Drug Design

Drug discovery

In this course we will discuss different approaches used today in the **initial stages** of the pharmaceutical research.

These stages don't aim at producing directly a drug candidate leading immediately to a clinical protocol.

They aim at finding "hits" which may result in "leads".
(When a molecule triggers an interaction with the protein receptor, it registers as a "hit").

After a survey of the experimental methods used on a large scale in the pharmaceutical industry:

the **high-throughput screening** and the **combinatorial chemistry**,

the **computer-assisted methods** will be developed.

Drug discovery

There are two important aspects in drug design and drug strategies to improve :

1. **Pharmacodynamics properties**: to optimize the interaction of the drug with its target. (What the drug does to the body)

2. **Pharmacokinetics properties**: to improve the drug's ability to reach its target & to have acceptable lifetime. (what the body does to the drug)

Pharmacodynamics and pharmacokinetics should have equal priority in influencing which strategies are used and which analogues are synthesized

Drug discovery

What is a lead?

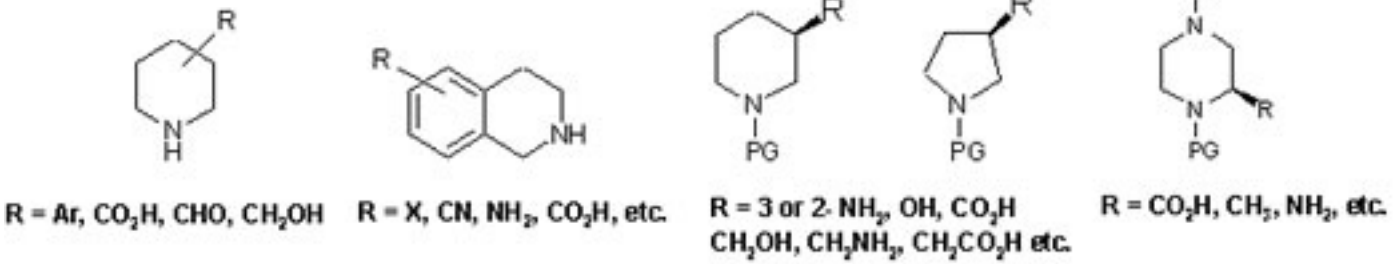
Many attempts have been made to define the properties that characterise a lead structure.

First of all, the compound must have some desirable biological activity, although it may be weak and even non-selective.

There must be related analogs, indicating that structural modification will modulate biological activity as well as other properties.

The lead structure must not be an extremely polar or lipophilic compound which may cause problems in bioavailability.

It should not contain toxic groups or groups that will produce toxic metabolites.



Drug discovery

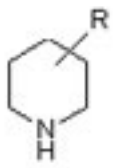
What is a lead?

Once the structure of lead compound is known, the medicinal chemist moves on to study its SAR.

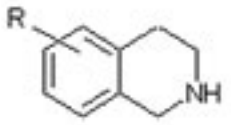
The aim is to discover which parts of the molecule are important to biological activity and which are not.

SAR is synthesizing compounds, where one particular functional group of the molecule is removed or altered.

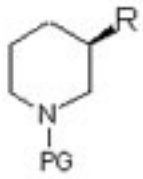
In this way it is possible to find out which groups are essential and which are not for biological effect.

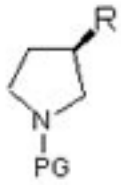


R = Ar, CO_2H, CHO, CH_2OH

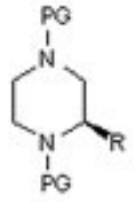


R = X, CN, NH_2, CO_2H, etc.





R = 3 or 2- NH_2, OH, CO_2H
CH_2OH, CH_2NH_2, CH_2CO_2H etc.



R = CO_2H, CH_2, NH_2, etc.

Drug discovery

What is a lead?

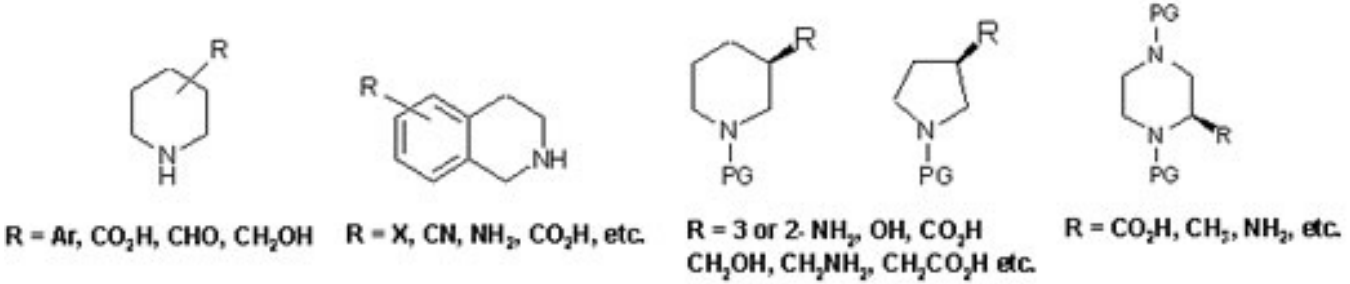
1- Functional groups such as alcohols,, amines, esters, amides, carboxylic acids, ketones can interact with binding sites by means of hydrogen bonding.

2- Functional groups such as amines and carboxylic acid can interact with binding sites by ionic bond.

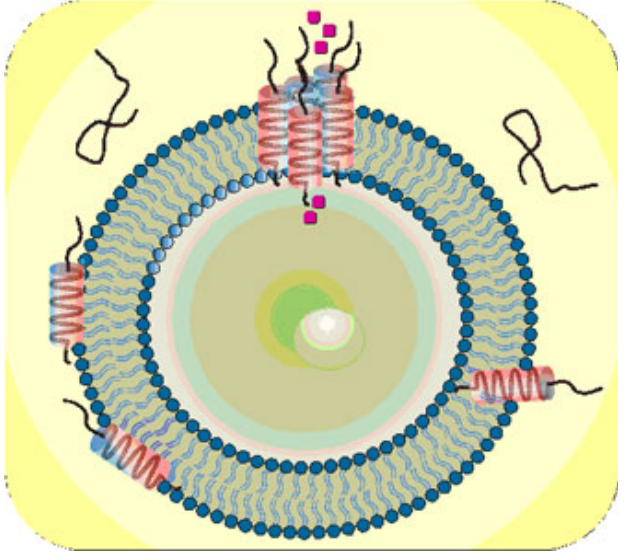
3- Functional groups such as alkenes and aromatic rings can interact with binding sites by means of Van der Waals interactions.

4- Alkyl substituents and the carbon skeleton of the lead compound can interact with hydrophobic regions of binding site.

6-Reactive functional groups such as alkyl halides may lead to irreversible covalent bonds being formed between a lead compound and its target.



Drug discovery



What is a lead?

Most important for the successful optimisation of a lead structure seems to be a certain molecular weight and lipophilicity range.

A recommendation has been that a lead should have a molecular weight < 350 and a lipophilicity, expressed by log P (P = n-octanol/water partition coefficient), smaller than 3.

The Lipinski "rule of five" demands that drugs should have a molecular weight < 500, a lipophilicity range of log P < 5, no more than 5 hydrogen bond donors, and no more than 10 N and O atoms in the molecule.

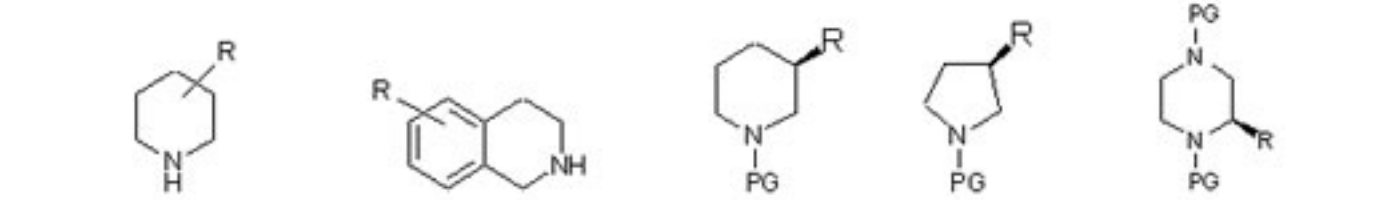
There is a high risk of poor bioavailability if two or more of these conditions are violated.

Drug discovery

What is a lead?

There are many exceptions to the empirical definitions of the lead structure properties, which are listed above, and that in special cases even “bad” leads can be successfully optimised to valuable drugs.

Lead structure optimisation is an evolutionary procedure, in which every minor or major improvement in certain properties leads to a new analog, which is further optimised until the final candidate has all desired properties to start its clinical investigation.



Drug discovery

Natural products

Natural products have been the richest source of drugs and lead structures.

About half of our drugs are still natural products, derivatives, or analogs of natural products.

Plants, fungi, insects, marine organisms and bacteria represent a rich sources of therapeutically useful compounds.

61% of 877 small-molecule New-Chemical Entities
(A new chemical entity is a compound not previously described in the literature) introduced worldwide from 1981 to 2002 can be traced to natural products.

78% of antibacterials and 74% of anticancer compounds are either natural products, or inspired by a natural-product model.

However, even though plant products still play a predominant role, nowadays several important classes of drugs are extracted or derived from microorganisms.

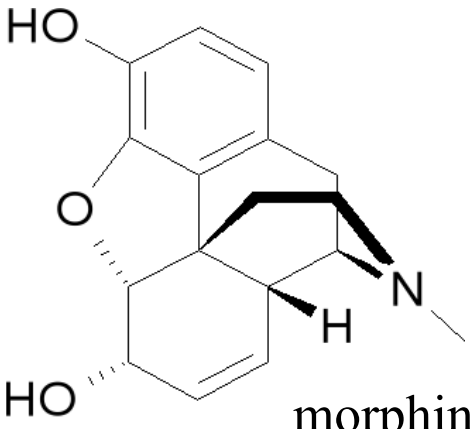
Drug discovery

Natural products

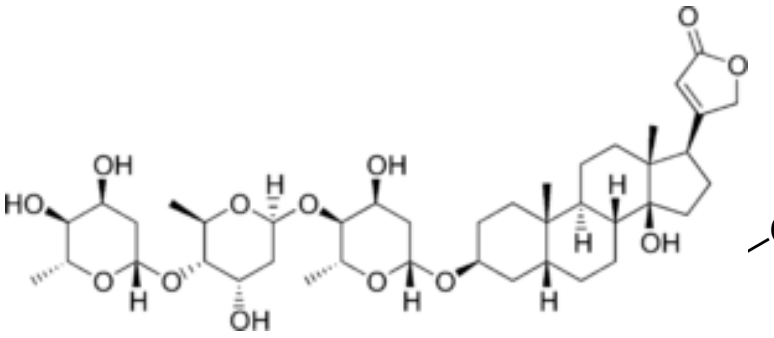
It all started with foxglove (Digitalis purpurea), morphine, quinine, and salicylic acid.

- Cardiac glycosides (heart failures), including analogs with improved pharmacokinetic properties, were extracted and derived from Digitalis species and other plants.

- Morphine found in opium turned out to be a valuable lead for major analgesics, some of them with much simpler chemical structures, antitussives, morphine antagonists, and neuroleptics.

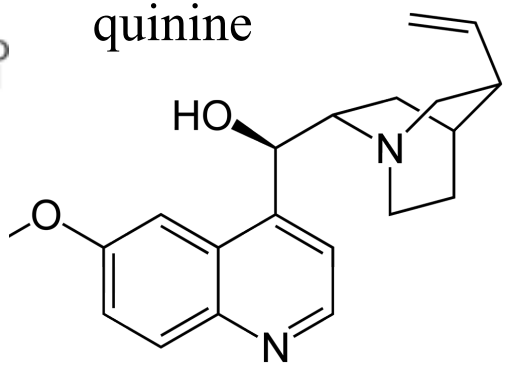


morphine



digitaline

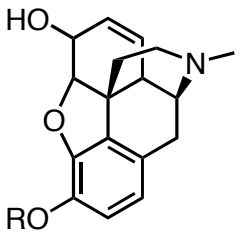
quinine



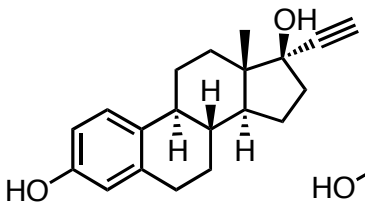
Drug discovery

Natural products

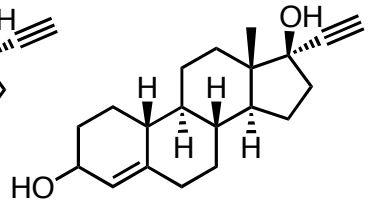
Cardiac glycosides, morphine, quinine, salicylic acid, taxol, camptothecin, penicillin, cyclosporin A, warfarin, artemisine….



R = H: Morphine
R = Me: Codeine
(pain killer)

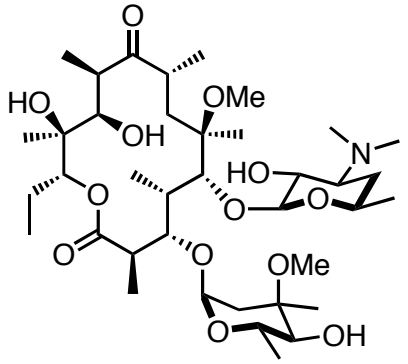


17-ethynylestradiol

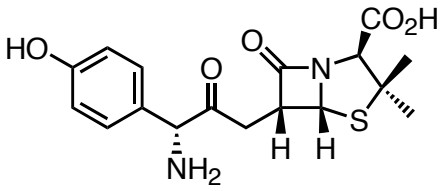


norethindrone

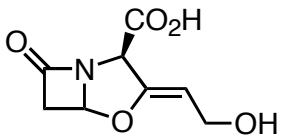
(the "Pill"; contraceptive)



Clarithromycin
(antibacterial)

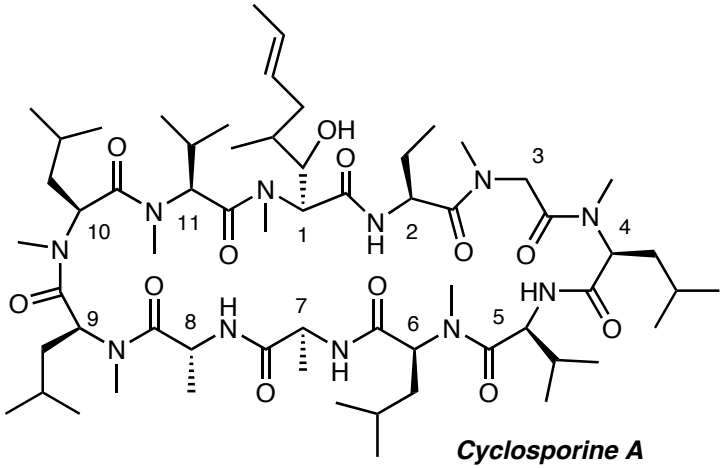


Ampicillin
(antibiotic)



Clavulanic acid
(β-lactamase inhibitor)

Augmentin
(antibiotic)



Cyclosporine A

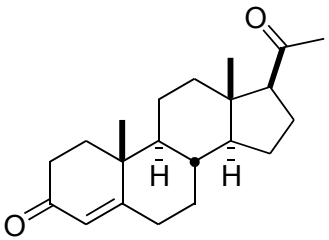
Examples of Natural Products as Leads & Drugs

Drug discovery

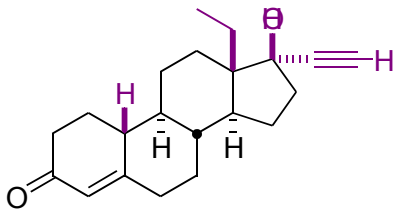
Leads in rational drug design are the *natural*: agonists, enzyme substrates, chemical messengers…

NATURAL LEAD | SYNTHETIC DRUG

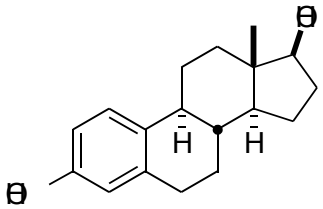
Oral Conctraceptives



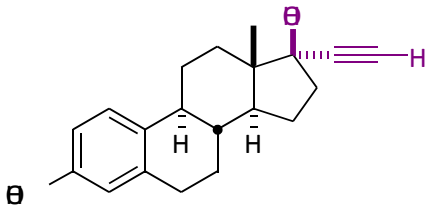
pregesterone



(+)-norgestrel



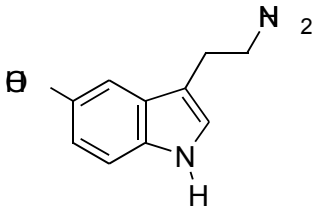
17b-estradiol



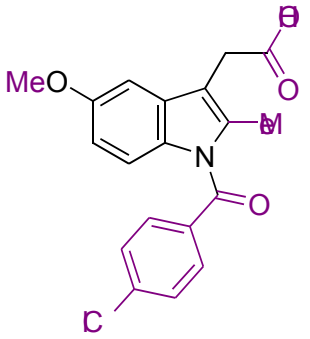
17a-ethynylestradiol

NATURAL LEAD | SYNTHETIC DRUG

Anti-inflammatory



serotonin

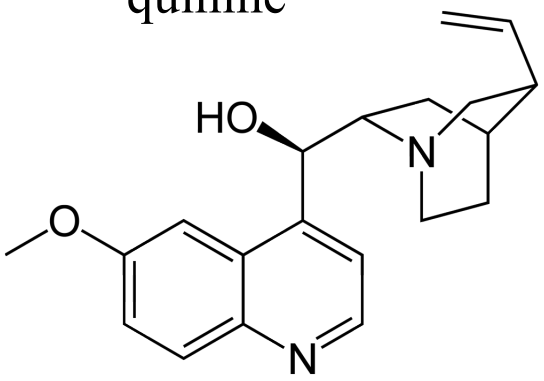


indomethacin

Drug discovery

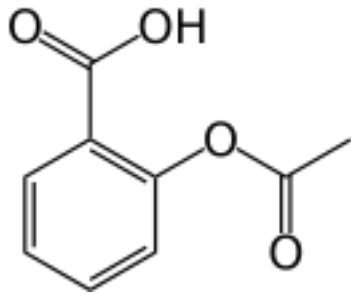
Natural products

quinine

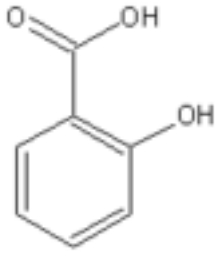


In the case of quinine (trunks of the cinchona tree have been beaten and bark peeled away to extract quinine), much simpler analogs could be derived from this complex natural product.

Salicylic acid (Salicylic acid is a white crystalline compound, which can be isolated from the bark of birch trees) is a natural product with weak antiinflammatory activity; its derivative acetylsalicylic acid (aspirin) is a more active compound and also suited for the prophylaxis of thrombotic diseases.



The antitumor drugs taxol and camptothecin, the anti-Alzheimer natural product huperzine, and the antimalarial drug artemisine are recent examples of plant products of therapeutical interest.



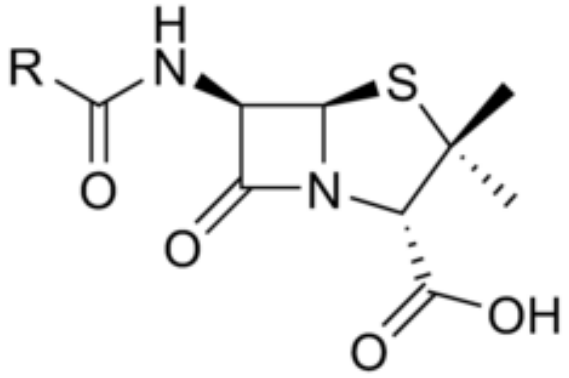
Salicylic acid

Drug discovery

Natural products

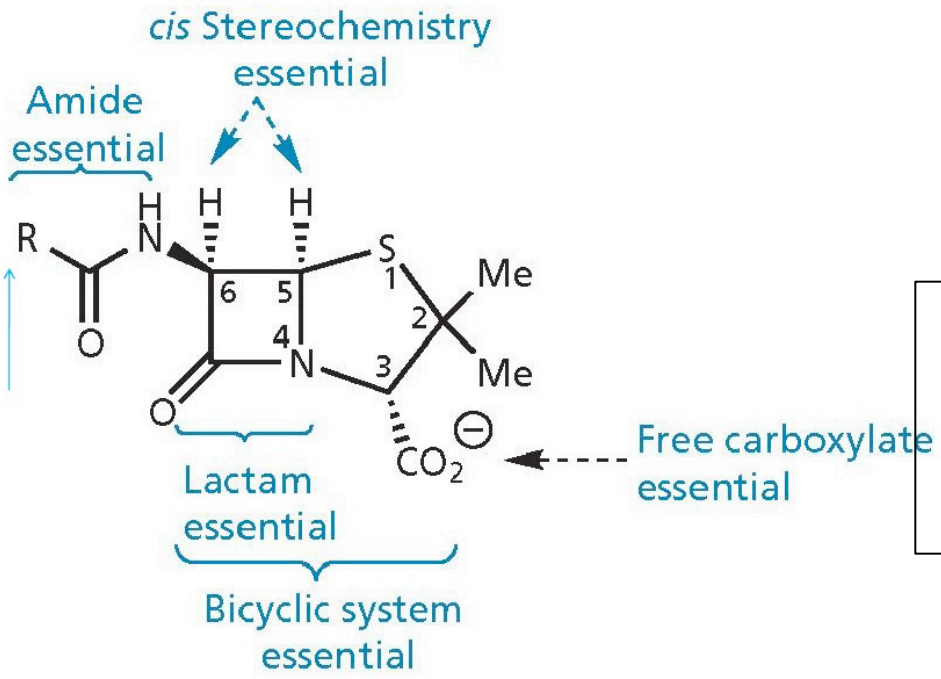
Since 1928, when Sir Alexander Fleming discovered the lysis of bacteria by a secretion product of a Penicillium strain, microorganisms have been a rich source of antibiotics.

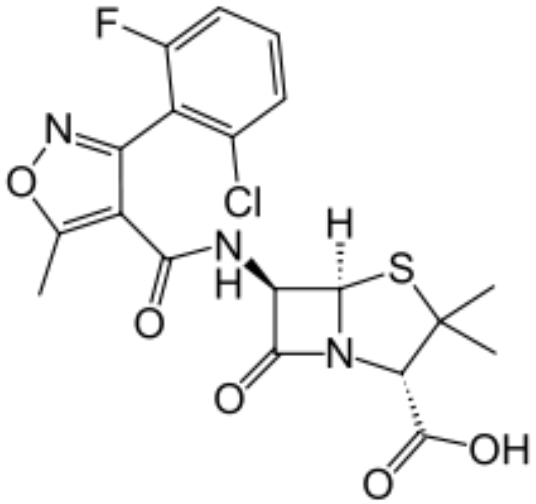
The original penicillin structure has been optimised, step by step, to bioavailable analogs, to broad spectrum antibiotics, and finally to lactamase-resistant derivatives.



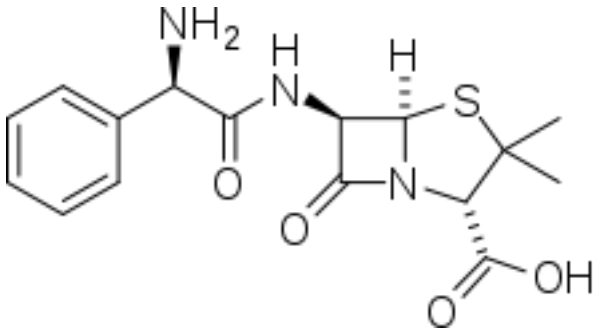
Drug discovery

Natural products





narrow-spectrum beta-lactam antibiotic, flucloxacillin has activity against beta-lactamase-producing organisms such as Staphylococcus aureus

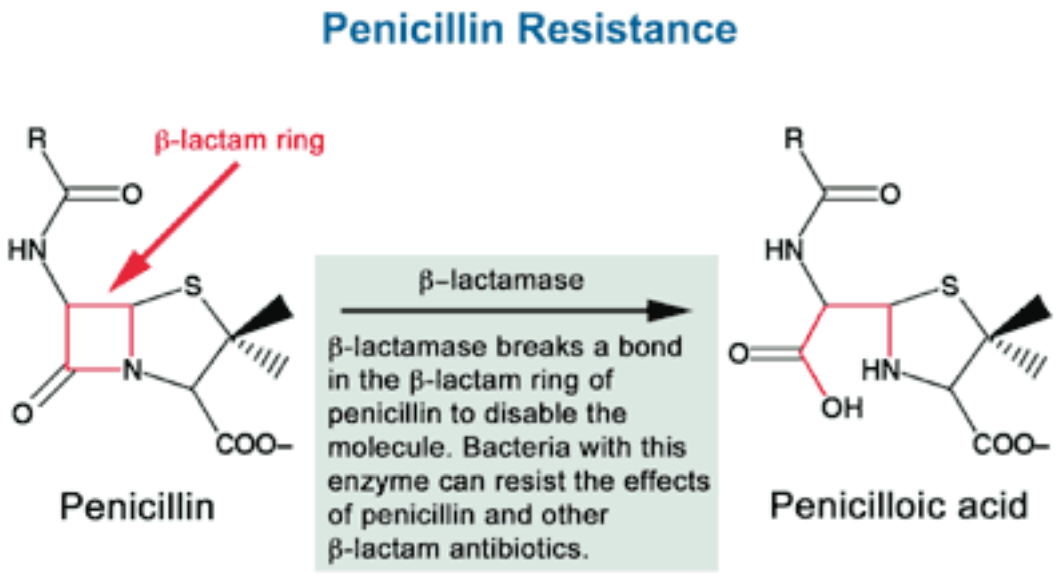


Ampicillin: broad spectrum penicillin analog

Drug discovery

Natural products

β-lactamases (Penicillinases) are enzymes produced by penicillin-resistant bacteria (e.g. Staphylococcus aureus) which can catalyze the reaction opening the ring

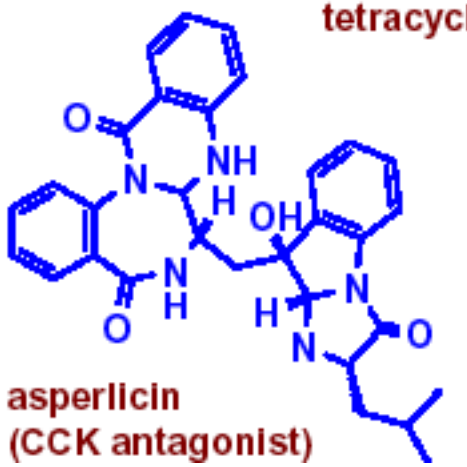


Drug discovery

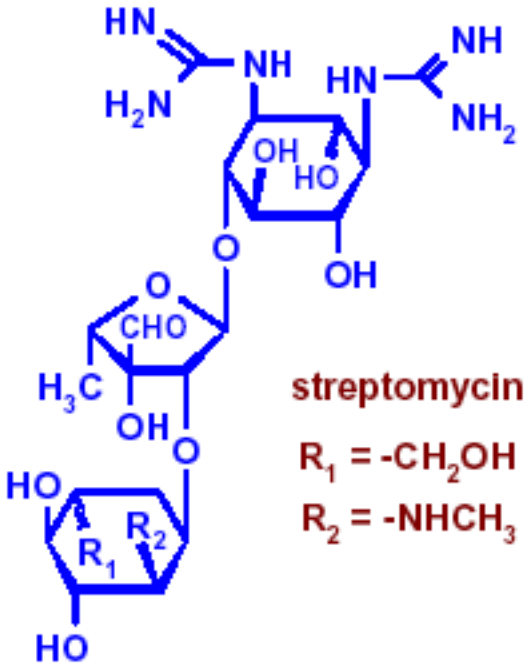
Natural products

Lead Structures: Microbial Natural Products

tetracyclin



asperlicin
(CCK antagonist)



streptomycin

R_1 = -CH_2OH

R_2 = -$NHCH_3$

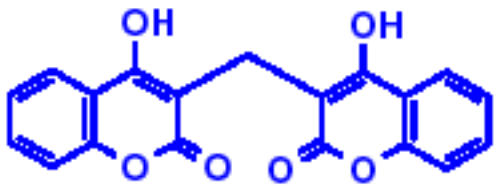
In addition to penicillin, the cephalosporins, tetracyclins, chloramphenicol, streptomycin, rifampicin, valinomycin, etc., turned out to be valuable lead structures or antibiotic drugs themselves.

Drug discovery

Natural products

But not only antibiotics resulted from microorganisms,
also cardiovascular drugs and the immunosuppressants cyclosporin A and tacrolimus, the antitumor principle epothilone
and the most important group of cholesterol biosynthesis-blocking statins.

Also the anticoagulant coumarins, like phenprocoumon and warfarin, were derived from dicoumarol, a microbial product first isolated from rotten hay.



dicoumarol

Drug discovery

Natural products

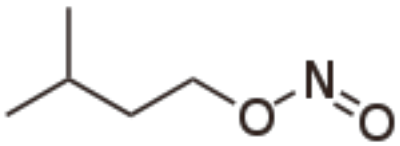
- Source of chemical diversity and biological functionality
- They are results of millions of years of combinatorial chemistry done by nature and their chemical diversity is complementary to that of synthetically derived compounds.
- The potential of natural products as a source for new drugs or as tools for target identification is by far not exhausted.
- Not required to know the structure of the drug nor that of the receptor
- But screening materials are complex
- Uncharacterised biological mixtures contain interfering substances
- Costly and time-consuming work to identify the active principle (but new innovative technologies)

Serendipitous drug discoveries

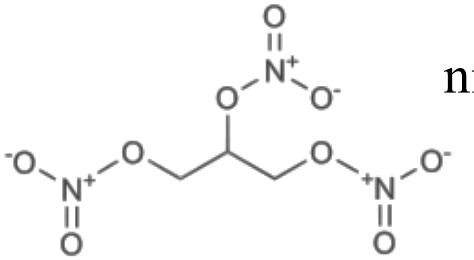
Some of the very first drugs were discovered by serendipity, already 150 years ago.

The use of nitrous oxide (N_2O - laughing gas) and ether $(CH_3CH_2)_2O$ as narcotic gases in surgery resulted from the observation that persons which inhaled these chemicals did not experience any pain after being injured.

The vasodilatory activity of amyl nitrite and nitroglycerin was also discovered by accident.
Chemists working with these organic nitrites experienced strong headache after inhaling or ingesting minor amounts.
Those suffering from angina or heart failure often experienced relief from chest pain during the work week.



amyl nitrite

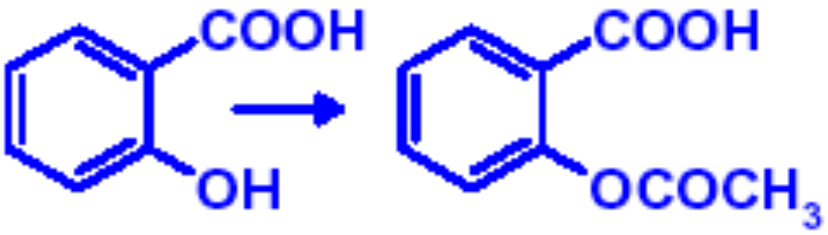


nitroglycerin

Serendipitous drug discoveries

Acetylsalicylic acid was considered to be just a better tolerable derivative, a prodrug, of salicylic acid but it turned out to have a unique mechanism of action.

Aspirin®
(Felix Hoffmann, 1897)



Serendipitous drug discoveries

Penicillin is one of the earliest discovered and widely used antibiotic agents, derived from the Penicillium mold.

The anticoagulants of the dicoumarol type resulted from the observation that cattle bled to death after being fed with rotten hay.

The anticoagulant warfarin
(synthetic derivative of coumarin which is found in many plants)
was originally used as a rat poison.
Its clinical applicability was confirmed, when a US soldier tried to commit suicide but survived.
Nowadays this “rat poison” is a most valuable drug in the prevention therapy after stroke and other thrombotic diseases.

Serendipitous drug discoveries

A closer inspection of drug discovery stories shows that serendipity and sagacity played an important role in many cases.

Fleming might have discarded his spoiled bacteria culture and Sternbach might have neglected the crystals of chlordiazepoxide (tranquilliser- benzodiazepine derivative) when he cleaned up his laboratory.

But they didn't because they were experienced investigators, according to the formulations:

"chance only favours the prepared mind" by Louis Pasteur

and

"discovery consists of seeing what everybody else has seen and thinking what nobody else has thought" by Albert Szent-Györgi, the discoverer of vitamin C.

Table 1. Incomplete list of serendipitous discoveries in drug research.

Compound	Accidental Discovery
Acetanilide	tested as internal antiseptic (instead of naphthalene)
Acetylsalicylic acid	irreversible enzyme inhibitor (*vs.* salicylic acid prodrug)
Aminoglutethimide	breast cancer treatment (instead of antiepileptic)
Amphetamine	stimulant (instead of nasal decongestant)
Chloral hydrate	prodrug of trichloroethanol (instead of chloroform)
Chlordiazepoxide	tranquillizer (unexpected chemical rearrangement)
Chlorpromazine	neuroleptic (tested to prevent surgical shock)
Cinnarizine	cardiovascular (predominant to antihistaminic) activity
Cisplatin	cytotoxic effect of electrolysis product
Clonidine	antihypertensive (instead of nasal decongestant)
Cromoglycate	antiallergic (accidental formation of chromone dimer)
Cyclosporin	immunosuppressant (instead of antifungal agent)
Dichloroisoprenaline	ß-blockade (instead of bronchodilation)
Dicoumarol	fatal cattle poisoning (bleeding) by moldy hay
Diethylstilbestrol	estrogenic impurity of anol (dimerization product)
Diphenhydramine	allergy treatment caused prevention of travel sickness
Diphenoxylate	antidiarrhoic (instead of analgesic)
Disulfiram	hypersensitivity to alcohol
Ether	anesthetic activity in inhalation party
Etomidate	anesthetic (instead of chemotherapeutic) activity
Griseofulvin	growth inhibition of conifers on certain soils
Guanethidine	antihypertensive (instead of antitrypanosomal drug)
Haloperidol	neuroleptic (instead of analgetic) activity
Heparin	deterioration of lipid coagulant unmasked anticoagulant
Imipramine	antidepressant (instead of neuroleptic) activity
Iproniazid	antidepressant (instead of tuberculostatic) activity
Isoniazid	tuberculostatic activity of organic intermediate
Levamisole	immunomodulating (instead of antiparasitic) agent
Lithium carbonate	antidepressant activity of lithium urate
Lysergide (LSD)	hallucinogenic (instead of cardiovascular) activity

Griseofulvin	growth inhibition of conifers on certain soils
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Lithium carbonate	antidepressant activity of lithium urate
Lysergide (LSD)	hallucinogenic (instead of cardiovascular) activity
Meprobamate	tranquillizer (instead of muscle relaxant)
Merbaphen	diuretic activity (of an antisyphilitic agent)
Methaqualone	hypnotic (instead of antimalarial activity)
Mifepristone	antiprogesterone (instead of glucocorticoid) activity
Naftifine	antifungal rearrangement product of CNS drug
Nalorphine	antagonism instead of respiratory stimulation
Nitrogen mustard	cytotoxicity observed after ship bombardment
Nitroglycerin	antianginal activity (headache after inhalation)
Nitrous oxide	accidental wounding in laughing gas session
Norethynodrel/Mestranol	estrogenic impurity in the first oral contraceptive
Penicillin	antibiotic activity of *Penicillium* infection
Pethidine (meperidine)	morphine agonist (instead of spasmolytic)
Phenylbutazone	antiinflammatory activity of solubility enhancer
Phenolphthalein	laxative (tested as label for cheap wines)
Praziquantel	antiparasitic agent (instead of antidepressant activity)
Prednisone	bacterial oxidation produced highly active analog
Propafenone	antiarrhythmic (instead of ß-blocker)
Sulphamidochrysoidine	prodrug of sulfanilamide (active only *in vivo*)
Sulfonamides, various	diuretic and antidiabetic side effects
Tamoxifen	antiestrogenic activity of *cis*-isomer
Urethane	hypnotic activity (instead of alcohol prodrug)
Valproic acid	anticonvulsant (solubility enhancer for various drugs)
Warfarin	low acute toxicity of rat poison in attempted suicide

Rational approaches: the golden age of drug research

Besides natural products from plants, endogenous neurotransmitters and steroid hormones have been the richest source of new drugs.

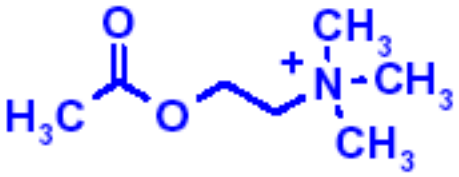
From the elucidation of the biochemical mechanisms underlying the transmission of nerve impulses and the deeper understanding of hormone effects, a large number of therapeutically useful drugs resulted.

This phase of drug research may be considered as its golden age (1950s and 1960s).

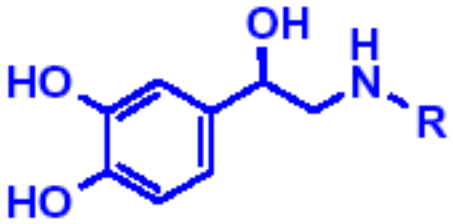
Neurotransmitters are small molecular weight molecules which diffuse easily across the synapse.

Rational approaches: the golden age of drug research

Lead Structures: Endogenous Neurotransmitters

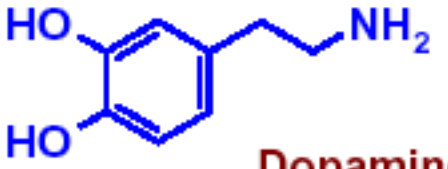


Acetylcholine

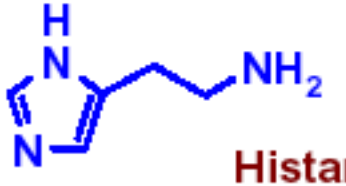


Epinephrine, R = CH_3

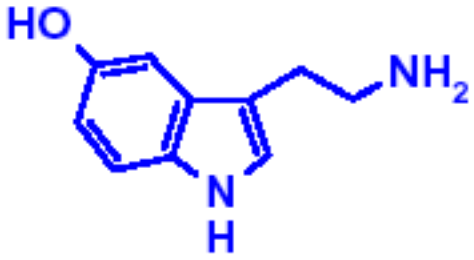
Norepinephrine, R = H



Dopamine



Histamine



Serotonin (5-HT)

Nearly every <u>modification of dopamine, serotonin, histamine or acetylcholine</u>, using the modification strategies of classical medicinal chemistry, resulted in a compound with modified activity and selectivity, most often in a drug candidate.

<u>A broad repertoire of drugs</u>, some of them still being used today, resulted from this period of the 1950's and 1960's.

Me Too Research

Copying existing drugs, with only minor chemical variations, is designated as **“me too” research**.

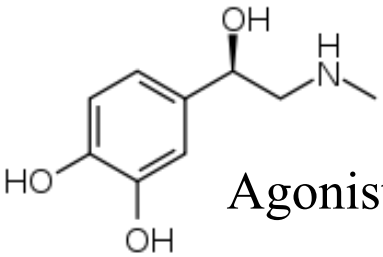
Many examples demonstrate that many analogs show indeed major advantages:

- the bioavailable, broad-spectrum, and lactamase-resistant penicillins.

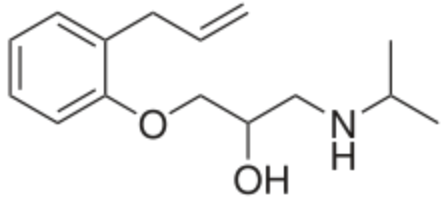
- Diuretic and antidiabetic sulfonamides derived from antibacterial sulfonamides

- polar H1 antihistaminics without sedative side effects

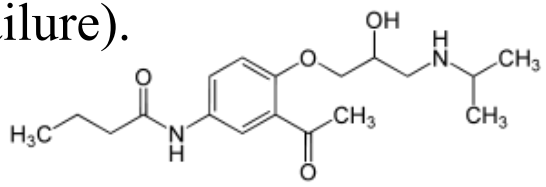
- B1-specific antagonists compared to the original nonspecific B1- and B2-inhibiting betablockers (to reduce high blood pressure, relieve angina, regulate heart rhythm or for treatment of heart failure).



Agonist: epinephrine



Non selective beta-blocker



Specific beta-blocker

Me Too Research

Sometimes a second drug in the market has some therapeutic advantage that immediately puts it in first place.

Ranitidine vs. Cimetidine (gastric ulcers, stomach acidity)
Ranitidine was found to have a far-improved tolerability profile (i.e. fewer adverse reactions), longer-lasting action, and ten times the activity of cimetidine.

Enalapril vs. Captopril (antihypertensives)

Despite the chances of improvement of an existent drug, “me too” research is nowadays only performed if blockbuster drugs may result.

Experimental Methods

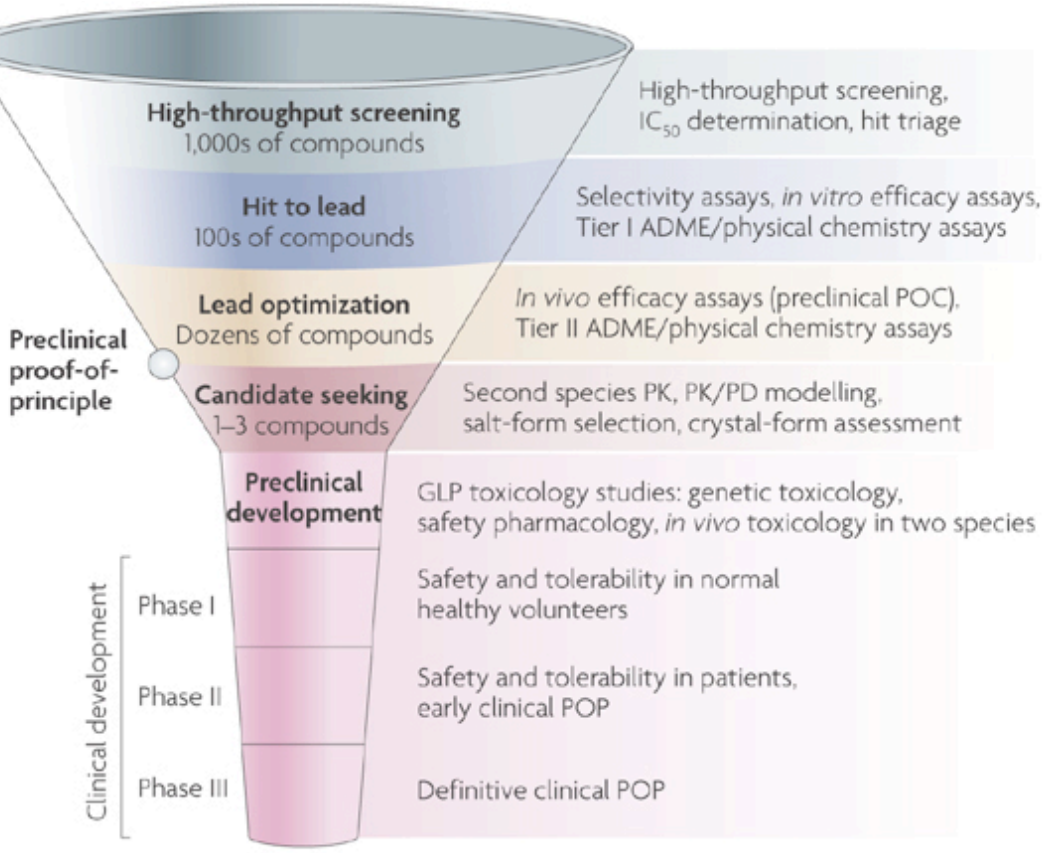
In parallel to the global move aiming at a greater rationale in the process of finding new active molecules, the development of **high throughput screening** (HTS) and **combinatorial chemistry** led to a different approach in the research of "lead" molecules.

In this approach the rationale behind drug design is sometimes replaced by the **magic of large numbers**.

It has been said that the benefits of this new approach are not yet noticeable and that these technologies have not yet provided potentially marketable molecules.

Experimental Methods

The main purpose or goal of this technique is to hasten the drug discovery process by screening the large compound libraries with a speed which may exceed a few thousand compounds per day or per week.



Screening and High Throughput screening

Benzodiazepines (anxiety relief and insomnia treatment), naftifine (skin infection), cyclosporin A (immunosuppressive agent), coumarins as HIV protease inhibitors, and several non-peptidic antagonists of peptide G protein-coupled receptors, resulted from screening.

Thus, there is no question that screening contributed to the discovery of many valuable leads.

However, with automated high-throughput screening, the situation is more difficult.

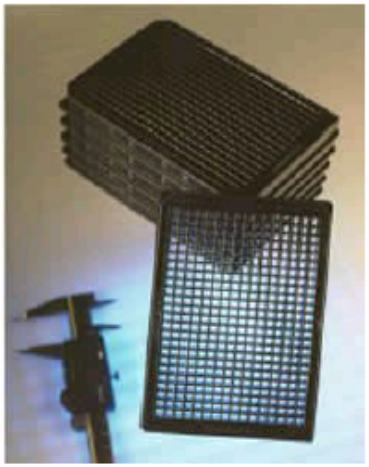
High Throughput Screening (HTS)

HTS is a technique which has got into the laboratories in 1997.

It is based on the **use of robotics to screen large libraries of compounds** onto an isolated protein target, a cell or a tissue so as to identify the molecules able to bind.

The more advanced techniques enable to screen 100,000 to 2,000,000 compounds per day.

HTS depends on the development of quantitative tests which are pharmaceutically significant and adapted to the target and which can be reproduced on a large number of samples.



384 wells

High Throughput Screening (HTS)

This screening involves the use of complex laboratory automation
but assumes no prior knowledge of the nature of the chemotype likely to have activity at the target protein.

- plethora of assay formats

HTS assays can be divided into two categories, namely biochemical assays and cell-based assay

Often target-based biochemical assays, mainly enzyme inhibition and receptor-ligand binding assays are used

BUT

are limited because not all targets can be purified.

High Throughput Screening (HTS)

1. Pharmacological relevance of the assay.
2. Reproducibility of the assay.
3. Assay costs.
4. Assay quality.
5. Effects of compounds assay.

Combinatorial chemistry

The advent of HTS revealed an inadequacy
between the time required by the medicinal chemists to synthesise the molecules and the rate authorised by the screening.

Combinatorial chemistry provides a large number of molecules that can be tested by automated high throughput screening systems.

The use of a robot for synthesis has greatly contributed to this acceleration.

Combinatorial chemistry

Combinatorial chemistry is the synthesis of a large number of compounds, a library, combining in a systematic way, the representatives of two or more families of compounds.

For example, combining a family of 87 aldehydes with a family of 34 amines and one family of 91 carboxylic acids can result in an ensemble of 269178 compounds.

This discipline originates to 1963 when Merrifield presented the sequential synthesis of a tetrapeptide (solid-phase synthesis in which molecules are bound on a resin bead and synthesized step-by-step in a reactant solution).

The method was then extended to the organic, organo-metallic and inorganic chemistry with industrial applications in pharmacochemistry, catalysis, material sciences, dyes.

High Throughput Screening (HTS)

Today, big pharmaceutical companies can screen up to 100,000–300,000 per screen to produce approximately 100–300 hits.

On average, one or two of these will become lead compound series

Larger screens of up to 1,000,000 compounds in several months may be required to generate something closer to five leads.

Despite its promises this young technology had trouble at the start to satisfy the expectations it has aroused.

However several compounds currently in clinical phase have been provided by HTS.

High Throughput Screening (HTS)

Drugs that evolved from structures discovered through HTS:
- nevirapine, delavirdine , efavirenz (HIV non-nucleoside RT inhibitors)
- bosentan (Tracleer, endothelin receptor antagonist; pulmonary arterial hypertension) –
gefitinib (Iressa, tyrosin kinase inhibitor; antineoplastic, lung cancer)

At the beginning companies have been aware that the original concept does not deliver to the expected extent.

Problems were: Limited solubility, deposition after dilution, compound decomposition, as well as unknown concentrations, coloured impurities, fluorescence of some compounds, etc.,
produce legions of **false negatives and false positives**.

In many cases, re-testing did not confirm any primary hits.

In other cases, re-testing of analogs uncovered their activity, although they were initially found to be inactive.

Combinatorial chemistry

More disappointing than HTS results
was the success rate of combinatorial libraries, especially in the early years.

Huge libraries of ill-defined mixtures of most often lipophilic and too large compounds were tested, without any positive result.

The hit rate of libraries generally decreases with an increase in the number of "over-decorated", i.e. too large and too complex molecules.

Only after introduction of the Lipinski rule of five and other virtual screening techniques people became aware of the importance of certain drug properties, like appropriate molecular weight and balanced lipophilicity.

Combinatorial chemistry

It was then proposed to change strategies in the synthesis of libraries.

Combinatorial chemistry developed into automated parallel synthesis of much smaller libraries of single and pure (or purified) compounds of biological interest.

Combinatorial chemistry

Better recommendations for the synthesis combinatorial libraries of natural product analogs have been given.

The synthesis of high-diversity libraries, based on multi-component reactions that generate a multitude of different scaffolds.

A convincing example of the proper application of combinatorial chemistry in early lead profiling is, e.g. the discovery of nanomolar somatostatin receptor subtype-selective ligands (cancer therapy) in several libraries, with up to 350,000 members per library.

Somatostatin receptors (SSTRs), especially SSTR subtype 2, are found expressed at relatively higher levels in many tumor cells

Combinatorial chemistry

Despite its advantages in terms of synthesis easiness and of the number of potential molecules solid-phase chemistry does not allow to use all synthesis ways which are accessible in solution, limiting the possibilities of this approach.

The initial frenzy took over to a research of quality instead of quantity.

New approaches have been developed based on the structure of the target in conjunction with the use of retro-synthesis tools to guide the automated synthesis (introduction of rationality in “chance” methods).

Drug design and instrumentation

Structure-based design requires precise structural information.

NMR and Xray diffraction are two techniques which are used to determine the structure of a ligand-receptor complex or to show that certain residues of the receptor interact with the ligand.

Progress these last years have enable to commonly use these techniques in the process of drug discovery.