ROLE AND APPLICATIONS OF COMBINATORIAL CHEMISTRY IN MODERN DRUG DISCOVERY

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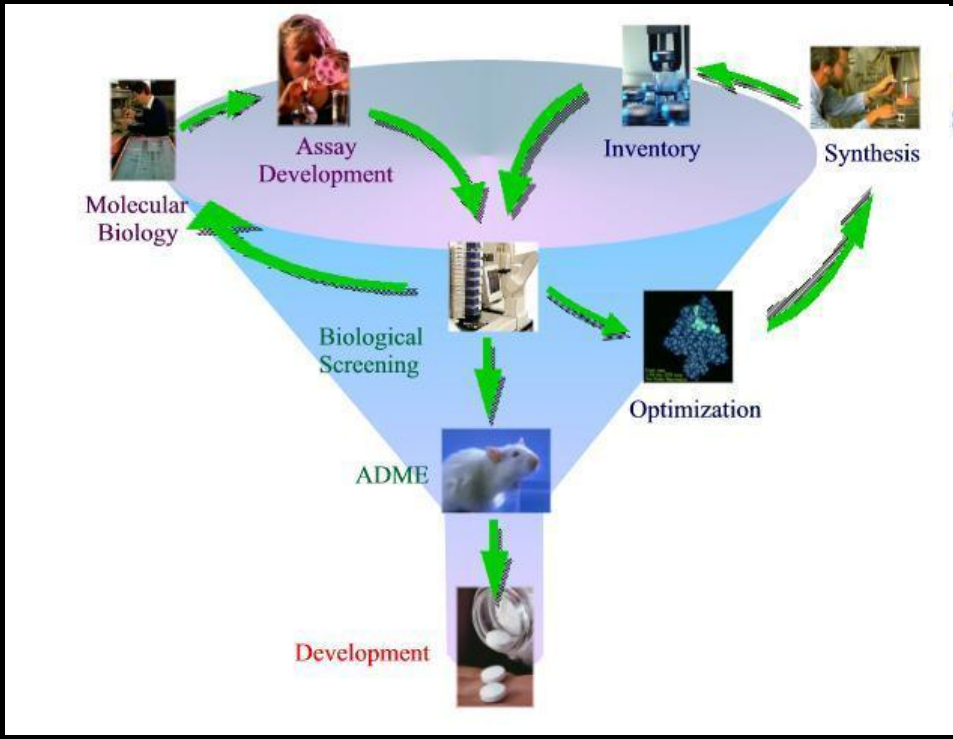
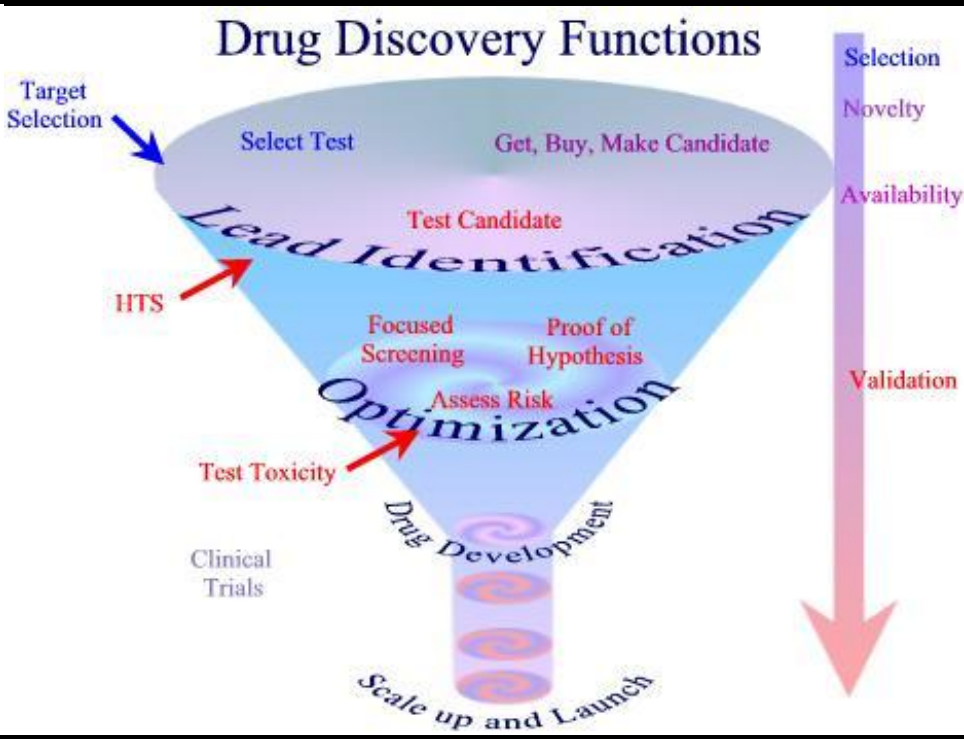
Drug Discovery-A concerted effort



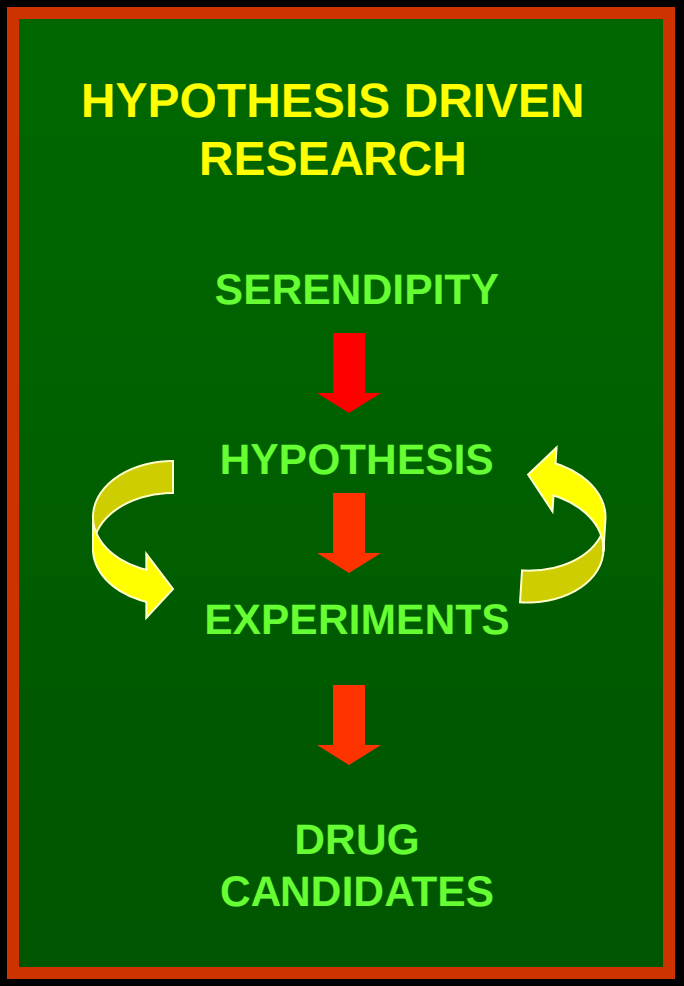


DRUG DISCOVERY RESEARCH

Early drug discovery paradigm

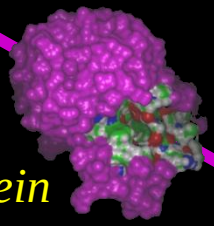
Drug discovery has always been difficult, and to a great extent, dependent upon serendipity. Drug discovery has required brilliant minds to toil to isolate and identify cures for disease.

Physiology-based approach has been the traditional way.

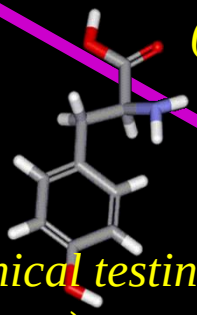


DRUG DISCOVERY & DEVELOPMENT

Identify disease



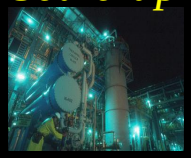
Isolate protein involved in disease (2-5 years)



Find a drug effective against disease protein (2-5 years)

Preclinical testing (1-3 years)

Scale-up



File IND

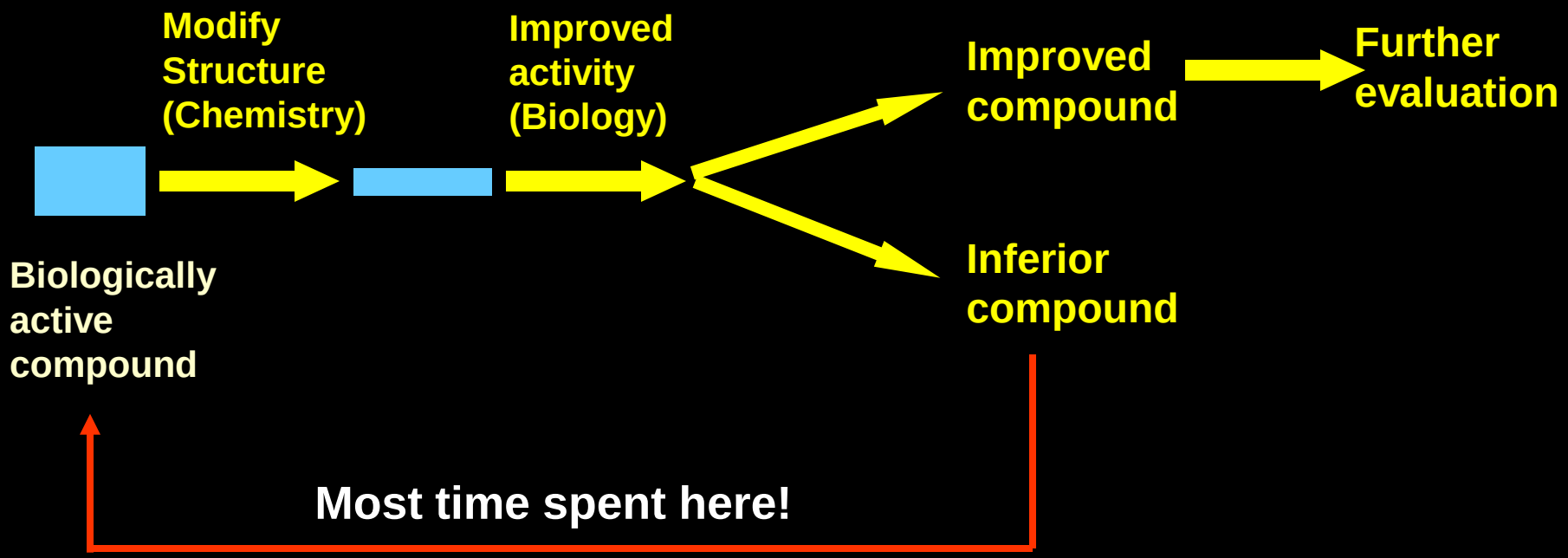
Human clinical trials (2-10 years)

Formulation

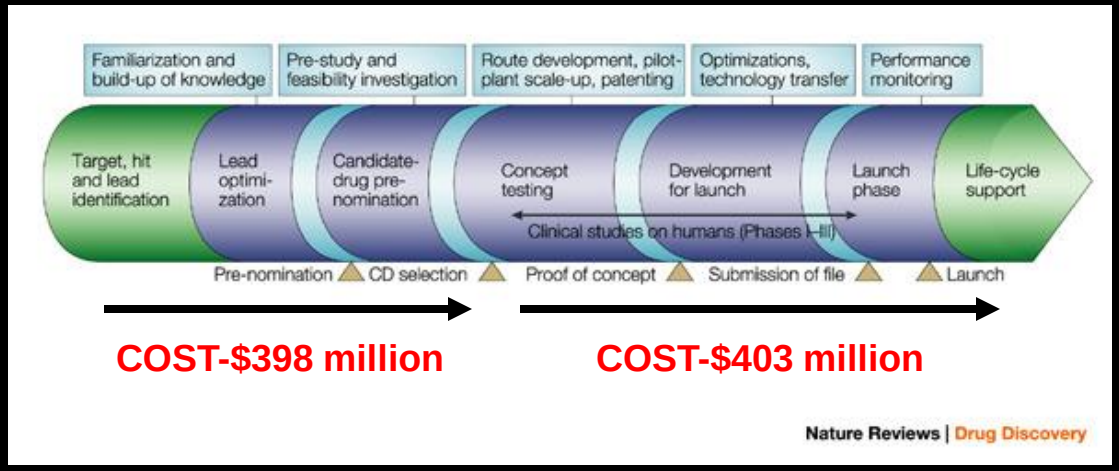
File NDA

FDA approval (2-3 years)

TRADITIONAL DRUG DISCOVERY



COST OF NEW DRUG



1995-
400 m

2002-
800 m

2004-
1.2 b

Infrastructure

Elaborate chemical and biological databases

Better & Hi-Tech facilities Automation

Executive's expenditures

TECHNOLOGY IS IMPACTING THIS PROCESS

Identify disease

GENOMICS, PROTEOMICS & BIOPHARM.

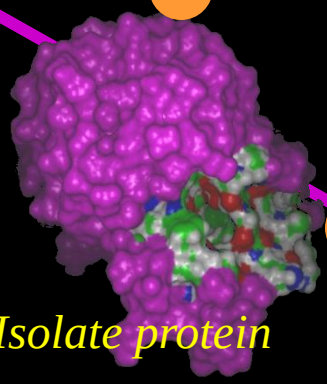
Potentially producing many more targets and "personalized" targets

HIGH THROUGHPUT SCREENING

Screening up to 100,000 compounds a day for activity against a target protein

VIRTUAL SCREENING

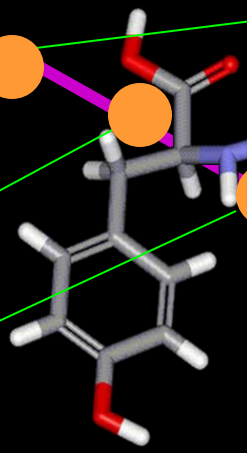
Using a computer to predict activity



Isolate protein

COMBINATORIAL CHEMISTRY

Rapidly producing vast numbers of compounds



Find drug

MOLECULAR MODELING

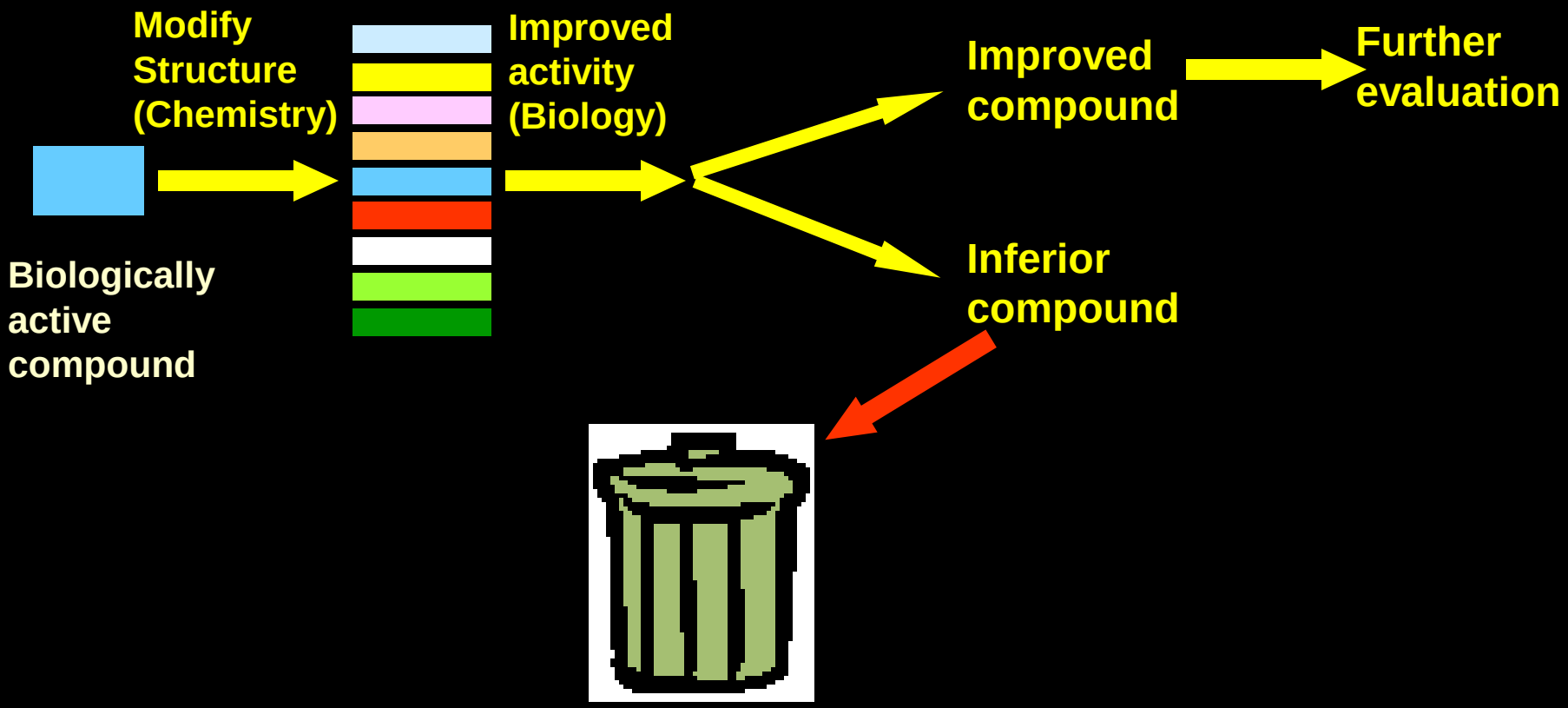
Computer graphics & models help improve activity

Preclinical testing

IN VITRO & IN SILICO ADME MODELS

Tissue and computer models begin to replace animal testing

MODERN DRUG DISCOVERY



MODERN PARADIGM- TARGET-BASED DRUG DISCOVERY

- **Target is defined as a single gene, gene product or molecular mechanism that has been identified on the basis of genetic analysis or biological observation.**
  - **Genetic targets- represent genes or gene products that, in specific diseases, have been found to carry mutations or that confers a higher disease risks.**
  - **Mechanistic targets- represent receptors, genes, enzymes, and so on that usually are not genetically different from normal population. Originate from biological observations**

STEPS FOR TARGET-BASED DRUG DISCOVERY

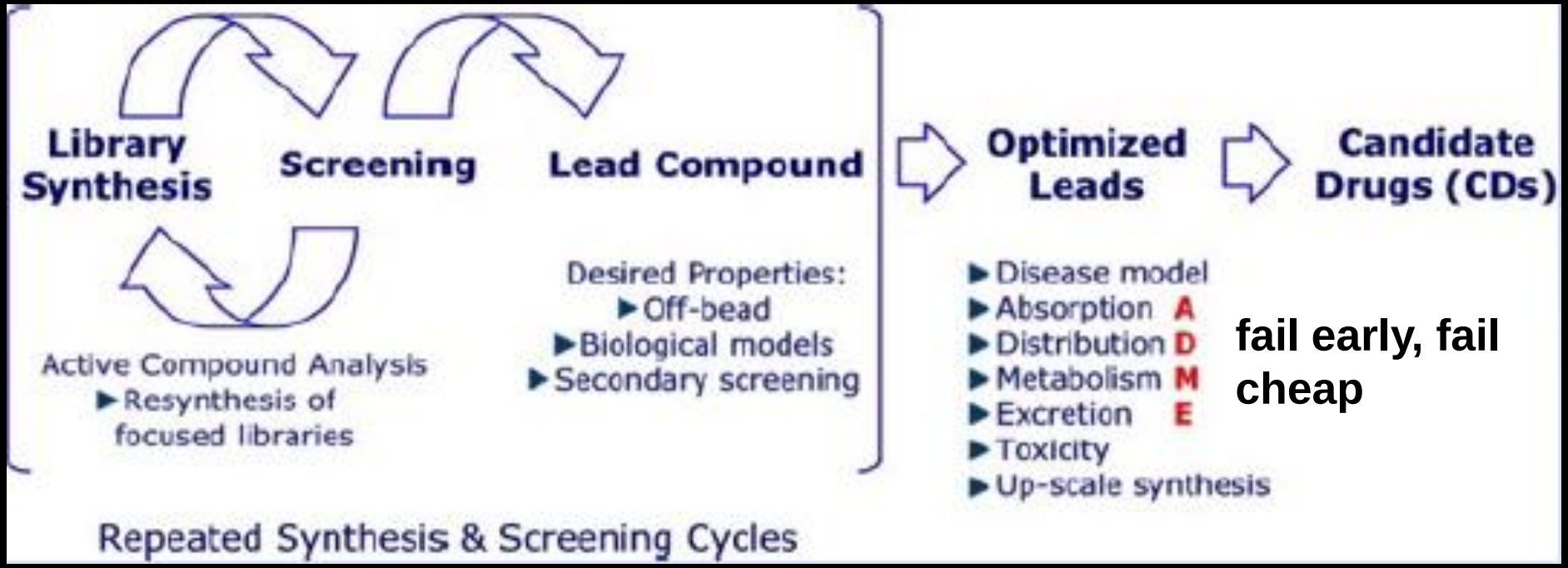
- **Target identification**: where the exact target and the specific population is identified.
- **Target validation:** where the therapeutic value of the target in the specified patient population is determined.
- **Assay development:** where the target is expressed in a HTS system.
- **Lead identification:** where compound libraries are screened to identify target-selective compounds.
- **Lead optimization:** where the lead structures are optimized for target affinity and selectivity.

MOLECULAR TARGETS OF KNOWN DRUGS

Target	Number of drugs
GPCRs	217
Enzymes	135
Hormones and factors	53
Unknown	34
Ion channels	24
Nuclear receptors	10
DNA	10

- **With the unraveling of human genome the number of targets for drug discovery was believed to increase.**
- **Human genome has 30,000 genes which is equivalent to that of *Drosophila*.**

FUNDAMENTALS IN NEW AGE



RULE OF FIVE OR LIPINSKI'S RULE

Compound has poor absorption/permeation properties, if:

- **There are more than 5 H-bond donors**
- **There are more than 10 H-bond acceptors**
- **The Molecular Mass is over 500Da**
- **logP value is over 5**

(Ref. Lipinski et al., *Advanced Drug Delivery Reviews* 1997, 23, 3-25)

SCREENING

- Ability to detect changes in infectivity, enzyme activity etc. constitute the basis of screen.
- Screens usually involve the simultaneous evaluation of **range of molecules** that are likely to exert a desirable effect on a biological process.
- Screens are performed in parallel arrays.
- The more molecules you have the more likely you are identify a ‘HIT’.

Where do these come from?

THE PERFECT SCREENING PROGRAM

- Unlimited collection of unique compounds
- Unlimited access to novel, well defined biological targets
- High Speed automation for maximum throughput
- Unlimited computational resources

THE REALITY

- Limited size of compound collections
  - Conventional collections in the range of 10^6
- Limitations in automation
- Small number of well characterized targets available for screening
- Less than perfect computational resources

THE KEY TO SCREENING SUCCESS

- A large, diverse compound collection
- Validated targets
- A collection of screens optimized for high throughput
- Appropriate automation to handle large scale screening
- Well designed information gathering and analysis system

AUTOMATION AND HTS

- Modern HTS involves screening >100K samples in < 1 month
- HTS is not amenable to manual execution
- Screening assays must be formatted in micro-titer plates (MTP's) (typically 96 or 384 well format)
- Automation usually provides for better precision and speed, at a lower cost (better, faster, cheaper)

CHEMICAL LIBRARIES

Big Pharma-company has compound collection of more than 500,000 compounds

- **As historical collections prepared by traditional synthetic protocols**
- **Large libraries prepared as mixtures**
- **Discrete arrays prepared by parallel synthesis method**
- **Acquiring compounds from universities**

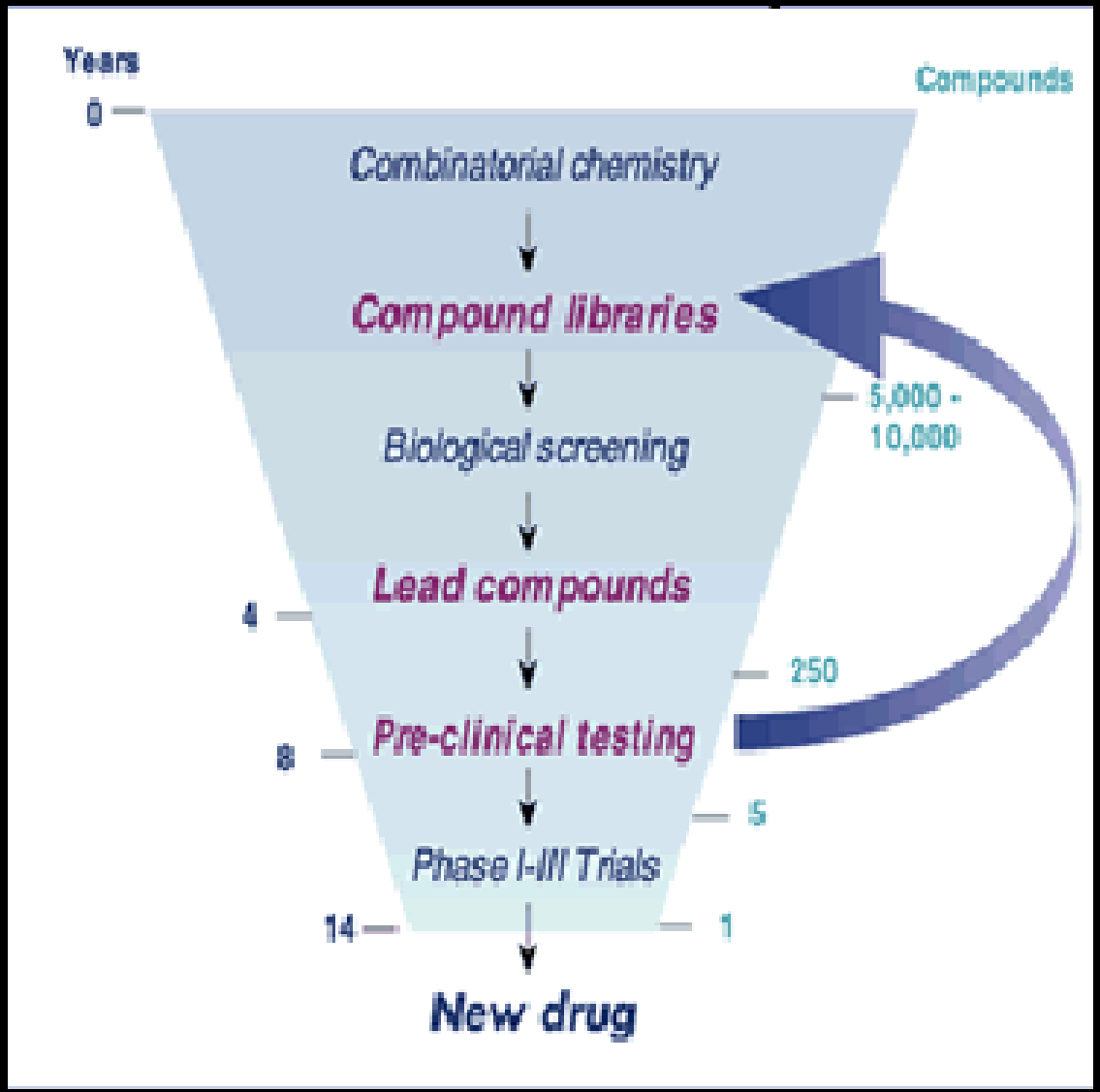
CANDIDATE DRUG MOLECULES

- Available from
  - Natural Sources

    Plants, Microbes,
    Sponges…….
  - Synthetic Sources
    - Compound Collections
    - Multiple molecule Synthesis

Combinatorial Chemistry

CONCEPT OF COMBICHEM



GENERAL CONSIDERATIONS

- Why should I consider combinatorial chemistry?
- What is combinatorial chemistry and why is it useful?
- What types of libraries?
- Design of libraries

THE DRUG DISCOVERY PROCESS- INDUSTRY PRESSURES

- Pharma companies must maximize revenue from patented drugs
- Estimated that large pharmaceutical needs 3 new drugs per year (£300m)
- Efficiencies in drug discovery necessary

THE DRUG DISCOVERY PROCESS - COMBINATORIAL CHEMISTRY

- Combinatorial chemistry can provide vast increases in productivity
- Prevents chemistry ‘bottleneck’ in drug discovery process
- Allows companies to patent sooner
- Increases time for revenue generation
- Offers cost savings to pharmaceutical drug programs

ADVANTAGES TO THE MEDICINAL CHEMIST

- Can impact drug discovery at several stages
- Possible to cover more pharmaceutically relevant space in one library
- Easier to identify multiple activity regions
- Rapidly explore active series
- Can refine compounds to find best pharmacokinetic profile
- Provides many patent examples

MODERN DRUG DISCOVERY

- **Library-size isn’t everything.**
- **Now small more focused libraries are preferred.**

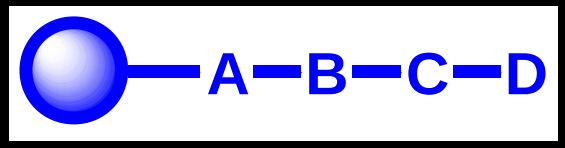
LARGE LIBRARIES

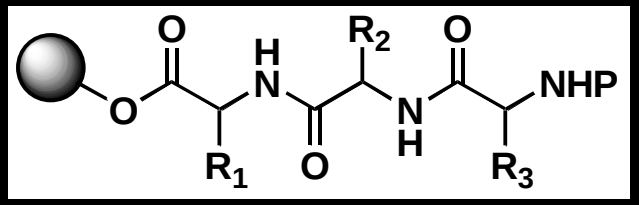
Benefits	Limitations
• Large numbers of compounds produced	• Very small quantities produced
• Cover many permutations	• Frequent deconvolution problems
• Comprehensive template 3D space coverage	• Limited chemistry available
• Good VFM – Efficient use of time and reagents	• Almost exclusively solid phase
	• Validation time is long

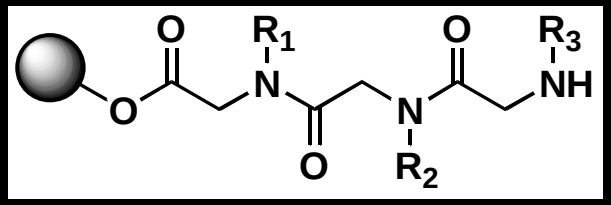
SMALL LIBRARIES

Benefits	Limitations
• Generally 1 compound per well	• Expensive
• Solution / solid phase compatibility	• Can leave diversity ‘gaps’
• More chemistry applicable	• Only covers small regions of 3D space for template
• Makes more of each compound	

LIBRARY TYPES - LINEAR



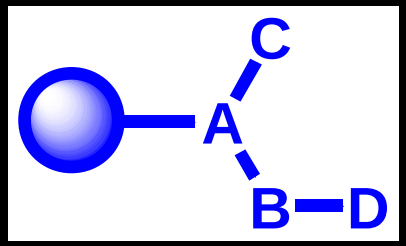


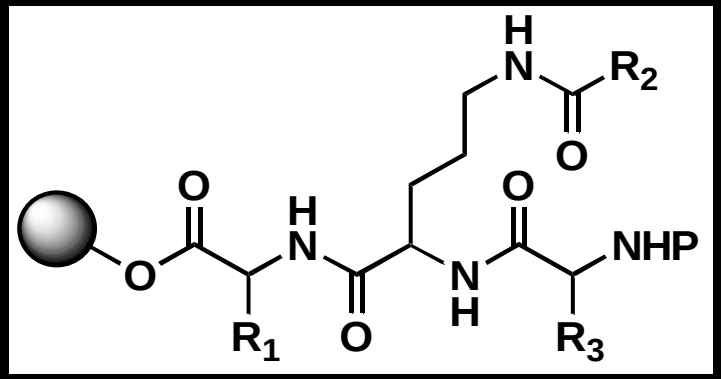


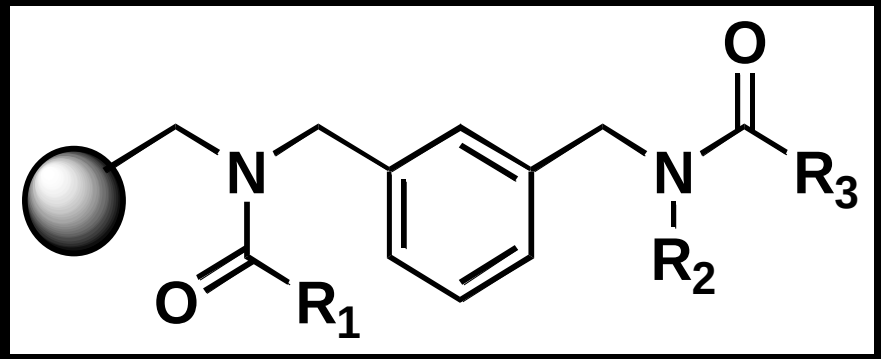
- Peptides
- Most common linear library

- Peptoids
- Synthetically straightforward

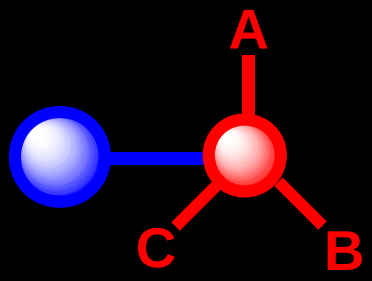
LIBRARY TYPES - BRANCHED

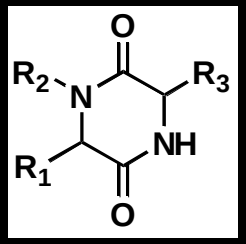




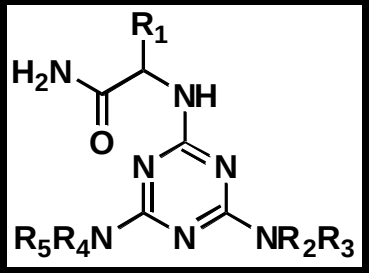


LIBRARY TYPES - TEMPLATES

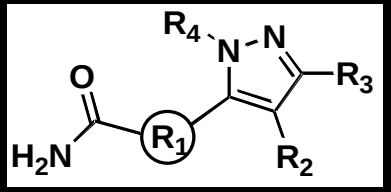




Gordon; *Bioorg. Med. Chem. Lett.*, **1995**, 5, 47



Lebl; *Molecular Diversity, **1996**, 2, 75*



Felder; Tet. Lett. **1996**, 37(7), 1003

LIBRARY TYPES - SYNTHESIS OPTIONS

- **Iterative approach**
- **Positional scanning**

Both susceptible to additive activity.
Multiple motifs may ‘scramble’ info.

- **One-bead-one compound**

Tagging strategy or deconvolution required.
Huge numbers may be synthesised

- **Spatial array**

Relatively small nos. produced. Always know compound identity by position in array

LIBRARY TYPES - ITERATIVE APPROACH

O = one of 20 amino acids

X = all 20 amino acids

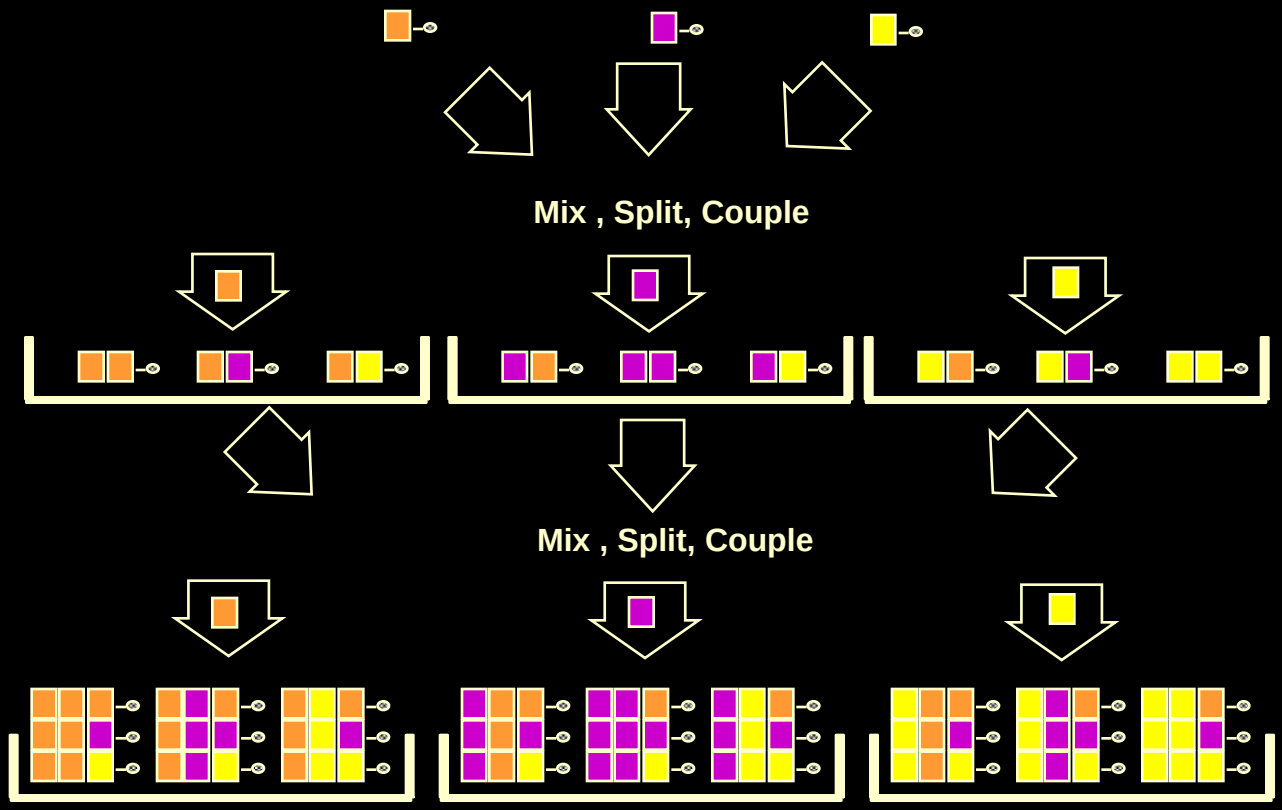
LIBRARY TYPES - POSITIONAL SCANNING

O = one of 20 amino acids

X = all 20 amino acids

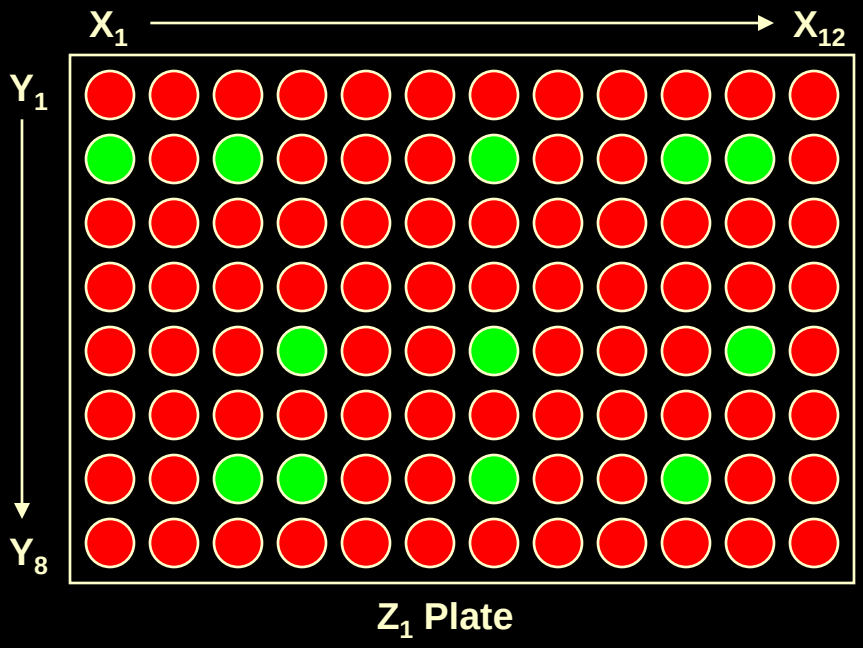
LIBRARY TYPES - ONE-BEAD-ONE COMPOUND

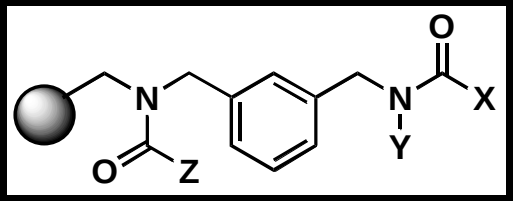
Deconvolution or tagging can be employed to identify the hit depending on the way the compounds are screened



Furka et al- *Int. J Peptide Protein Res,* (1991), 37, 487-493.

LIBRARY TYPES - SPATIAL ARRAYS





THINGS TO CONSIDER

- Development takes longer than you might expect
  - Solid phase routes are difficult to optimize
  - Solution phase usually needs to be purified
  - The interesting monomers usually cause all the problems
- LCMS sometimes gives misleading results
- Always a balance of time versus cost

DESIGN OF LIBRARIES - DIVERSITY

- Plenty of products on the market to measure diversity and display results
- Measure diversity by m+n or mn approach
- Is diversity the best guide to a good library?
- Medicinal chemistry input critical to library success
- Consider biological screen

STRATEGIES USED TO FIND LEAD USING HTT

- Random or diversity driven screening
- Screening of thematic libraries
- Screening of project-directed/ knowledge based/focused libraries
- Virtual synthesis and virtual screening (prior to sample preparation and assay)

RANDOM OR DIVERSITY-DRIVEN SCREENING

A traditional HTS approach

- **Hundreds of thousands of compounds screened**
- **The strategy is most appropriate when little is known about the function or binding requirements of biological targets. (*especially with the advent of whole genome sequencing)***

Drawbacks

- **Highly expensive due to involvement of large volumes of disposables.**

HTS OF THEMATIC LIBRARIES

Libraries are directed towards the biochemical function of the target

- Peptidomimetics as receptor agonists/antagonists
- Scaffolds related to the mechanistic classes of enzymes as potential inhibitors

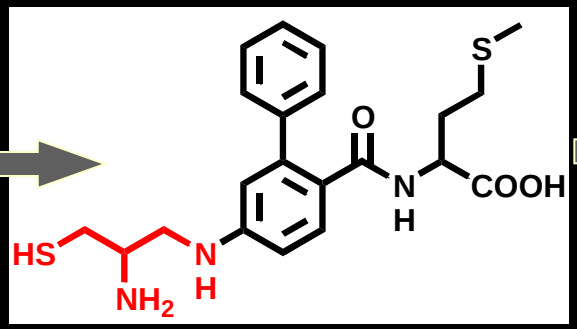
HTS OF PROJECT-DIRECTED LIBRARIES

- Knowledge-based or focused screening
- Most commonly used Combinatorial chemistry strategy by medicinal chemist
- Library selection or design is generally based on the chemical structure of hit/substrate

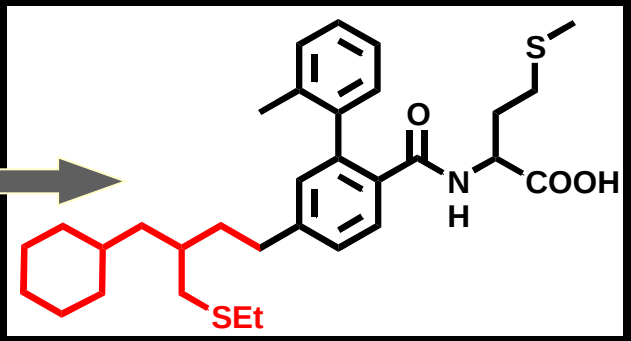
Library of benzylic amines

(Abott Lab)

HTS



Hit



Lead

VIRTUAL SCREENING AND SYNTHESIS

In-silico screening of 350 000 compounds

3000 compounds

HTS for DNA gyrase assay

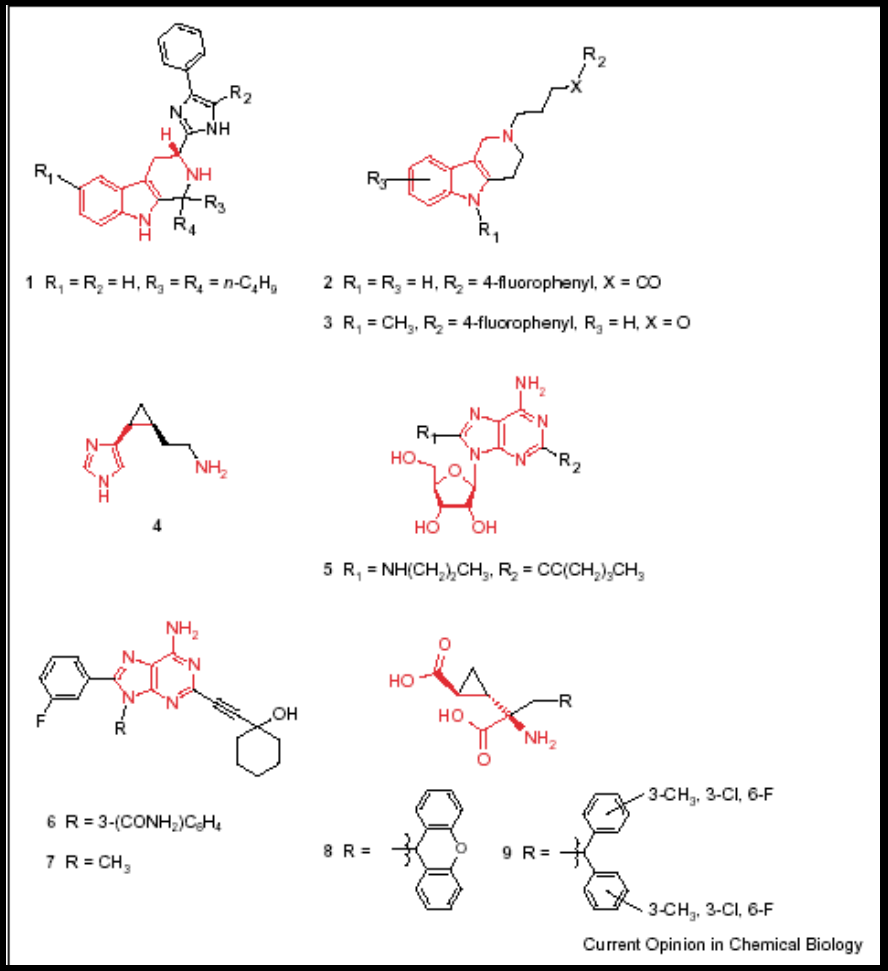
150 hits in 14 classes

7 classes validated as true novel DNA gyrase inhibitor

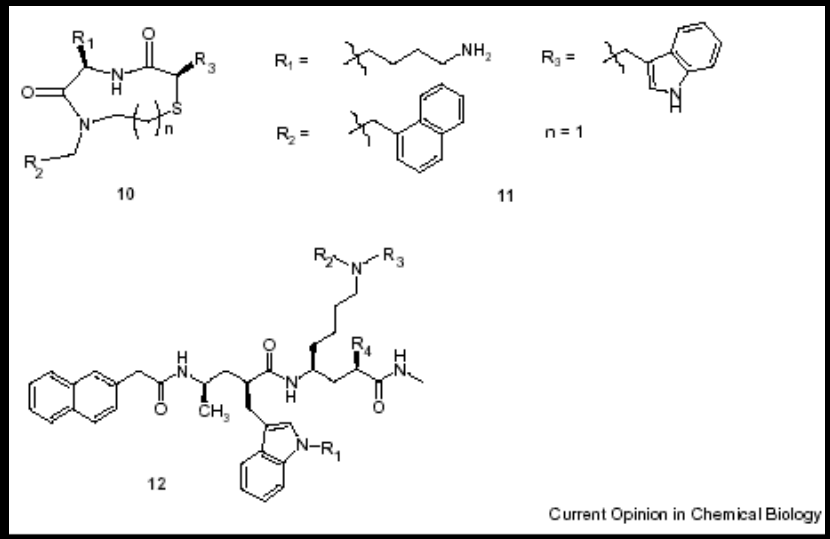
Leads 10 times more potent than Novobiocin

LEAD-GENERATION

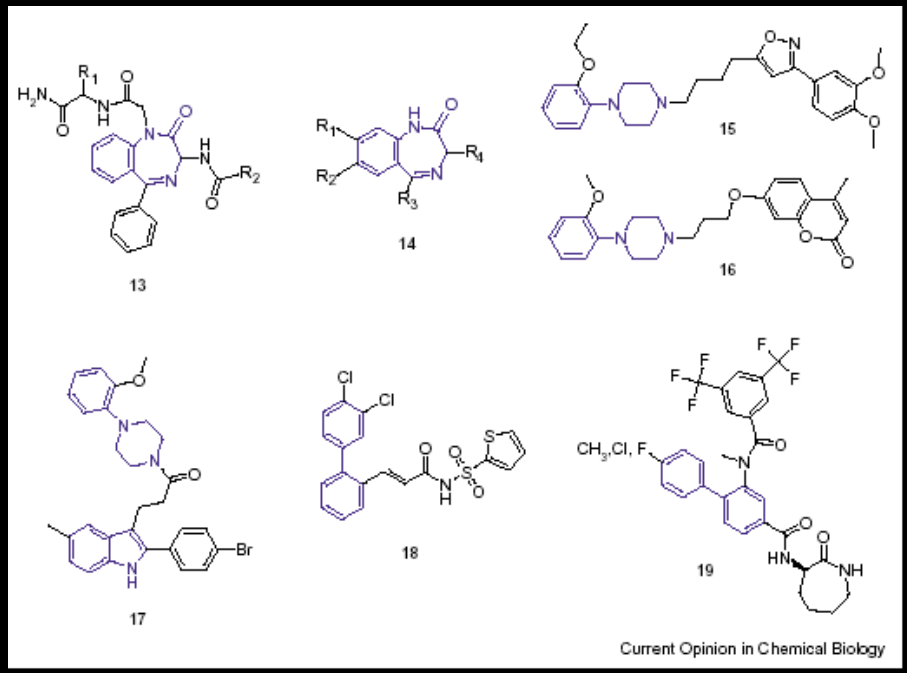
- Ligand-based
  - Ligand elaboration
  - Pharmacophore elaboration
- Privileged structures
  - Templates
  - Fragments
- De-novo design
- Virtual screening
  - 2D ligand-based similarity searches
  - 3D pharmacophore screening
  - Receptor based virtual screening



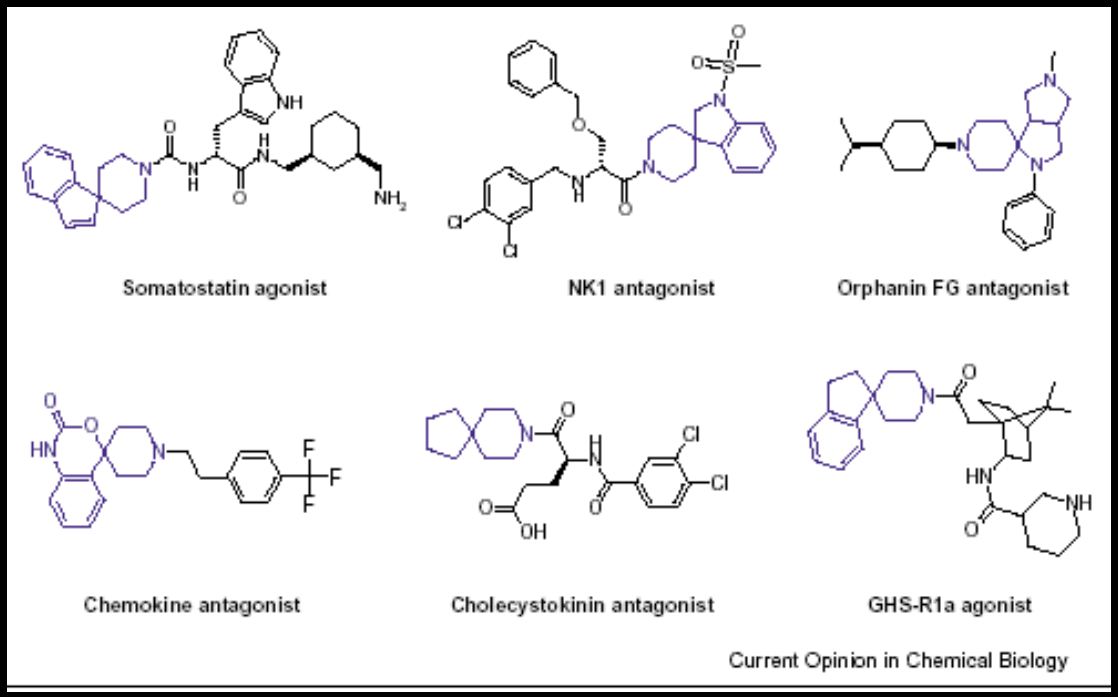
GPCR modulators incorporating endogenous ligands

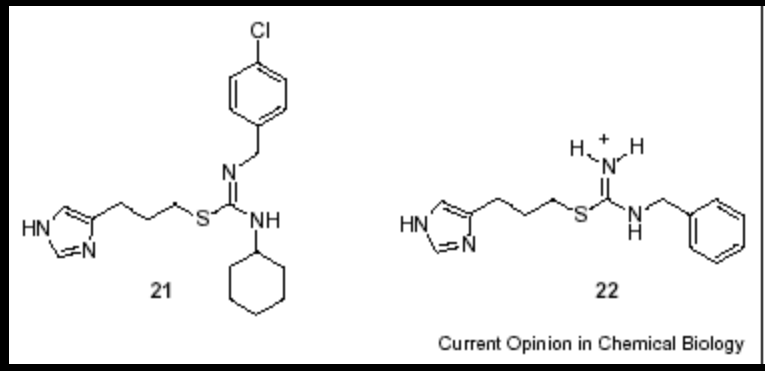


GPCR modulators based on β-turn mimetics



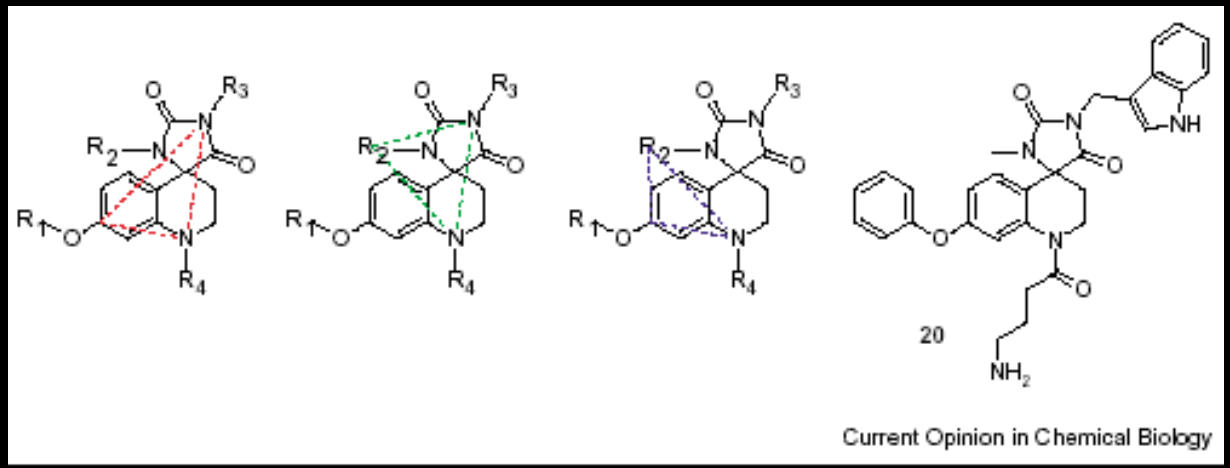
GPCR modulators incorporating privileged structures





Molecular structure of H3 antagonist 21 derived from clobenpropit (22)

Spiropiperidines have been recognized as one type of privileged structures

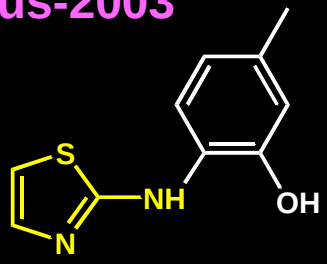


Matching of Garland–Dean geometries onto the selected scaffold and its decorated analogue 20 showing agonist activity for somatostatin subtypes SST1-4.

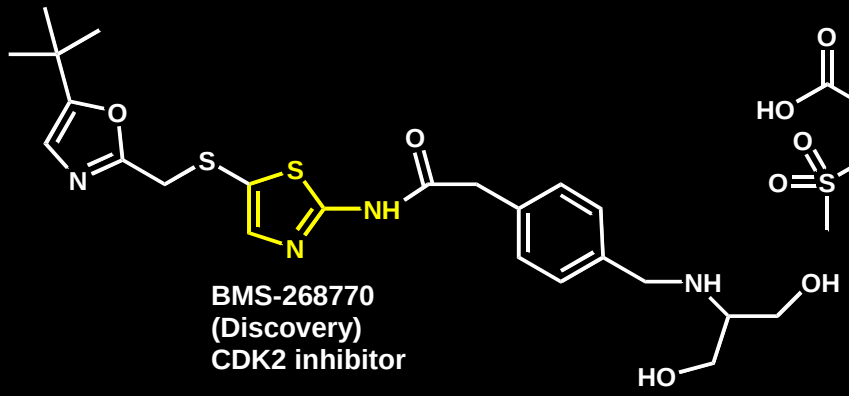
PRIVILEGED STRUCTURES

- Termed by Evans et al. 1988
- Substructural element with a proven correlation with the target family based on a single or variety of key structural elements that account for a target family wide commonality in molecular recognition (Single molecular framework able to provide Ligands for diverse receptors)
- Classes can be based upon
  - Common template with action against different target proteins
  - Enzymes inhibitors and receptor antagonists that structurally follow a common underlying template
  - Fragments from crystallographically derived structures (compounds in complex with target proteins)

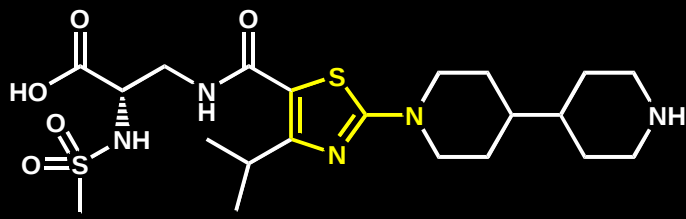
Status-2003



CBS-113-A (Clinical)
COX, 5-Lipoygenase

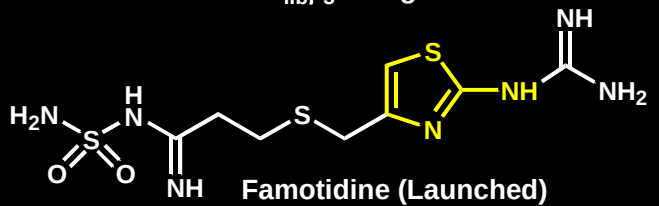


BMS-268770
(Discovery)
CDK2 inhibitor

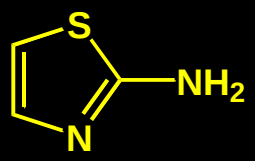


UR-12947
(Discovery)
$\alpha_{IIb}\beta_3$ antagonist

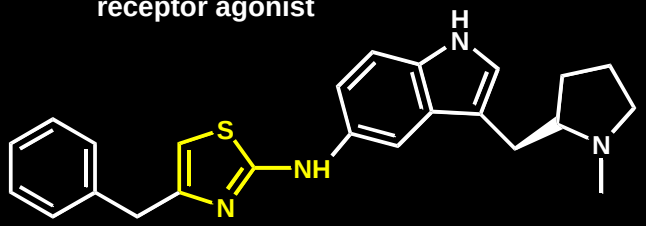
Pramipexole (Launched)
Dopamine D2 and D3
receptor agonist



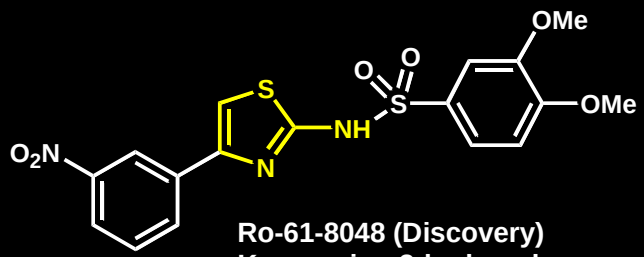
Famotidine (Launched)
Histamine H_2 antagonist



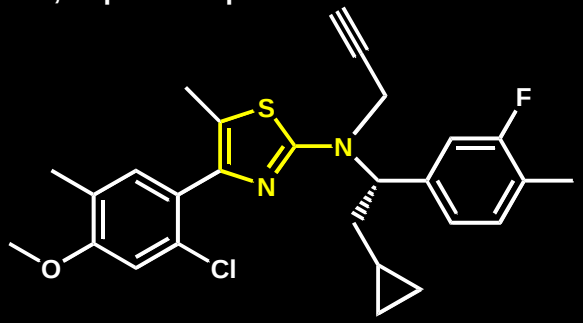
2-amino thiazole containing
compounds displaying biological
activity at diverse target proteins



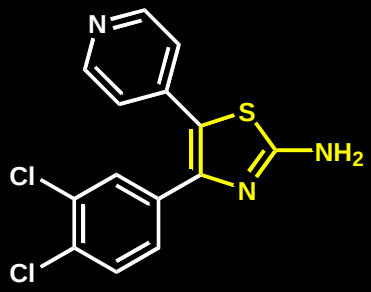
CP-146662 (Discovery) 5-HT_{1A}
agonist, dopamine uptake



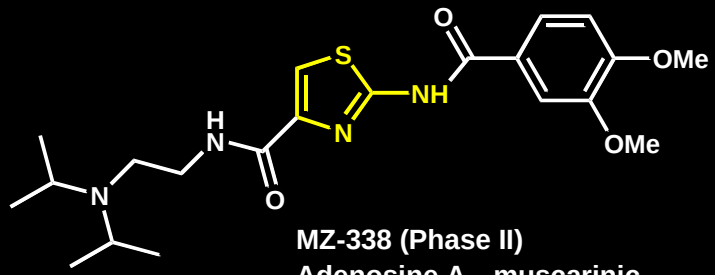
Ro-61-8048 (Discovery)
Kynurenine-3-hydroxylase



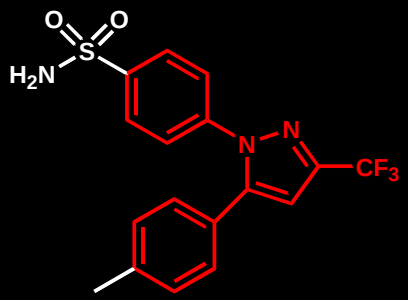
SSR-125543 (Discovery)
CRF-1 agonist



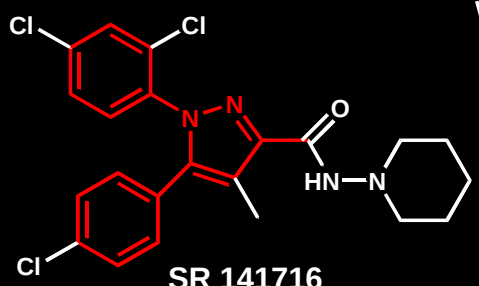
CGS-2466 (Discovery)
Adenosine A_3 antagonist,
PDE-4, p38 MAP



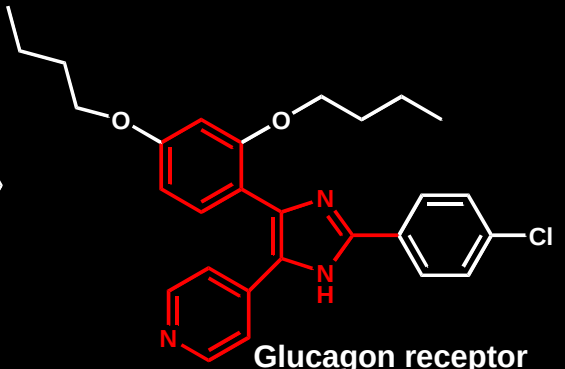
MZ-338 (Phase II)
Adenosine A_1, muscarinic
M1 and M2 antagonist,
AChE inhibitor



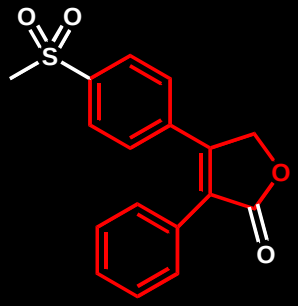
Celecoxib (SC-58635)
Celebrex- Searle & Co



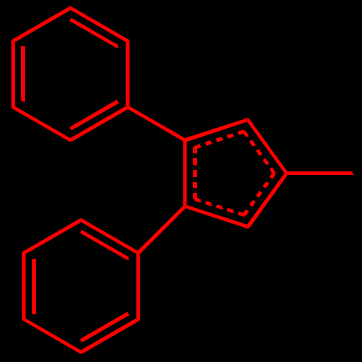
SR 141716
CB1 antagonist

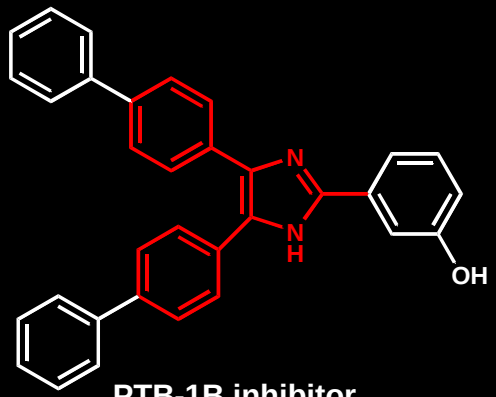


Glucagon receptor
antagonist

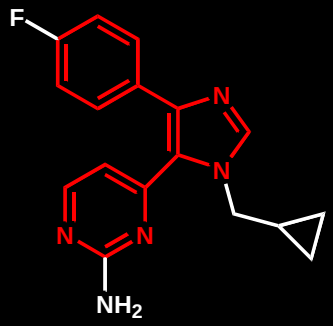


Rofecoxib (MK-966)
Vioxx-Merck Frosst

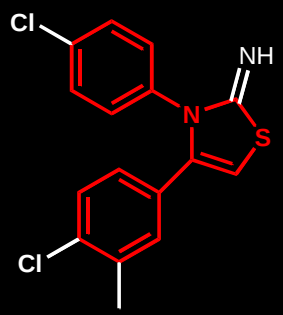




PTB-1B inhibitor

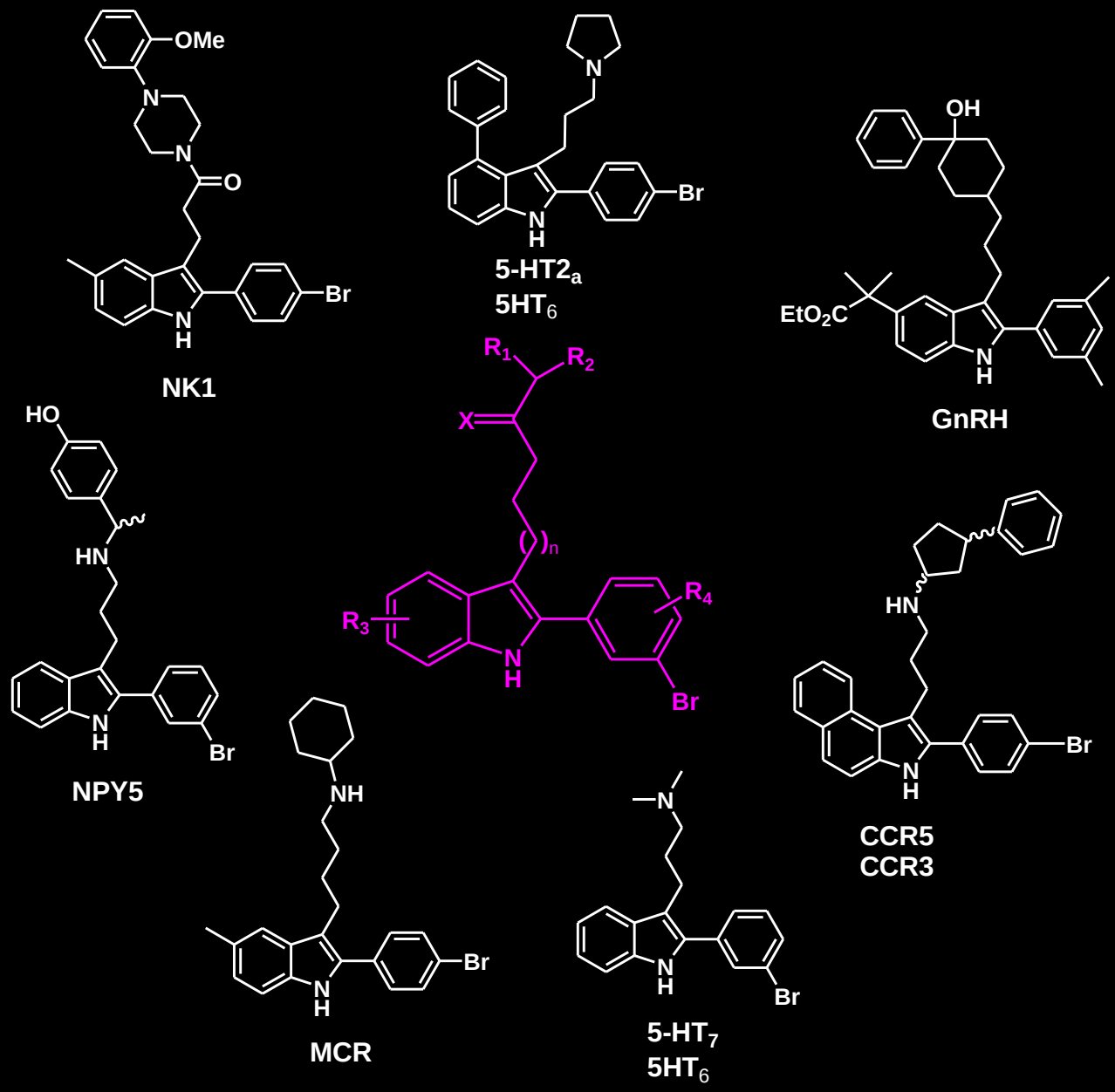


SB-218655
p38 MAP kinase



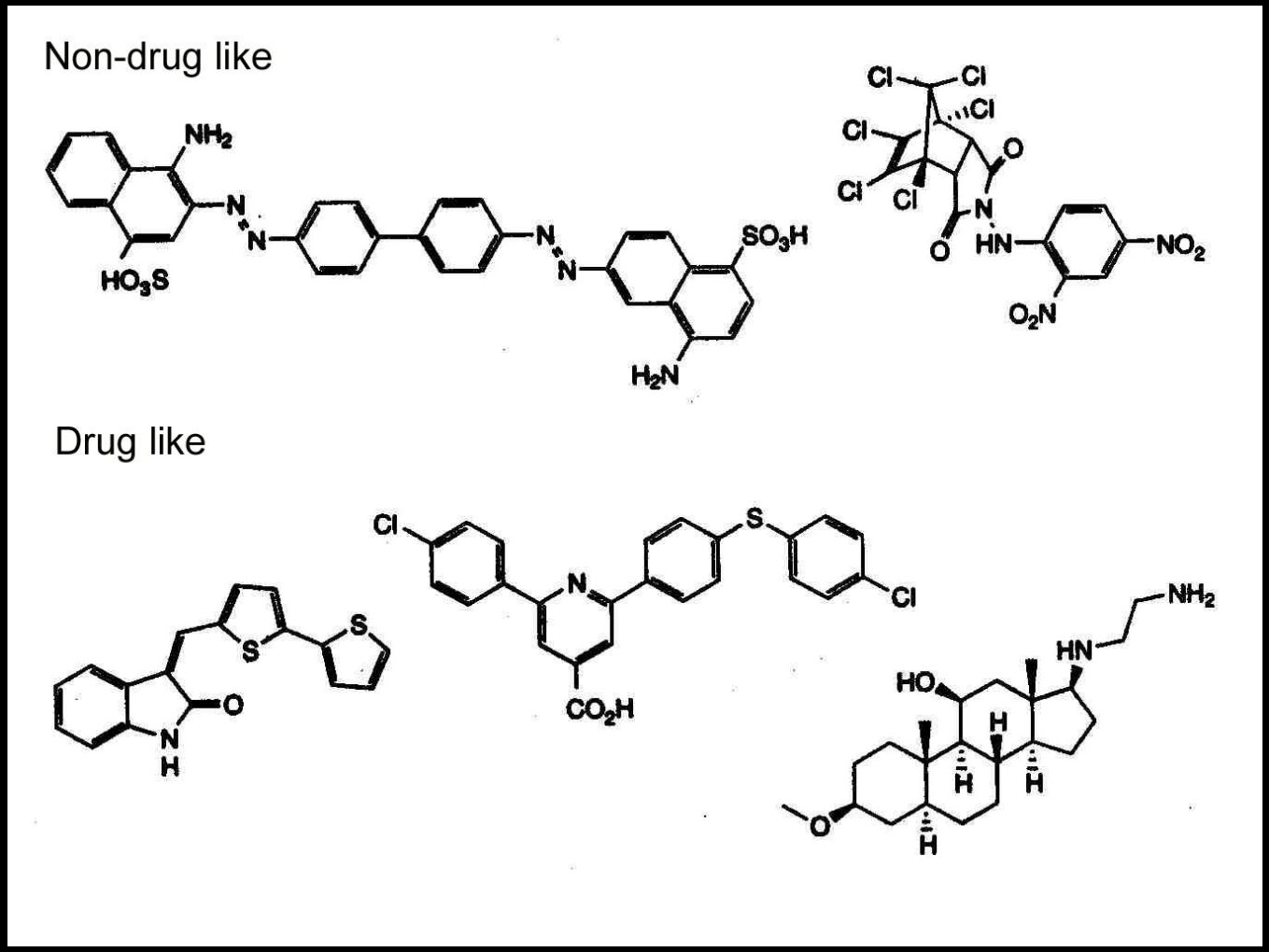
Dopamine
transporter inhibitor

Enzyme inhibitors and receptor antagonists that structurally follow a common underlying template



GPCR targeted library producing highly active and selective compounds targeted towards specific members of a gene family

NON-PRIVILEGED STRUCTURES



Chemical structures of compounds having the tendency to form molecular aggregates, thus interfering in a variety of biochemical assays, producing false positive results

SUCCESS STORIES-KINASES FOR CANCER

Tyrosine kinases in human cancer

Target	Indications	Development compounds
Overexpression of growth factors and their receptors EGFR family of TKs		
EGFR, HER-2, HER-3, HER-4, Mutant EGFRvIII	Breast, Lung, prostrate, colon, glioma	Iressa, Tarceva, CI1033, EKB569, GW2016, (PK1166)
VEGFR family of TKs		
KDR, Flt	Solid tumors (antiangiogenic, metastatic)	PTK787/ZK222584, ZD6474, SU6668, SU11248, CHR200131, CP547632, AG13736, CEP7055/5214
	AML (a type of leukemia)	KRN633
Flt-4 (VEGFR-III)		-
Tie-2		-
PDGFR	Giloma	SU6668, SU11248, AG13736, CHR200131
IGF-IR	Solid tumors	-
FGFR	Breast, Lung, Ovary	CP547632, CHR200131
Mutated/altered kinase levels and/or activities		
Bcr-Abl	CML (95%), ALL (15%)	Glivec (Gleevac)
c-Kit	GIST, SCLC, glioma	Glivec, SU11248, KRN633
c-Met	Renal cancer	-
Fit-3	AML	PKC412, SU11248, CT535518 (MLN518), CEP701
Various TKs		
Src family kinases (c-Src, Lck, Fyn)	Solid tumors	-
JAK	Leukemias	-
FAK	Metastatic diseases	-

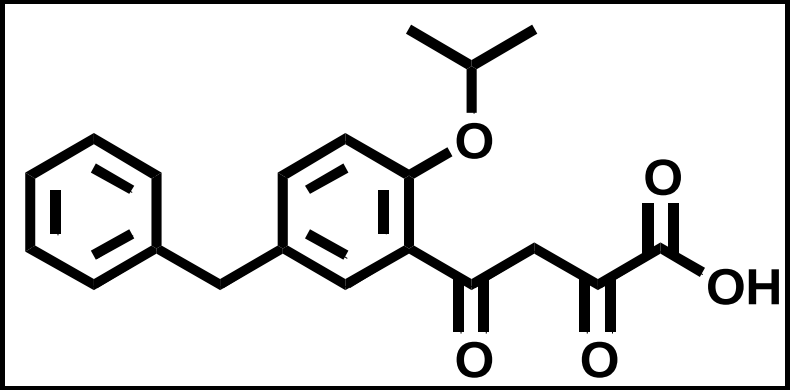
- Human genome encodes 90 tyrosine kinases out of which roughly 50 involved in cancer
- In the market
  - Monoclonal human epidermal growth factor receptor-2 antibody **HERCEPTIN** against breast cancer
  - Bcr-Abl inhibitor **GLIVEC** for chronic myelogenous leukemia
  - EGFR inhibitor **IRESSA** for several type of cancer

Small molecule tyrosine kinase inhibitors in clinical development

Compound	Company	Targets	Status	Indications
Glivec	Novartis	Bcr-Alb	Launched 2001	CML, ALL
		c-kit	Launched 2002	GIST
		PDGFR	Phase II	Solid tumors
Iressa	Astra-Zeneca	EGFR	Phase III (launched 2002)	Single agent/ combinations, NSCLC, breast, prostrate, ovarian
Tarceva	Roche/Genetech	EGFR	Phase III	Pancreas, head and neck, radiation
CI 1033	Pfizer	EGFR, ErB2	Phase II	Solid tumors
EKB 569	Wyeth-Ayerst	EGFR, ErB2	Phase II	Combination with sulindac (colon)
GW 2016	GlaxoSmithKline	EGFR, ErB2	Phase I	Solid tumors
PTK 787/ ZK 22584	Novartis/Schering	VEGFR	Phase I/II	Solid tumors
ZD6474	Astra Zeneca	VEGFR (EGFR)	Phase I	Solid tumors
SU 11248	Pharmacia/Sugen	VEGFR, PDGFR	Phase I	Solid tumors
CP 547,632	Pfizer	VEGFR	Phase I	Solid tumors
CHR 2001131	Chiron	VEGFR (Fit-4), FGFR, PDGFR	Phase I	Solid tumors
CEP 7055/5214	Cephalon	VEGFR, PDGFR	Phase I	Solid tumors
AG 13736	Pfizer/Agouron	VEGFR, PDGFR	Phase I	Solid tumors
PKC 412	Novartis	Fit-3	Phase II	AML
		PKC, (PDGFR, c-Kit)	Phase I	Solid tumors
CT 53518	Millenium	Fit-3	Phase I	AML
CEP 701	Cephion	Fit-3	Phase I	AML

Most of the structures of these compounds contain quinazoline, pyrrolo-pyrimidine, phenylamino-pyrimidine or indolinones

HIV suppressing compound

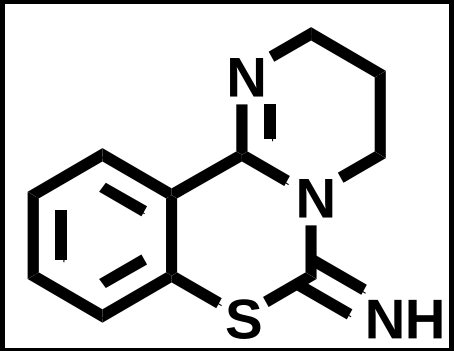


250,000 compounds

Activity: 2 fold less potent than Indinavir

(Merck Res Lab USA)

As Antibiotic



150,000 compounds

Activity: Inhibits KDO 8-P synthase

(SmithKline Beecham)

Clinical candidates identified using CC & HTS

OC 144093	Cancer/MDR	Clinical-I	P-glycoprotein inhibitor
ISIS 5320	HIV-1 infection	Clinical-I	GP120V3 loop binding
NX 1838	Angiogenesis	Clinical I	VEGF antagonist
3DP-4815	anticoagulant	Clinical I	Thrombin inhibitor
NGD 95-1	Antiobesity	Clinical I	NPY-antagonist
OGT-719	Cancer	Clinical II	Melanocortin IR Antagonist
Sch-59228	Cancer	Clinical II	Squalene synthetase inhibitor
SLV-305	Gastric motility	Clinical I	Motilin agonist

The industry perspective "High throughput Screening 2000: New Trends & Direction" reports 46 compounds in human trials

OBSERVATIONS

- Drug research is an evolutionary process
- Combinatorial chemistry speeds up drug discovery
- Drug-like properties are more important than chemical accessibility (Lipinski's rule of 5)

OBSERVATIONS

- Diversity is best considered as Dissimilarity
- A library 100 times larger than another library will not be 100 times more successful
- Virtual screening to aid library design will give smaller and better libraries

OBSERVATIONS

- Combinatorial chemistry will not replace classical organic synthesis
- Combinatorial chemistry is a tool to produce information about a drug target
- A successful combinatorial approach is one that ultimately leads to a drug not one that gives hits

THANK YOU