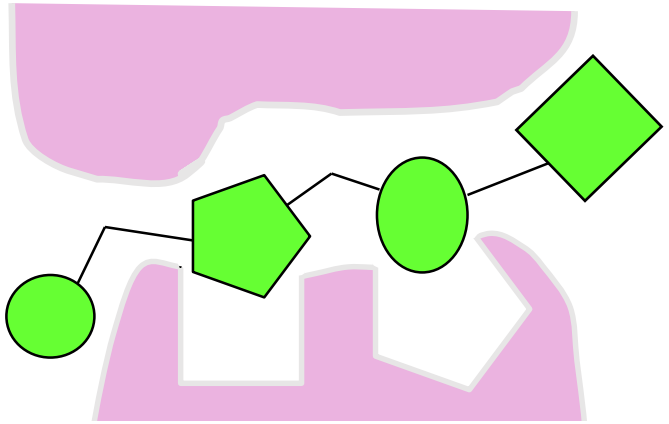
K_d>100µM

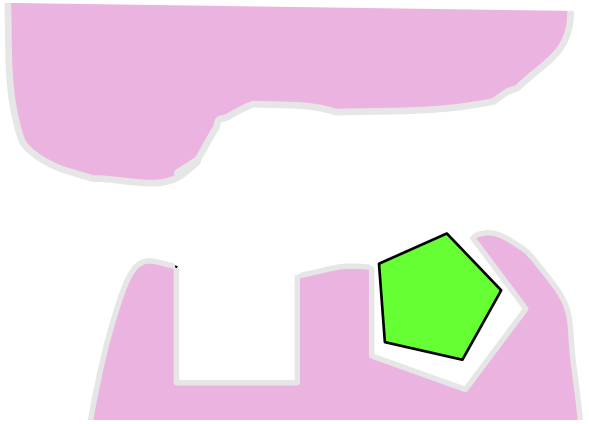
Erlason D.A, McDowell RS, O'Brien T. *J Med Chem.* **2004**, **47**:3463-82

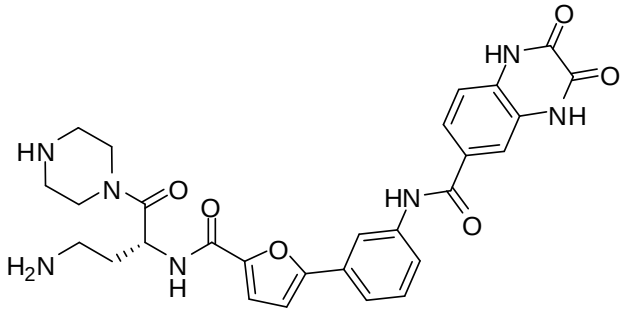
Lewis, W.G.et al *Angew. Chem. Int. Ed. Engl*. **2002**, 41,1053-1057

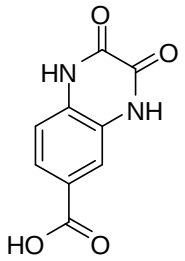
What Qualifies Compounds to be Fragments?

- Molecular Weight Mr ~300 Da
- H-bond donors (HBD) <3
- H-bond acceptors (HBA) <3





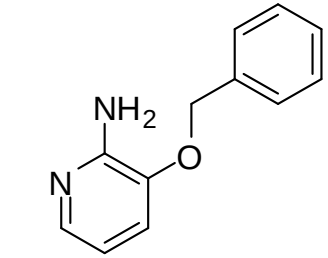




Congreve, M. et al. *Drug Discov.Today* **2003**,8, 876-877

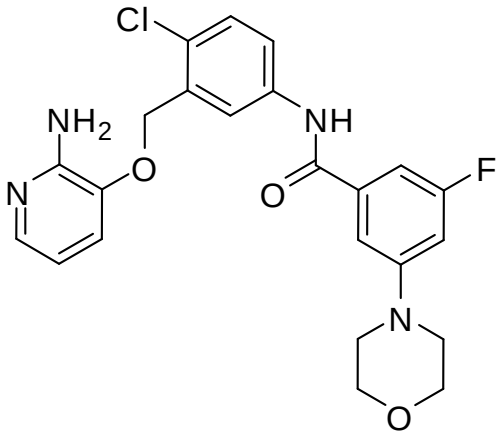
What Qualifies Compounds to be Fragments?

- Clog P <3
  - A measure of Lipophilicity of a compound
- Polar Surface Area (PSA) <60
  - A measure of permeability through the cell membrane.



Fragment

Clog P=1.92
PSA=48.14

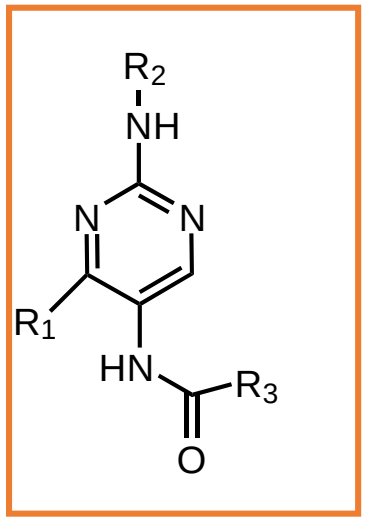


Lead for protein kinase inhibitor

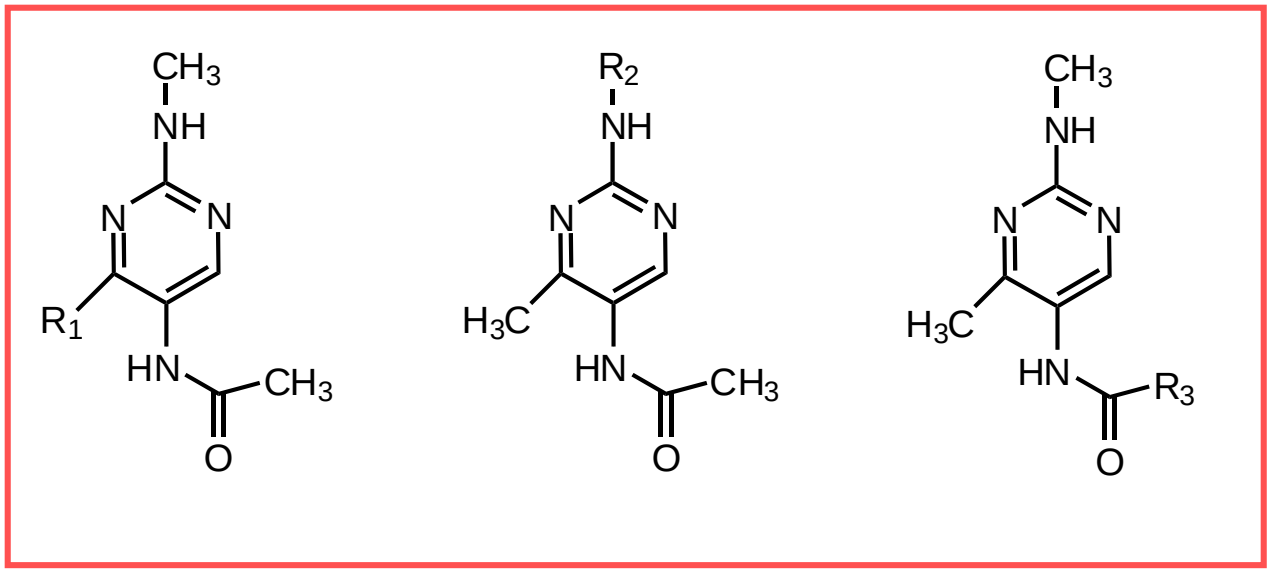
Clog P=3.07
PSA= 77.6

Congreve, M. et al. *Drug Discov.Today* **2003**,8, 876-877
Ertl, P.et. al. *J.Med.Chem*. **2000**, 43,3714-3717

Conventional HTS vs. Fragonomics Based on Central Scaffold



100 X R_1
100 X R_2, and
100 X R_3 yields

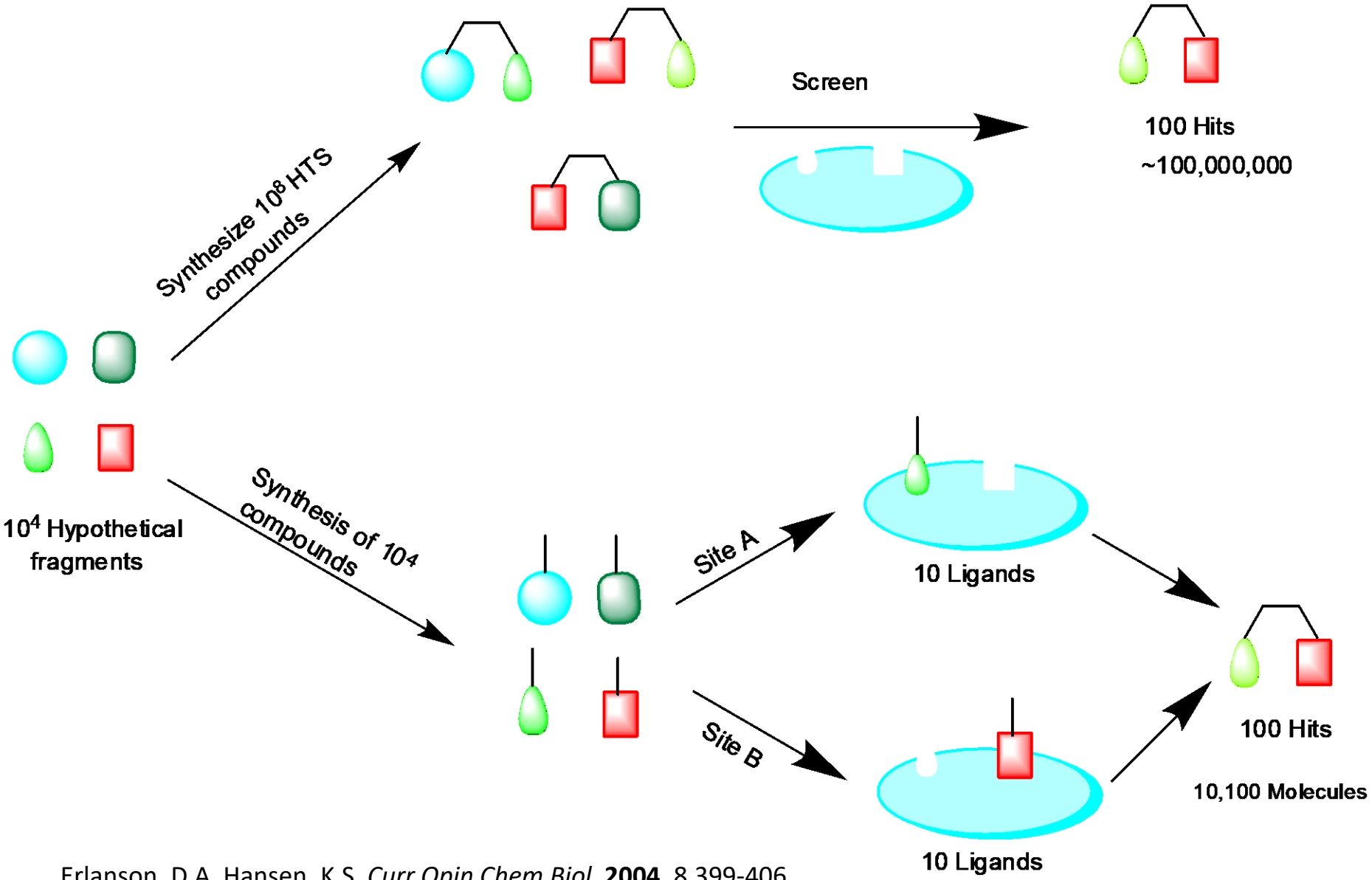


A library with 1 million compounds

Variations yield a library of only 300 compounds

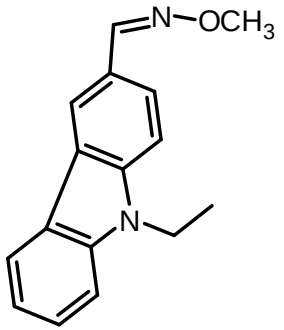
Carr, R, and Hann, M. *Modern Drug Discov*. **2002,** 45-48

Conventional (HTS) and Fragment Based Drug Design



Erlanson, D.A, Hansen, K.S. *Curr Opin Chem Biol*. **2004**, 8,399-406.

Fragment-Based Design : Protein Kinase Inhibition



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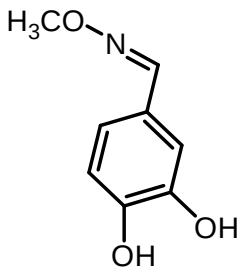

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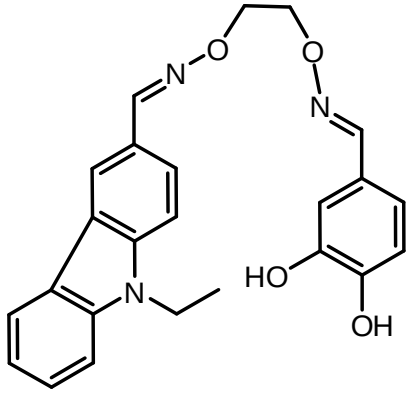
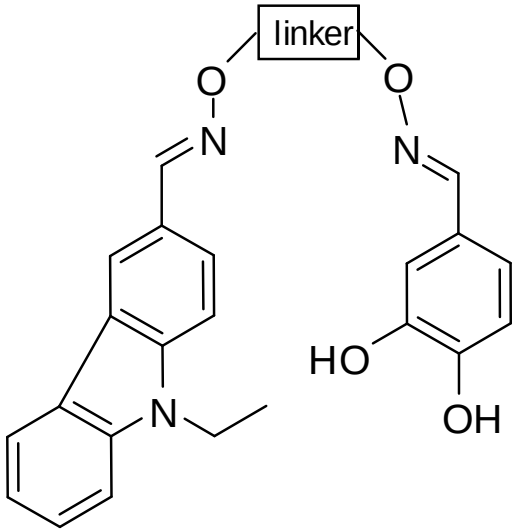


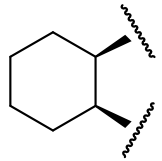
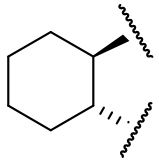
The **half maximal inhibitory concentration (IC_{50})** is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit an enzyme by half.

Compound	IC_{50}μM			
	C-Src	Fyn	Lyn	Lck
[7]	41± 5	>1000	>1000	>1000
[16]	40± 16	64± 50	400± 170	>500
[7,16]	0.064± 0.038	5.0± 2.4	13 ± 2.4	>250

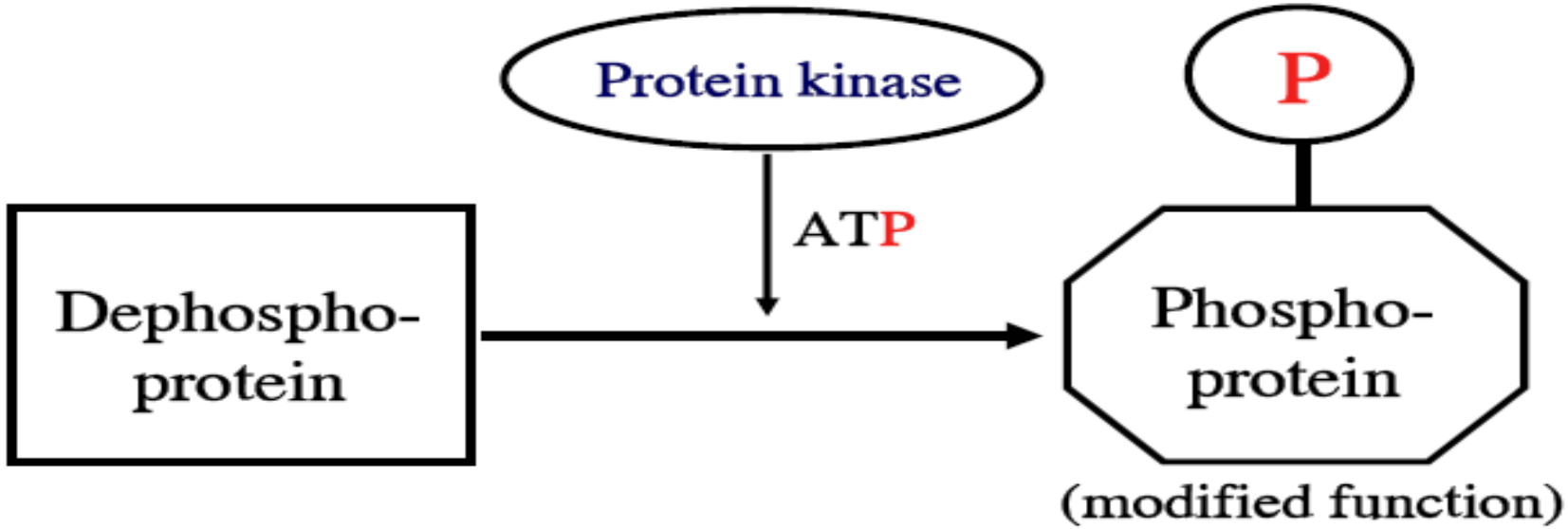
D.J.Maly, I.C.Choong and J.A, Ellman,Proc.Natl.Acad.Sci.USA,**2000**,97,2419-2424

Correlation of linker structure with IC_{50} values for c-Src Inhibition



Entry	Compound	Linker	c-Src IC_{50}, μM
1	7,16, n=2	$-(CH_2)_n-$	0.064±0.038
2	7,16, n=3	$-(CH_2)_n-$	1.1±0.2
3	7,16, n=4	$-(CH_2)_n-$	6.5±3.0
4	7,16, n=5	$-(CH_2)_n-$	6.5±0.8
6	7,16, n=6	$-(CH_2)_n-$	5.3±2.1
7	7,16, cis		1.2±0.6
8	7,16, trans (1R,2R)		0.62±0.02

Proteína Quinase



Proteína Quinase

- Tirosina, serina/treonine e histidina quinases.

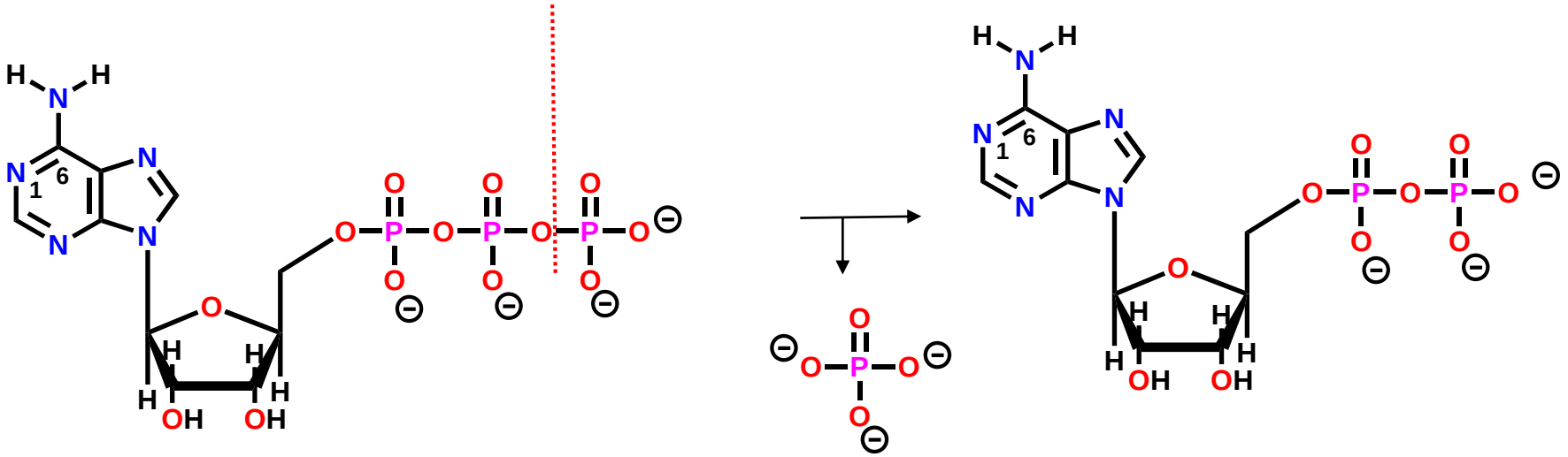
- O ATP é um cofactor.

- Enzimas que catalisam as reações de fosforilação de proteínas.

- Aproximadamente 500 genes codificando para proteina quinases.

- Proteina quinases podem estar no citoplasma ao acoplados a receptors (fatores de crescimento).

- A Superexpressão pode estar correlacionada com cancer.



Proteína Quinase

Sítio Ativo

- Contém o sítio de ligação do substrato.
- Contém o sítio de ligação do co-fator ATP.
- O sítio de ligação do ATP é semelhante mas não identico para todas as quinases.
- Permite a seletividade dos inibidores.

Small molecule inhibitors

Various protein tyrosine kinase inhibitors

TYRosine PHOSPHorylation INhibitors

↓

tyrophosphins

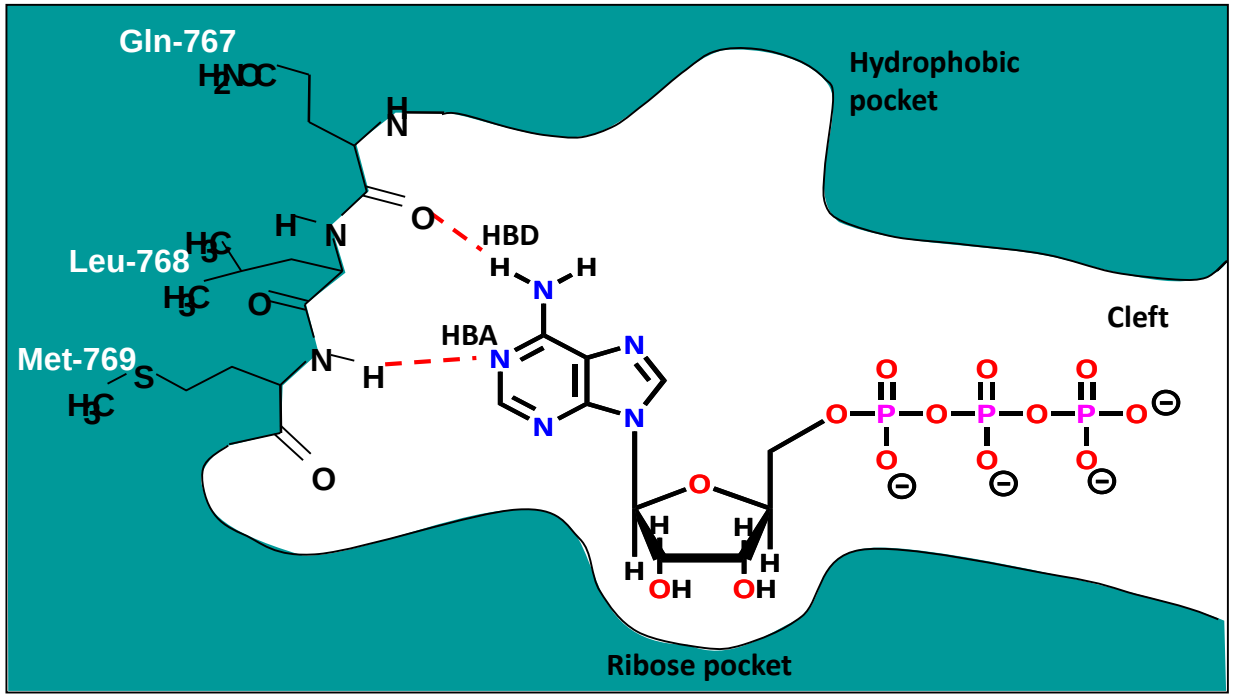
Competitive with substrate (eg.Itaconic acid).

Competitive with ATP (Quinolines).

Proteína Quinase

Sítio de ligação do ATP

- *Hydrophobic pocket* em frente ao *pocket* de ligação da ribose.
- O gatekeeper residue é um amino ácido na entrada do *Hydrophobic pocket* .
- O tamanho desse resíduo é importante para o *desenho* de drogas.
- Os amino ácidos do binding *pocket são importantes para o desenho* de drogas.



Inibidores de Proteína Quinase

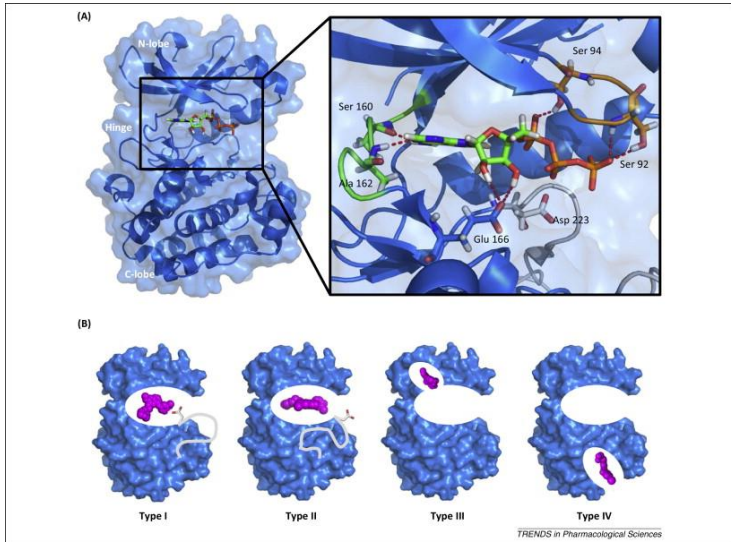
- Tipo I atuam sobre a conformação ativa da enzima.
- Tipo I ligam o sítio de ligação do ATP bloqueando o acesso ao ATP
- Tipo II atuam sobre a conformação inativa da enzima.
- Tipo II estabilizam a conformação inativa.
- Tipo II são mais seletivos

Type I inhibitors

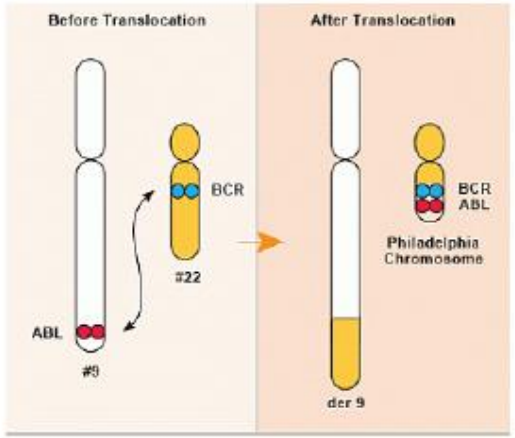
Gefitinib, erlotinib, SU11248 and seliciclib

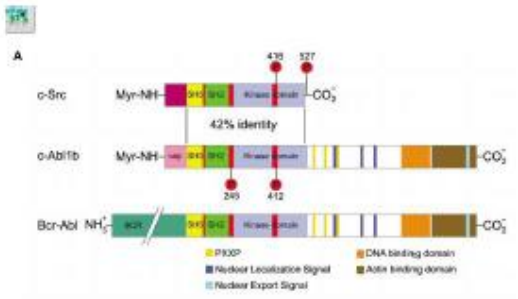
Type II inhibitors

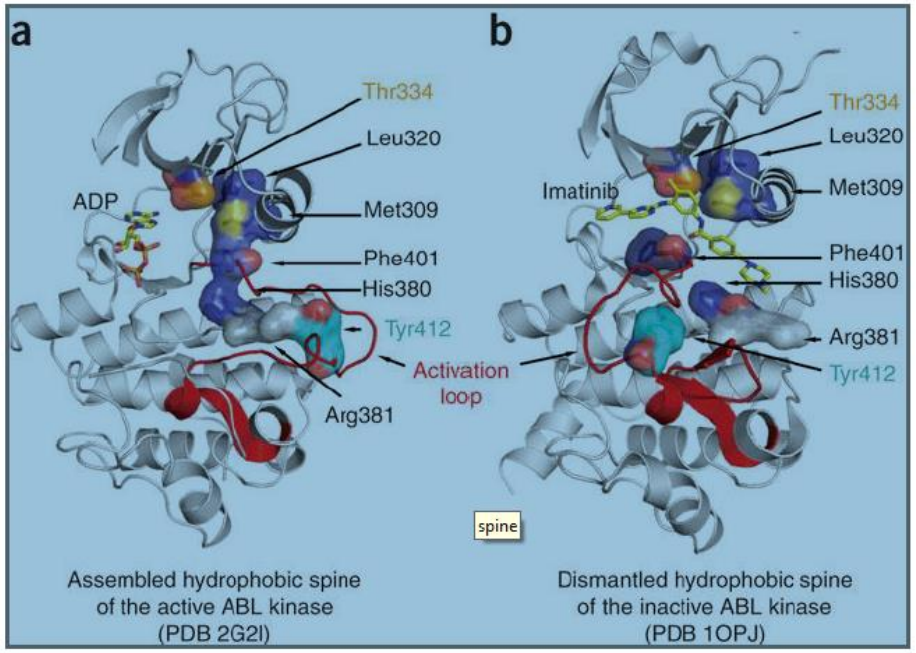
Imatinib, lapatinib, sorafenib and vatalanib



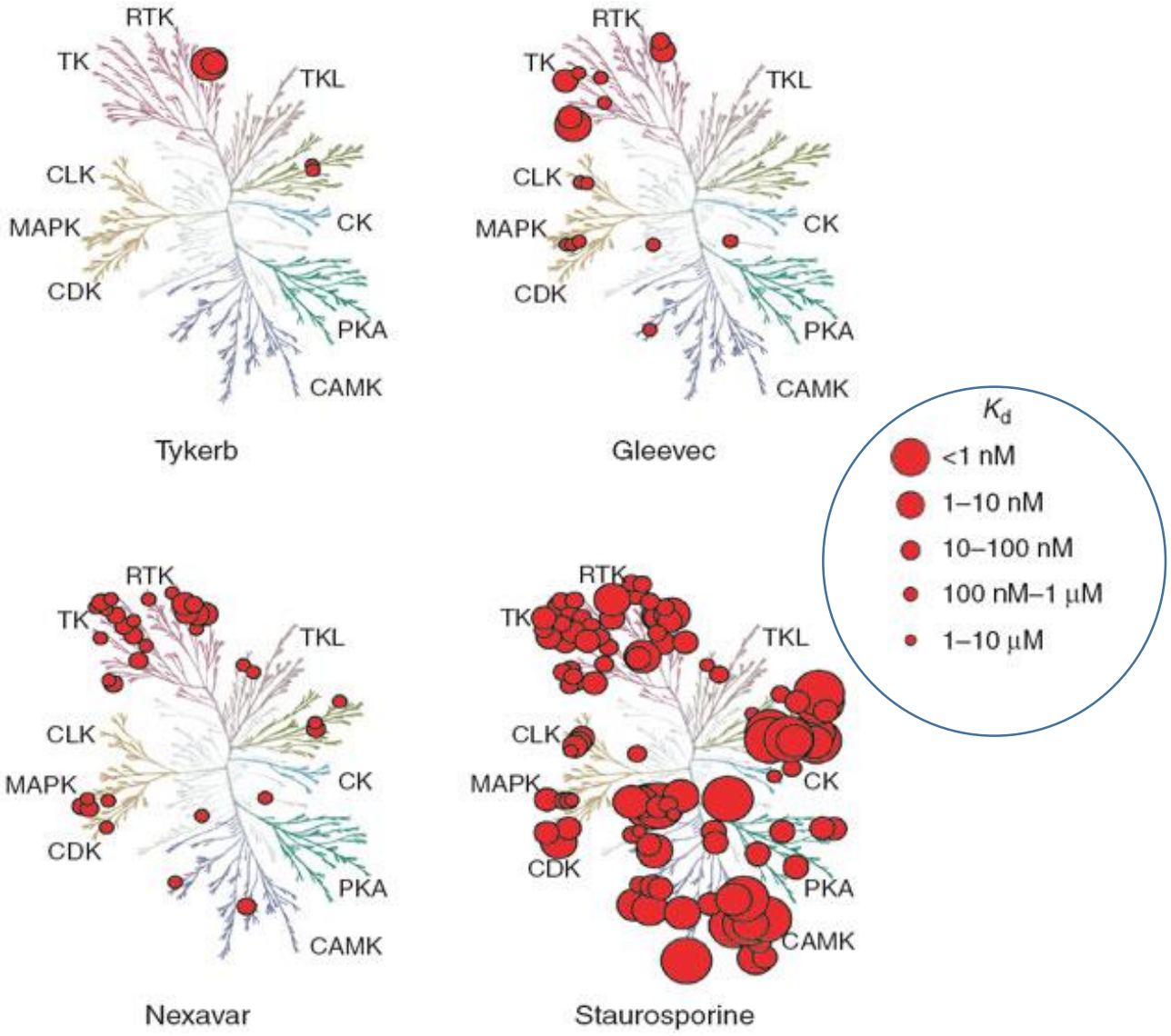
Leucemia mieloide crônica







One Drug Binds to Multiple Targets

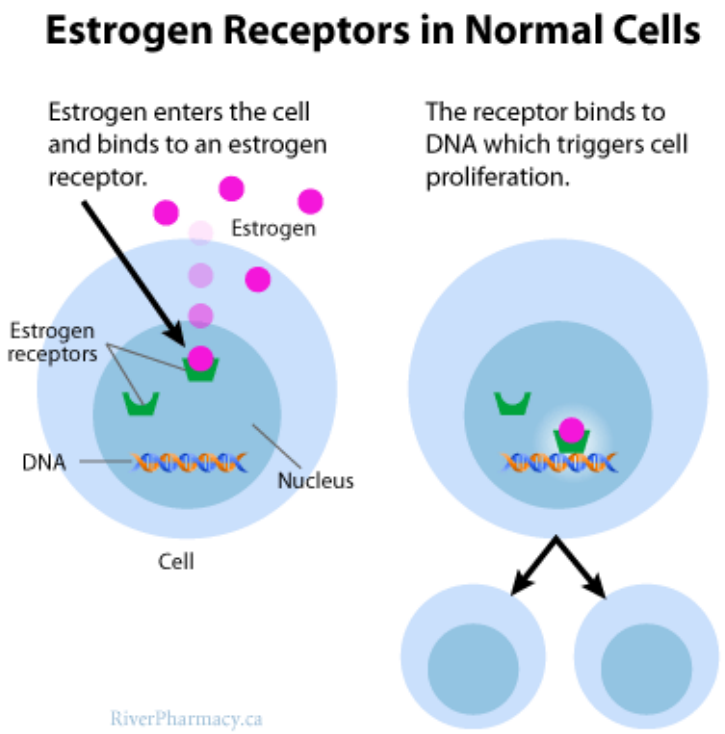


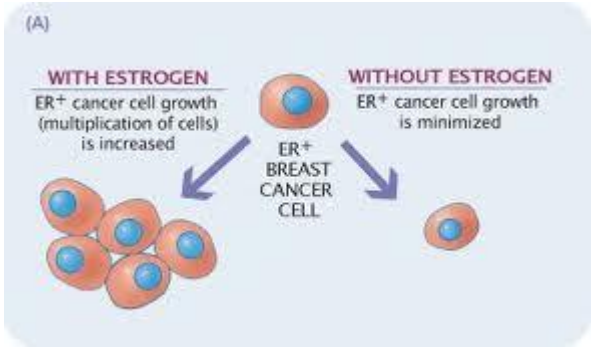
- Tykerb – Breast cancer
- Gleevac – Leukemia, GI cancers
- Nexavar – Kidney and liver cancer
- Staurosporine – natural product – alkaloid – uses many e.g., antifungal antihypertensive

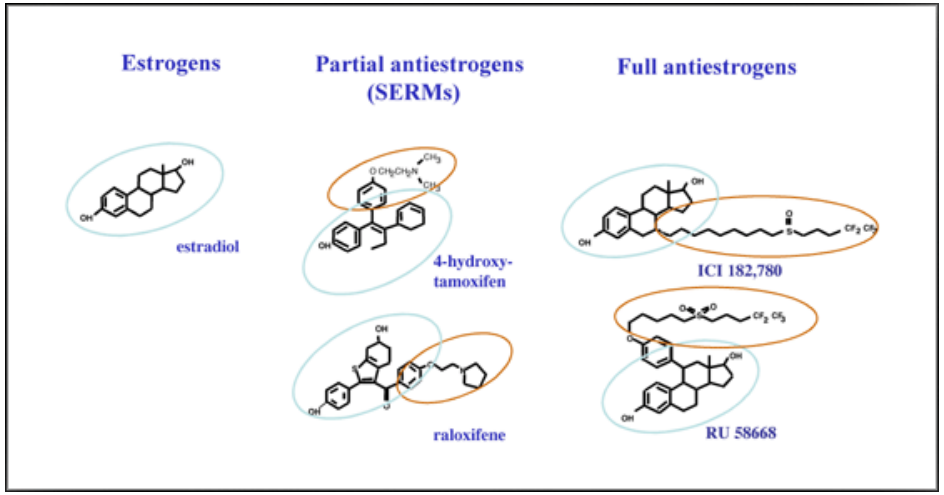
Collins and Workman 2006
Nature Chemical Biology 2 689-700

Especificidade das drogas

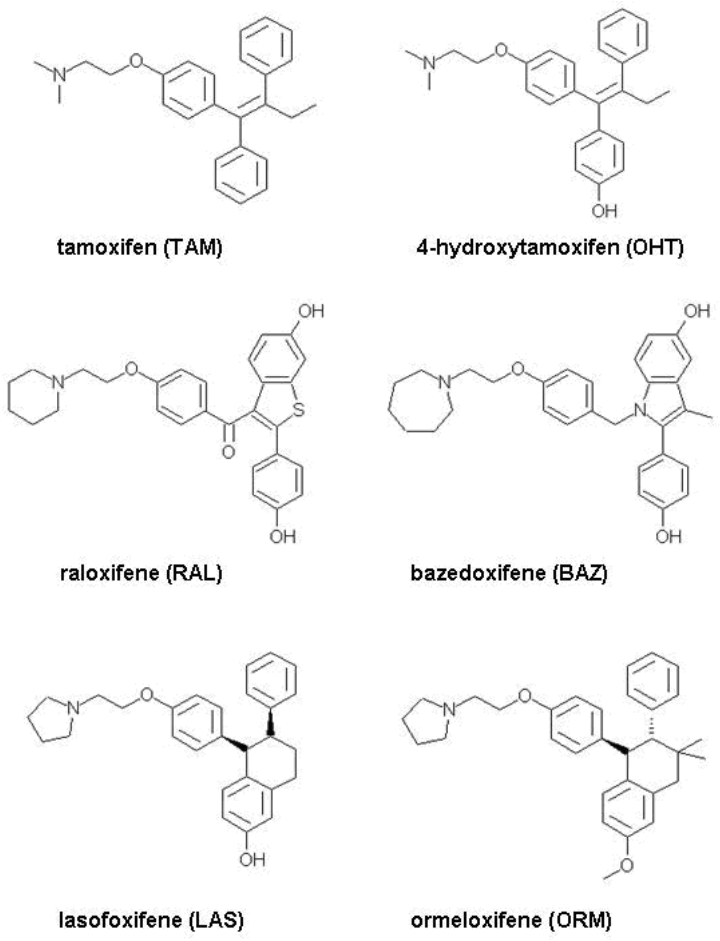
Selective Estrogen Receptor Modulators (SERM)







Selective Estrogen Receptor Modulators (SERM)

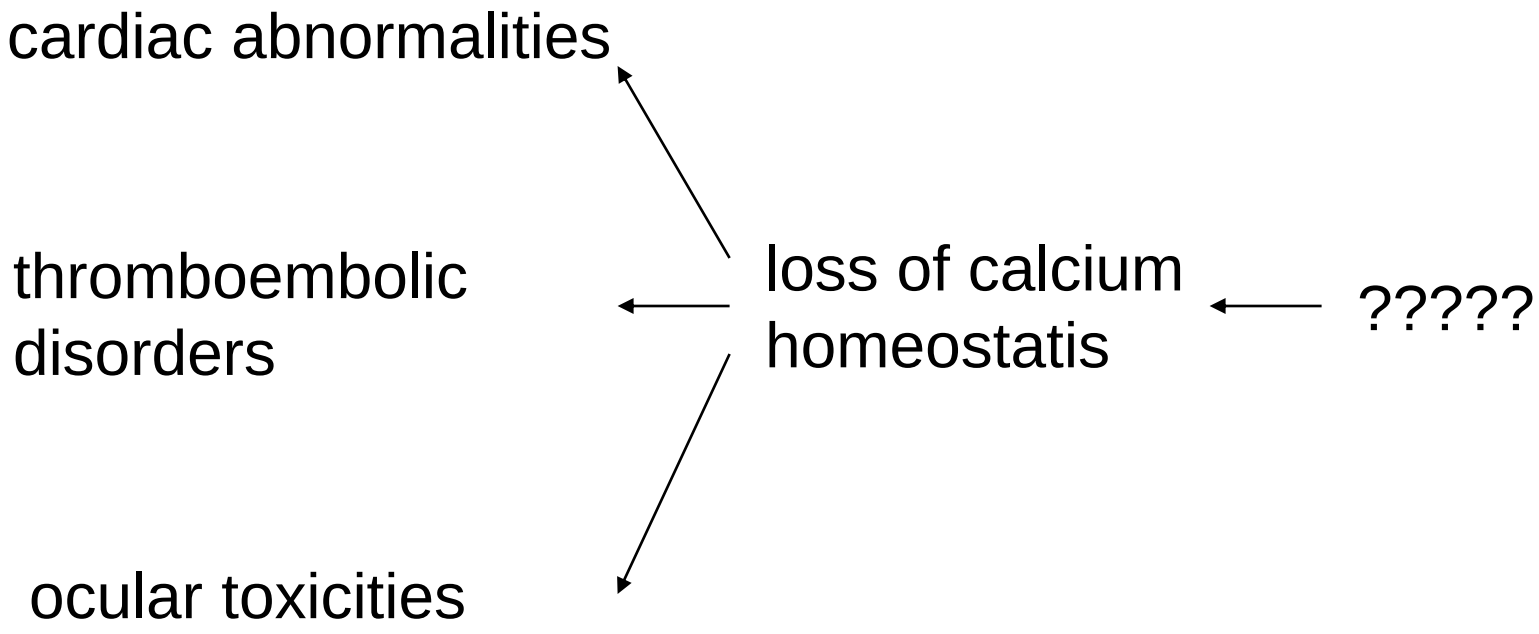


- One of the largest classes of drugs
- Breast cancer, osteoporosis, birth control etc.
- Amine and benzine moiety

Side Effects - The Tamoxifen Story

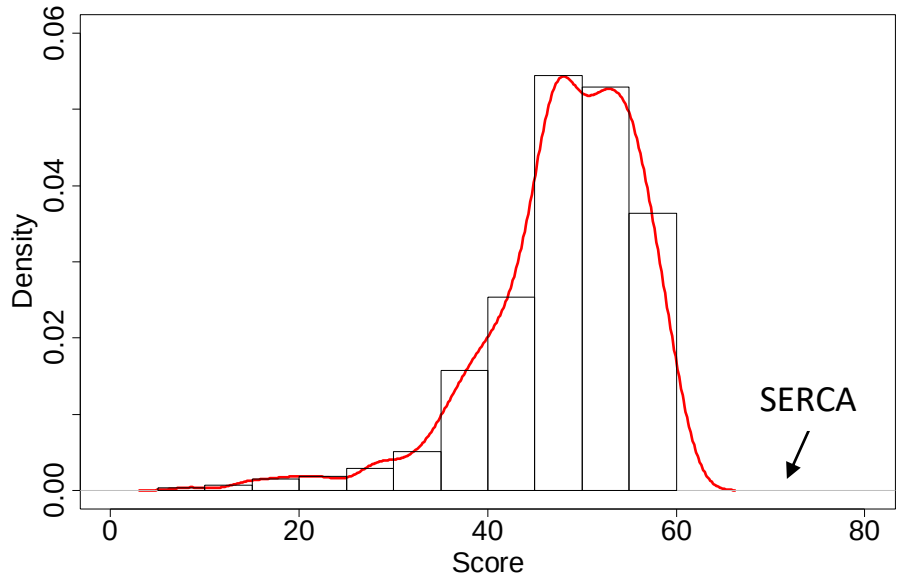
PLoS Comp. Biol., 2007 3(11) e217

Adverse Effects of SERMs



PLoS Comp. Biol., 2007 3(11) e217

Ligand Binding Site Similarity Search On a Proteome Scale

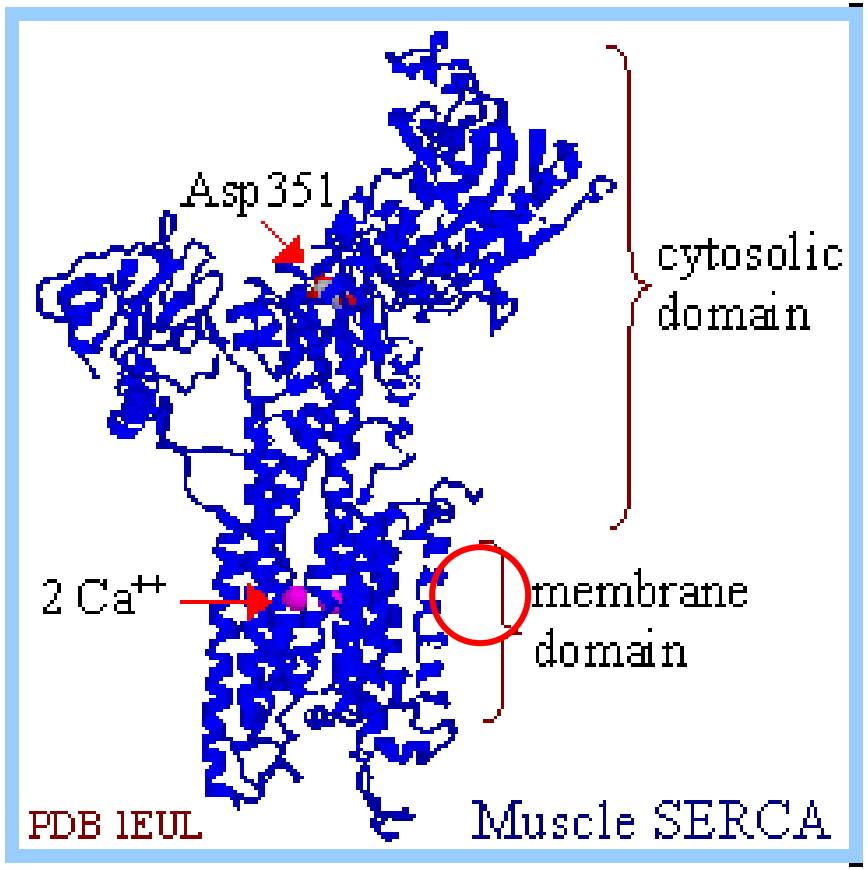


- Searching human proteins covering ~38% of the drugable genome against SERM (selective estrogen receptor modulators) binding site
- Matching ***Sacroplasmic Reticulum* (SR) Ca2+ ion channel** ATPase (SERCA) TG1 inhibitor site
- ERα ranked top with p-value<0.0001 from reversed search against SERCA

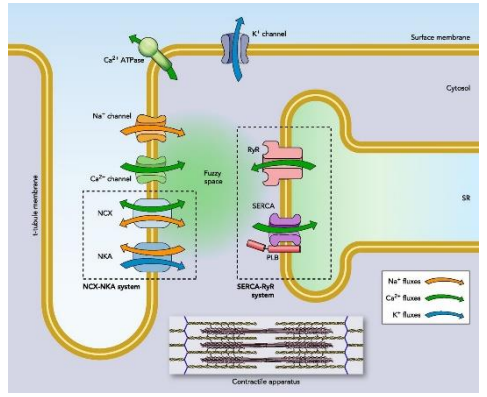
Side Effects - The Tamoxifen Story

PLoS Comp. Biol., 2007 3(11) e217

Structure and Function of SERCA



- Regulating cytosolic calcium levels in cardiac and skeletal muscle
- Cytosolic and transmembrane domains
- Predicted SERM binding site locates in the TM, inhibiting Ca2+ uptake



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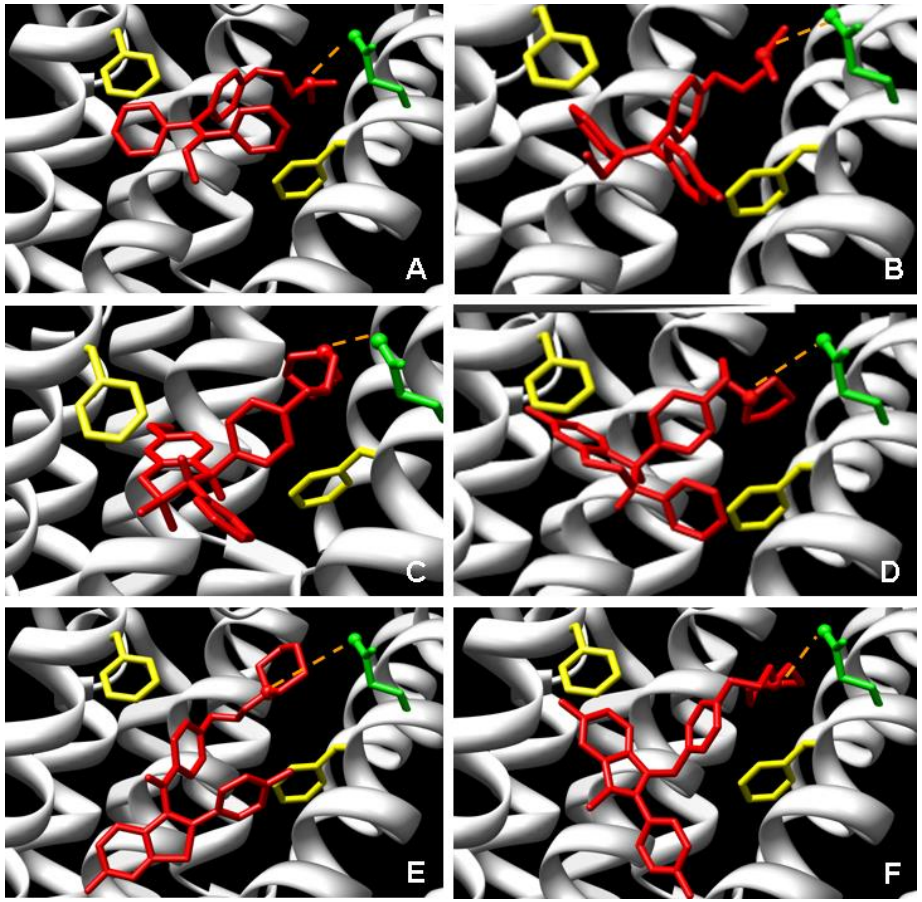
Binding Poses of SERMs in SERCA from Docking Studies



6 SERMS A-F (red)

- Salt bridge interaction between amine group and GLU
- Aromatic interactions for both N-, and C-moiety

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The Challenge

- Design modified SERMs that bind as strongly to estrogen receptors but do not have strong binding to SERCA, yet maintain other characteristics of the activity profile

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