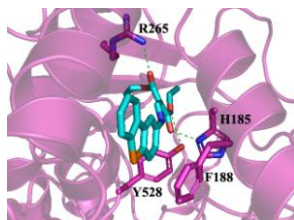


Introduction to Drug Discovery

06509 / 58300

Dr A.N. Boa



1

Course and Learning Outcomes

- **Lecture 1: Introduction**
 - Describe the decision-making processes relating to the selection of a new drug discovery programme.
- **Lecture 2: Starting in the lab**
 - Discuss critically the approaches used for the identification of lead compounds.
- **Lecture 3: Combinatorial Chemistry (CombiChem)**
 - Compare and contrast CombiChem techniques relevant to drug discovery. Apply knowledge to show how a simple compound library may be constructed from a given lead compound.
- **Lecture 4: Rational Drug Discovery**
 - Discuss critically approaches used in the process of 'rational drug design'.
- **Lecture 5: Drug Discovery**
 - Describe selected approaches used during the development of a drug candidate.
- **Lecture 6: Inhibitors of Angiotensin-Converting Enzyme : A case study**

2

Pre-20th century medicine

Man has found, by trial and error, which berries, roots, leaves and barks could be used for “medicinal purposes” to alleviate symptoms of illness.

- * All ancient civilisations made discoveries in this field
- * Chinese herbal remedies are probably the most well known

The Doctrine of Signatures:

(propounded by Paracelsus 1493-1541 and Jakob Böhme 1575-1624)

introduced the idea that God had “specially marked everything to reveal its purpose”

Iris petals - for treating bruises

Liverwort - for treating liver ailments

Goldenrod - for treating jaundice

3

Willow bark and salicylic acid

Doctrine of Signatures

The Rev Edward Stone (1760s) searched along a riverbank (i.e. a cold and wet place) for a plant-based cure for the fevers associated with influenza. Found that the bark of the willow was effective in reducing fever.



Native American Cherokees used willow bark for such purposes for centuries.

- * willow bark contains **salicin** →
- * metabolized *in vivo* to the **active agent salicylic acid** →
- * salicylic acid and more **tolerable “prodrug” aspirin** made in late 19th century →
- * **mechanism of action** not discovered until the 1970s.

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Quinine

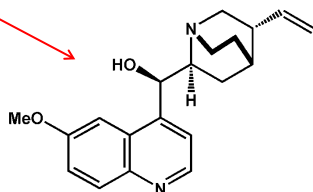


Powdered dried bark of the **cinchona tree**, a native of South America, was made into a drink and used by the **Quechua Indians** of Peru to treat fevers.



"Discovered" by Jesuit priests in the 1620s, Barnabé de Cobo takes cinchona bark to Europe in 1632 to treat malaria.

Quinine isolated in 1820 by **Pierre Joseph Pelletier and Joseph Caventou**



Kills parasites causing malaria. Mode of action complex and still not 100% worked out.

First total synthesis: (1943) **RB Woodward** and WE von Doering

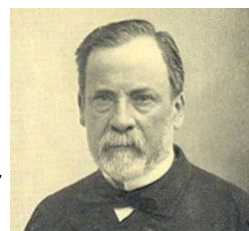


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Early milestones in development of medicinal chemistry



Anton van Leeuwenhoek
1632-1723



Louis Pasteur
1822-1895



Robert Koch
1843-1910



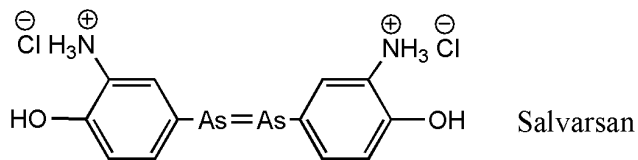
Paul Ehrlich
1854-1915

6

Paul Ehrlich (1854-1915)

The so-called 'father of modern chemotherapy'.

Original proponent of the "magic bullet" he aimed to use chemicals to treat disease. In 1910 the first fully synthetic drug was made: 'Salvarsan' which contained arsenic!



Used for treating sleeping sickness (trypanosomiasis) and syphilis (caused by *Treponema pallidum*).

The Nobel Prize for Medicine 1908

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Modern drug discovery

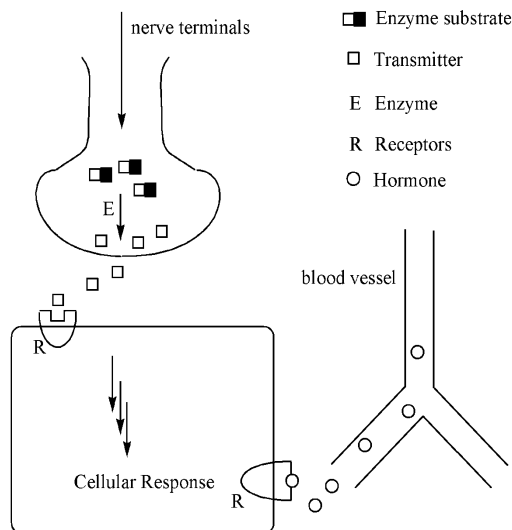
Key stages:

- Programme selection (choosing a disease to work on)
- Identification and validation a drug target
- Assay development
- Identification of a "lead compound"
- Lead optimization
- Identification of a drug candidate
- Clinical trials
- Release of the drug
- Follow-up monitoring

Some of these areas will not be covered in any detail, and some will be covered in other lecture courses.

8

Principles of drug action



Proteins (enzyme, receptor or ion channel) and nucleic acids form critical links in all "biochemical processes".

When one of these links is malfunctioning then a disease state may arise.

Modulating a malfunctioning biochemical process may alleviate the symptoms ("cure") of a disease state.

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Drug targets

Drug targets are most often **proteins**, but **nucleic acids** may also be attractive targets for some diseases.

TARGET	MECHANISM
• Enzyme	Inhibitor - reversible or irreversible
• Receptor*	Agonist or antagonist
• Nucleic acid	Intercalator (binder), modifier (alkylating agent) or substrate mimic.
• Ion channels*	Blockers or openers
• Transporters*	Uptake inhibitors

*present in the cell membranes

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Drug target validation

A bio(macro)molecule may be involved in a disease process, but to be a **drug target** it has to be **validated**. In other words shown to be critical in the disease process.

Useful techniques available to validate a target are:

Gene knockout: does removal of the gene that encodes the target protein result in, for example, the death of a pathogen (disease causing microorganism)?

RNA interference (RNAi): involves double-stranded ribonucleic acid (dsRNA) interfering with the expression of genes with sequences complementary to the dsRNA. Results in a reduction of the production of the protein (target) in question.

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Drug - target interactions

How specific does the interaction between a drug molecule and its target have to be? A rough and ready 'back of the envelope' calculation shows:

Consider: an active compound **MW 200 g mol⁻¹**
1 mole = 6×10^{23}

Therefore **1 mg** substance gives
 $(6 \times 10^{23}) \times (10^{-3}/200) = 3 \times 10^{18}$ **molecules**

A human has approximately 3×10^{13} cells giving.....
 $3 \times 10^{18} / 3 \times 10^{13} = 1 \times 10^5$ **molecules of active substance per cell**

An erythrocyte (a typical cell) contains approx 10^{10} molecules
 \therefore 1 molecule of active substance per 100,000 cellular molecules

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Programme selection

- Development of new medicines is **complex**, **time consuming** and **very expensive** (£300-400 million for a completely new drug)
 - Success rate in getting from an **initial compound** to an approved and commercially available product is very low.
 - < 2% of new compounds investigated may show suitable biological activity
 - Modification of an existing drug can yield as little as 1% suitable compounds
 - < 10% of these compounds result in successful human clinical trials and reaches the market place

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Deciding on an area for research

Before experimental work starts a clear strategy is needed

Selection of a disease target:

- In the 'early days' there were many therapeutic opportunities:
Infectious diseases, cardiovascular....etc
- whereas acceptable therapies are available today for many conditions, e.g.
Antibiotics for bacterial diseases etc, paracetamol / ibuprofen etc. for moderate pain relief

Nowadays **acceptable therapies are available for many conditions**.
New agents must have statistically proven **clear advantages over existing therapy** (not just that it is clinically effective).

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Programme selection: decisions

In proposing a new research project one should consider:

A) the medical need

- life threatening or self-limiting condition?

B) availability of current therapy

- is level of satisfaction high or low?

A new drug may have advantages as it provides a **new dosage** form which results in a particular advantage to the patient (e.g. oral formulation vs. creams) or which **requires less frequent dosing** (once a day tablet).

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Programme selection: decisions

- C) **competitor activity**. Will the proposed new drug:
- show increased **selectivity** for a particular biological mechanism?
 - permit a **novel approach** to the management of the disease?

Optimum agents of a particular class may have been identified next therapeutic advance requires an alternative pharmacological approach. Must choose whether to seek improvements within an existing drug class or follow a novel approach.

D) commercial opportunity

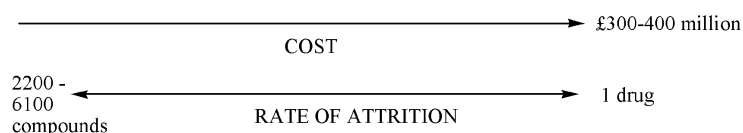
- potential market (patient numbers)?
- duration of the proposed therapy?
- is the condition acute or chronic?

The product must of course be commercially viable!

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Example of research and development timescales

SYNTHESIS	IND*	NDA ^S	LAUNCH	PATENT EXPIRY
1982	1986	1992	1994	2002
DISCOVERY RESEARCH	CLINICAL DEVELOPMENT	REGULATORY REVIEW	POST MARKETING DEVELOPMENT	



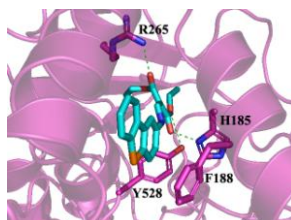
*INVESTIGATIONAL NEW DRUG APPLICATION

^SNEW DRUG APPLICATION

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Finding a Lead Compound

Lecture 2



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Lead compounds

- New projects can be divided into those which have “**lead compounds**” on which to base the design of novel analogues, and those which do not.
- A *lead compound* is:
 - “a compound from a series of related compounds that has some of a desired biological activity. This molecule can be characterised, and modified to produce another molecule with a better profile of wanted properties to unwanted side effects”
 - A **lead compound** is a first foothold on the drug discovery ladder
 - It takes much more effort to make a **lead compound into a drug candidate**

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Starting in the lab

- Without some **reliable measure of potency** it is impossible to conduct a systematic analysis of possible new drugs

Screening or assaying

- “The testing of a (series of) molecule(s) against a known biological target that correlates with a cellular or pharmacological activity is known as screening - e.g. enzyme inhibition or receptor binding”

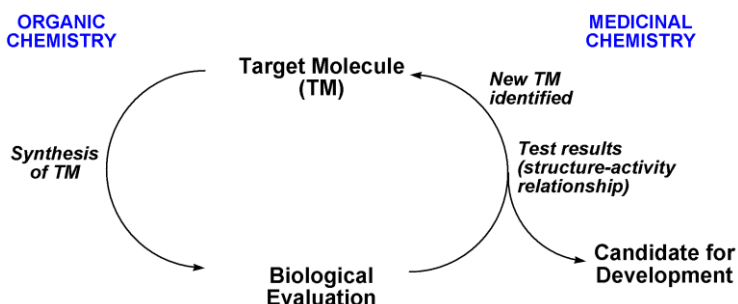
This is now made possible / easier in modern research because:

- Macromolecular targets (proteins, e.g. enzymes / receptors) can be identified
- Targets are now available in large quantities using molecular biology
- Automated (high-throughput) screening technologies available

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Compound design cycle

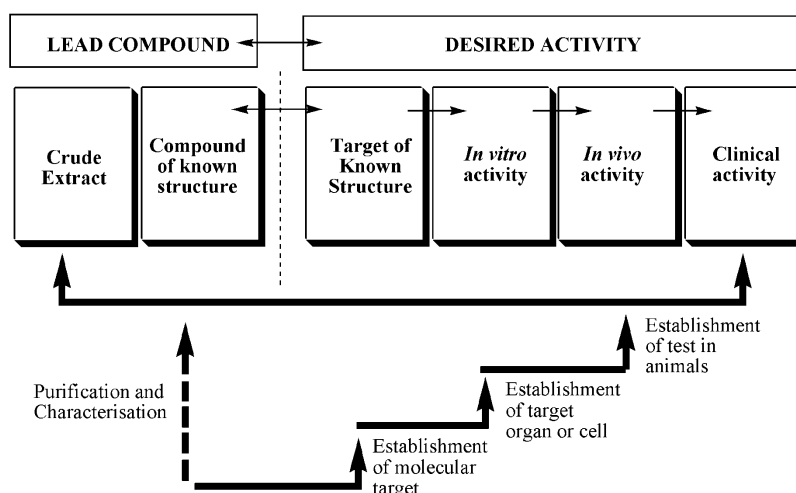
The start of a drug discovery project relies on a “make and test” cycle.



To make meaningful progress, a quick entry into this cycle must be gained through identification of a “lead compound” with some of the desired biological activity.

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Discovery and development cycle

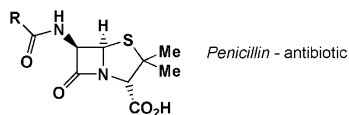


22

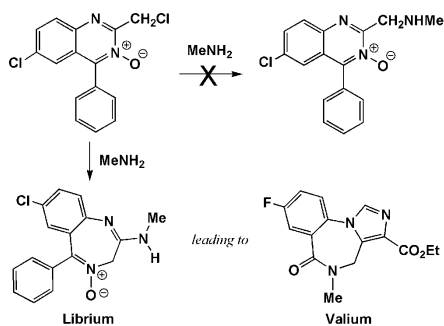
Identification of lead compounds 1

Lead compounds may be identified by chance, e.g.:

Penicillin (antibiotic) – discovered by Fleming (and others).



Librium (anxiolytic) – discovered by Sternbach.



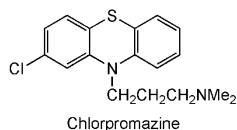
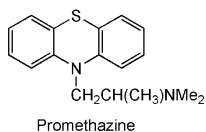
An inactive compound's structure was misassigned and "shelved" and the project was wound up. The compound was unearthed in a lab tidy some years later and tested in a new project. Was found to be effective as a treatment for short-term anxiety. First in the class of benzodiazepines. 23

Identification of lead compounds 2

Historically, drugs in the clinic have been found to have side effects. Structures can be modified to reduce the primary indication and optimize side effects.....

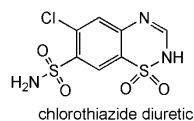
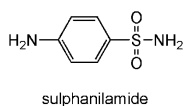
Chlorpromazine (antipsychotic).

Phenothiazines were being developed as antihistamines, but the French navy surgeon Laborit noticed the relaxed nature of patients about to undergo the knife.



Chlorothiazide (diuretic).

Sulphanilamide, the active metabolite of an early class of antibiotics had diuretic side effects.



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Identification of lead compounds 2

Cases of clinical observation of a side effect has lead to the **SOSA** approach to lead discovery: the **Selective Optimisation of Side Activities**

The rationale for this approach to finding lead compounds is that almost all drugs exhibit one or more side effects: i.e. they are capable of exerting more than one “biological action”.

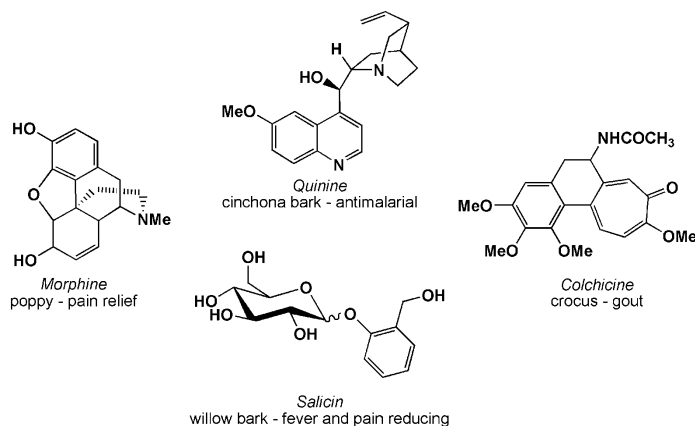
The aim is **to screen all new pharmacological (drug) targets against a limited set of existing drugs.**

The Prestwick Chemical library consists of 1120 compounds **of wide chemical and pharmacological diversity** as well as **known bioavailability and safety** in humans. 85% of the collection is made up of well established drugs, with the remaining 15% being bioactive alkaloids (natural products containing a basic nitrogen).

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Identification of lead compounds 3

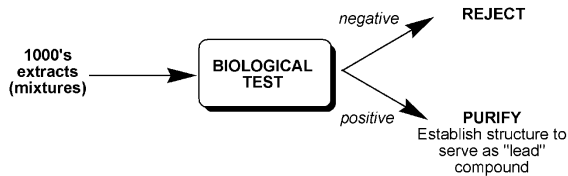
Lead compounds have been identified by isolation of active ingredients of folklore / traditional remedies, e.g.:



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Natural product screening 4

The isolation of many bioactive products from natural sources has led to the systematic screening of plant and animal extracts for activity.



- 80% of the world's population uses drugs exclusively from natural sources.
- 35% of drugs contain 'principles' (key structure elements) of natural origin.
- Less than 5% of the 500,000 higher plant species have undergone biological pharmacological screening.
- Each plant has potentially 10,000 different constituents.

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Bioactive agents from screening 4

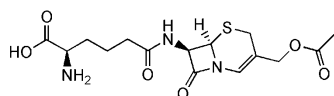
Life form	Species	Lead / Drug
Mould	<i>Cephalosporin acremonium</i>	cephalosporin C
Plant	yew tree	taxol
Marine organism	deep water sponge	discodermolide
Reptile	snake venoms	teproptide

Problems with natural product screening

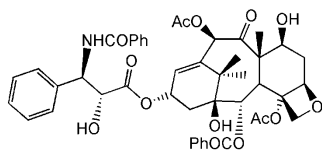
- Isolation of an active component present in a very small amount can be problematic given a large amount of background "rubbish".
- The mixtures are often very complex and contain many large macromolecules. These can often "hide" biological activity.
- Compound isolation and structure determination difficult.
- Structures often complex, therefore difficult to synthesise and identify the pharmacophore.

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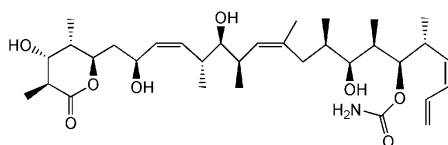
Bioactive agents from screening 4



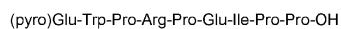
Cephalosporin C
mould - antibiotic



Taxol
yew tree - antitumour



Discodermolide
marine sponge
antitumour / immunosuppressant



Teprotide
Venom from Brazilian viper
ACE inhibitor (lowers blood pressure)

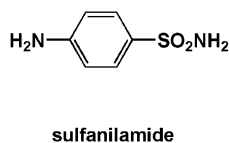
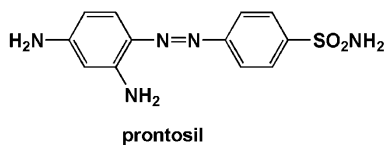
29

Screening of compound collections 5

Dedicated screening of single, characterized compounds has been around since the time of Ehrlich (salvarsan).

Prontosil (antibacterial):

Gerhard Domagk (IG Farbenindustrie, Germany, 1931) screened azo dyes in the search for compounds with biological activity.



This led in 1935 to the discovery of the first truly effective, fully synthetic antibacterial agent, a red dye called prontosil. Sulfanilamide is the active metabolite of the drug which is formed *in vivo*.

Nowadays, high-throughput screening is possible with robotic technology.

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Compound libraries 5

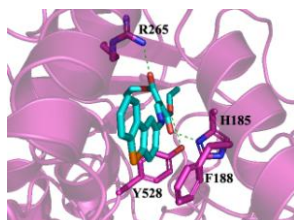
- A 'compound library' is a collection of compounds, just as we use 'library' for a collection of books. The variety (diversity) of compounds may be:
 - small and very limited diversity (e.g. departmental library),
 - big but relatively limited diversity (e.g. University academic library),
 - big and diverse (e.g. city library).
- Compound libraries from past projects are kept and may be screened for the biological activity you are looking for in a new project (c.f. Sternbach and librium).
- New compounds may also be made "in-house" but nowadays specialist chemical companies are often contracted to simply make NCEs (new chemical entities) for big pharmaceutical companies.
- A disadvantage of synthetic libraries is that they are often of limited structural diversity.

Twenty or so years ago a bottleneck in the modern drug discovery process was identified problem:

How can we access new compounds as quickly as possible? 31

Combinatorial Chemistry

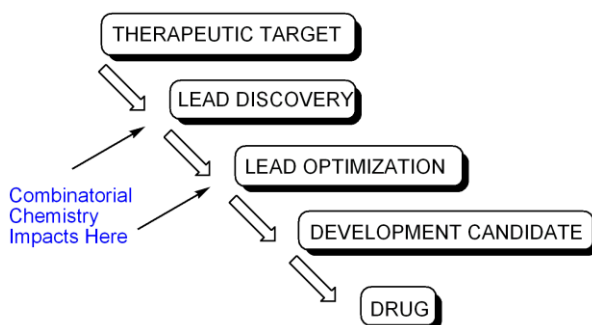
Lecture 3



Combinatorial chemistry

The aim of combinatorial chemistry ("CombiChem") is the **generation of (large) numbers of compounds very quickly**.

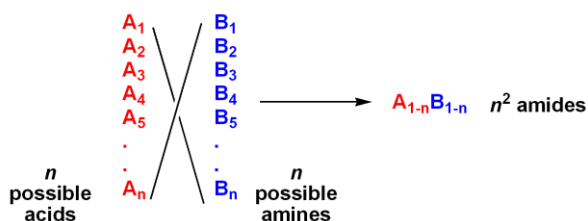
("combinatorial": of, relating to, or involving combinations).



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Example: making dipeptides

- Consider the synthesis of a dipeptide.
 - there are twenty 'DNA encoded' amino acids...
 - so there is the possibility of forming 20^2 dipeptides.



- A hexapeptide could give rise to 64 million different variations using the 20 building blocks! It would take forever to make these one at a time!

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CombiChem

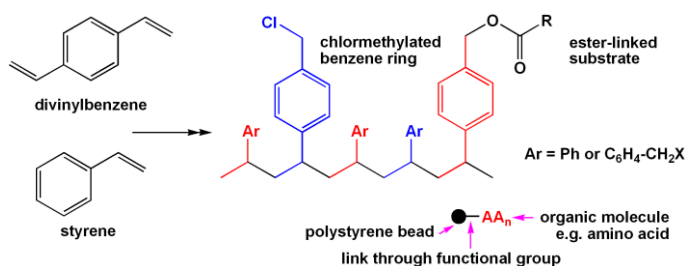
- The traditional method of medicinal chemistry concerned the preparation, purification and characterization of individual compounds on a relatively large scale.
- But only a few mgs are needed for a first run biological *in vitro* assay.
- Often target compounds are very similar (“analogues”) and so the chemistry to make them is, in many cases, identical.
- CombiChem allows us to make more than one compound at a time.
- There are two main approaches used in CombiChem:
 - Solid-phase organic chemistry (“SPOS”)
 - Solution-phase parallel synthesis

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SPOS

The essence of SPOS is to attach your substrate to a solid “support” (known as “beads” or “resin”) rendering the reactions heterogeneous. This facilitates the work-up and purification of your reaction products.

The resins are most commonly polystyrene cross-linked with 1-2% divinylbenzene to make insoluble porous beads a few microns in diameter.

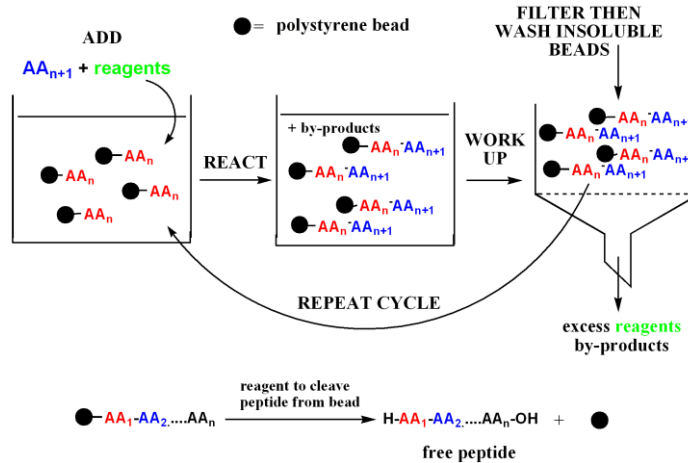


The polystyrene is derivatised, most commonly “chloromethylated”, to provide a functional group to which a small organic molecule may be attached either directly or via a linker group.

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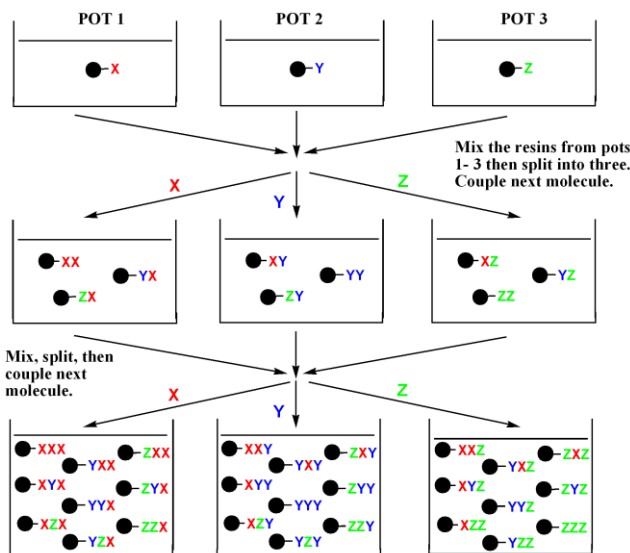
Merrifield peptide synthesis (1963)

SPOS has roots in R.B. Merrifield's (Nobel Prize 1984) method for the synthesis of peptides attached to microporous polystyrene beads.



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Divide Couple Recombine (DCR)



The DCR or "split-mix" approach was developed by Arpad Furka in the 1980s.

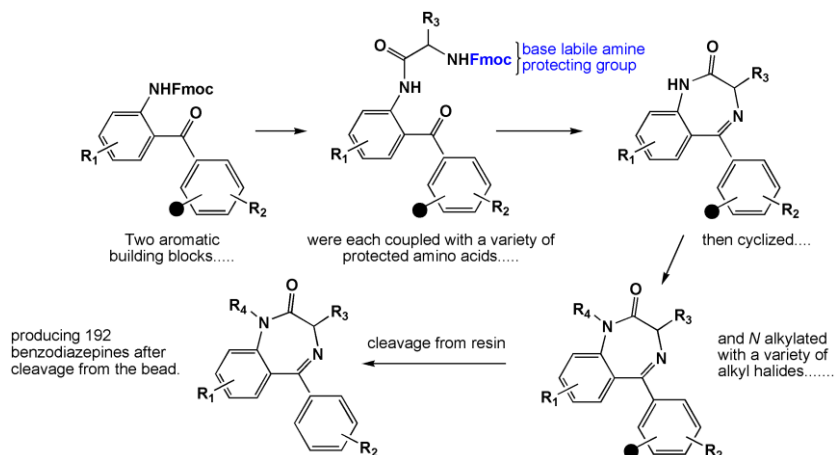
This is sometimes called the "one bead-one compound" approach.

Here the 27 possible tripeptides arising from three amino acids X , Y and Z are made in just two cycles.

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Solid phase synthesis of a benzodiazepine library

Prof J. Ellman (1992) applied this approach to non-peptidic molecules by making a library of 196 benzodiazepines.



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Advantages and disadvantages of SPOS

Disadvantages

- Quantities produced can be very low for very large libraries (maybe as low as 10s of nanomoles).
- Solution phase methods don't always work applied to the solid phase.
- Compounds will always have a "residual" functional group (e.g. alcohol) from the attachment point to the resins.
- Characterisation of intermediates difficult: how can we tell if our reaction has worked?
- How do you know *what* compound is attached to any one bead? ("deconvolution")

Advantages

- handling of material is easy and can be automated
- purification is easy - simple washing and filtration is usually all that is needed.

SPOS has evolved and now it is common to use **supported reagents** rather than **supported substrates**. Thus, the advantages of SPOS are retained with some of the major problems being removed.

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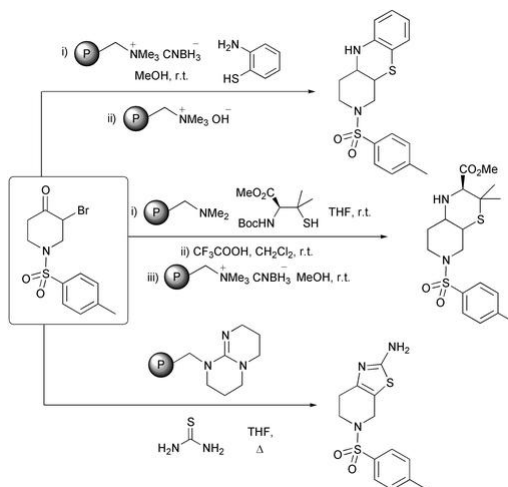
Supported reagents in synthesis

Journal of the Chemical Society, Perkin Transactions 1
DOI: 10.1039/b006588i

Review

"Multi-step organic synthesis using solid-supported reagents and scavengers: a new paradigm in chemical library generation"

S.V. Ley*, I.R. Baxendale, R.N. Bream, P.S. Jackson, A.G. Leach, D.A. Longbottom, M. Nesi, J.S. Scott, R.I. Storer and S.J. Taylor



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Parallel (array) solution-phase synthesis

- Another approach used more commonly now is to use solution-phase combinatorial synthesis where compounds are **synthesized in parallel** (at the same time) using an **array of reaction vessels**.
- In order to increase productivity it was not unheard of chemists in the past balancing half a dozen small round-bottom flasks on one stirrer-hotplate.

- Specialized multi-well reactors are now made by several companies, such as Radleys in the UK.



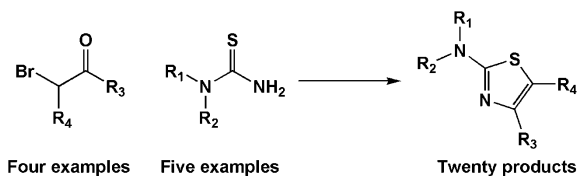
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Synthesis of aminothiazoles via the Hantzsch method

Glaxo-Wellcome
(1996)

In a 'proof of concept' study the reliable Hantzsch synthesis was used to prepare twenty thiazoles, including fanetizole [$R_1 = \text{Ph}(\text{CH}_2)_3$; $R_2 = \text{H}$; $R_3 = \text{Ph}$; $R_4 = \text{H}$] a known anti-inflammatory agent. The compounds were prepared using robotic system.

(70 °C, 5h in DMF; add diethylamine, solvent removed by nitrogen stream over 24 h. Structure analysis was performed by HPLC-mass spec.)



	B_1	B_2	B_3	B_4	B_5
A_1	A_1B_1	A_1B_2	A_1B_3	A_1B_4	A_1B_5
A_2	A_2B_1	A_2B_2	A_2B_3	A_2B_4	A_2B_5
A_3	A_3B_1	A_3B_2	A_3B_3	A_3B_4	A_3B_5
A_4	A_4B_1	A_4B_2	A_4B_3	A_4B_4	A_4B_5

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Parallel synthesis: advantages and disadvantages

Advantages:

- No synthesis development work needed (compared to SPOS).
- Easy to identify active hit as its position (X,Y coordinate) in the array encodes the reagents and thus structure of the product.
- New equipment, such as 'personal synthesisers' and 'multi vial apparatus,' allows for parallel (at the same time) synthesis of many compounds simply and quickly by one chemist.
- These techniques lend themselves to robotized technology.

Disadvantages:

- A build up of impurities can occur unless the by-products are volatile and the reactions very clean and high yielding.
- Most useful for one to three steps reactions only.
- Can only be used for making smaller (more focussed) libraries.

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“Automated synthesizers”



Emrys™ Optimizer EXP



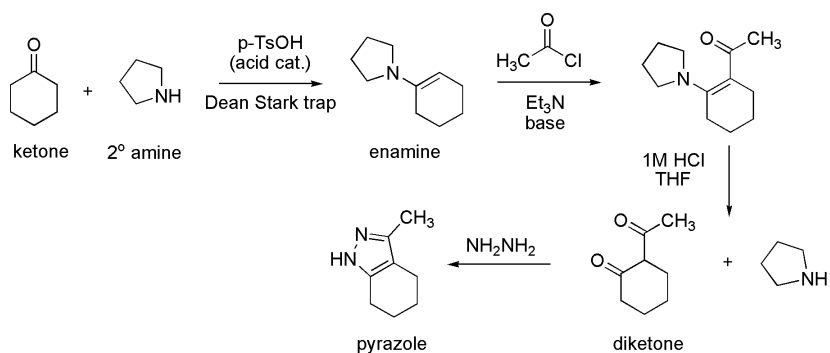
Advantage Series™ 3400 Process Chemistry Workstation

45

Designing a library (part 1)

Show how a simple reaction may be “modified” to take advantage of supported substrates (intermediates) for the synthesis of a library of pyrazoles:

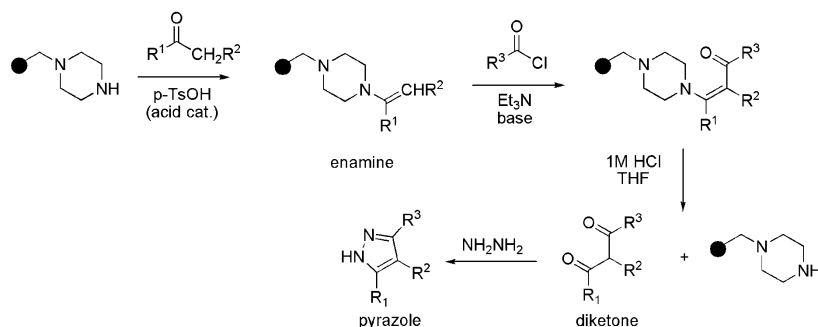
The [solution phase reaction](#), which is based on a Yr 2 Sem 2 Org Lab experiment, is as follows:



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Designing a library (part 2)

The solid-phase synthesis of a library of diketones has been reported (*Tetrahedron Lett.*, 2003, **44**, 1067-1069). The scheme below shows how the simple reaction from before may be adapted to take advantage of supported reagents and intermediates, in this case using a supported secondary amine.

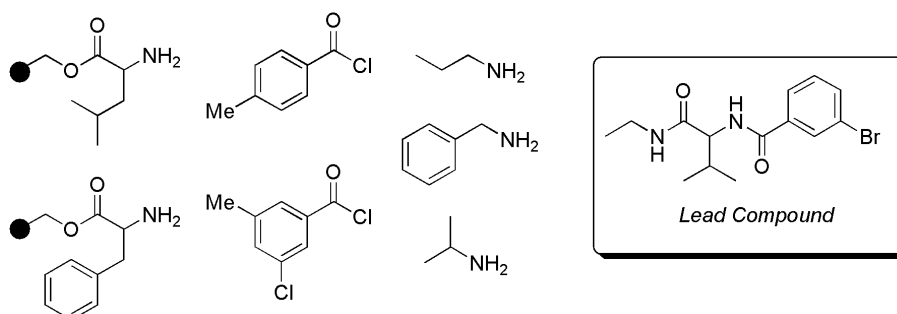


The split-mix (DCR) method allows for the preparation of a library of pyrazoles using this methodology.

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Problem

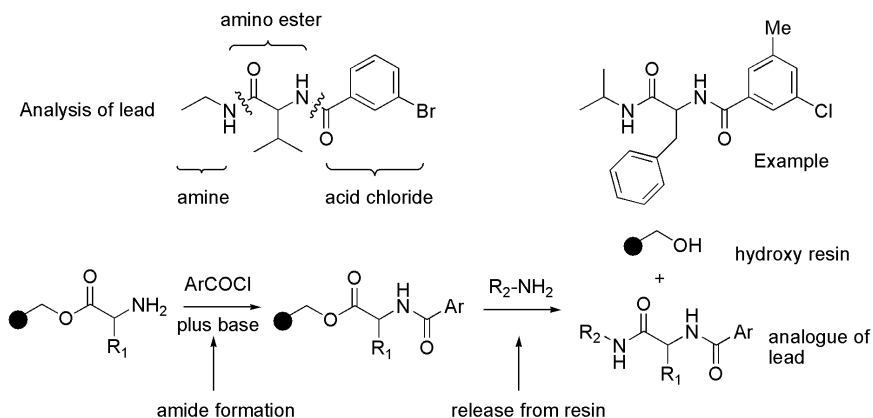
Question: Using the building blocks below, illustrate how you can construct a small library of analogues of the *lead compound* given using the Split-Mix Synthesis technique. (*Hint: ester + amine react to give amide + alcohol*)



How many library members will there be? Draw the structure of a representative member of the library. (*answer next week*)

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Answer



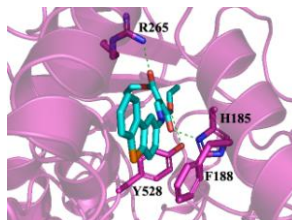
With two amino esters and two acid chlorides a total of four intermediates could be made. Release from resin with one of three amines gives a total of 12 analogues.

In the split-mix method there will be **two reaction "pots"** for the coupling of the **two acid chlorides** and **three pots** for reaction with the **three amines**.

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Rational Drug Design

Lecture 4

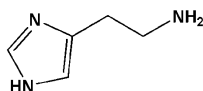


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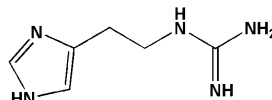
Physiopathological hypotheses

Detailed study / knowledge of biochemical pathways can lead to hypotheses for the discovery of new drug targets and lead compounds.

E.g. Gastric secretions are provoked by **histamine** and can lead to gastric ulcers. The antihistamines available at the time did not block the production of gastric secretions, leading to the hypothesis that an unknown sub-class of histamine receptor existed.

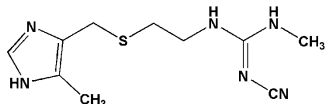


Histamine

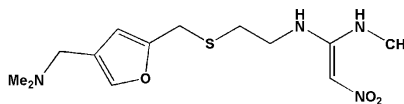


Guanylhistamine

Guanylhistamine was known as a weak antagonist of gastric secretion production. It served as the starting point to find a lead compound that ultimately led to the discovery of the H_2 -receptor antagonists cimetidine (Tagamet) and ranitidine (Zantac).



Cimetidine

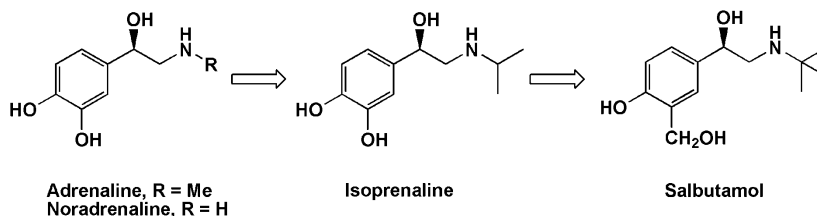


Ranitidine

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Substrate-based drug design

The **natural substrate** for a receptor or enzyme can serve as a **starting point for lead discovery**. For example salbutamol (albuterol), an analogue of adrenaline / noradrenaline, was developed to treat asthma.

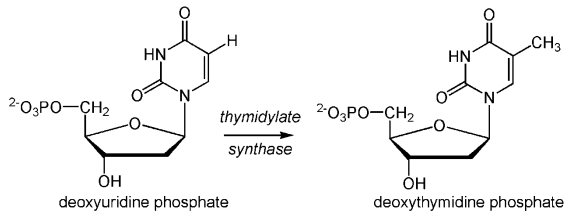


Adrenaline shows **no selectivity** for adrenergic receptor subtypes and so displays a range of cardiovascular side effects.

Isoprenaline shows selectivity for β - over I -receptor subtypes, but salbutamol is a **selective agonist** of the adrenergic β_2 -receptors (in lung tissue). It has the same potency as isoprenaline, but is 2000 times less active on the adrenergic β_1 -receptors (in heart tissue).

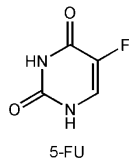
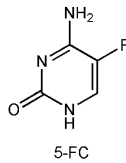
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Substrate based drug design: nucleoside analogues



Uracil is joined to a sugar group to make deoxyuridine. This is converted to deoxythymidine, an essential building block for DNA.

With the structure of the heterocyclic bases in mind, 5-fluorocytosine (5-FC) and 5-fluorouridine (5-FU), synthetic pyrimidines, were made as a prospective antitumour agents (5-FC actually has antifungal properties).

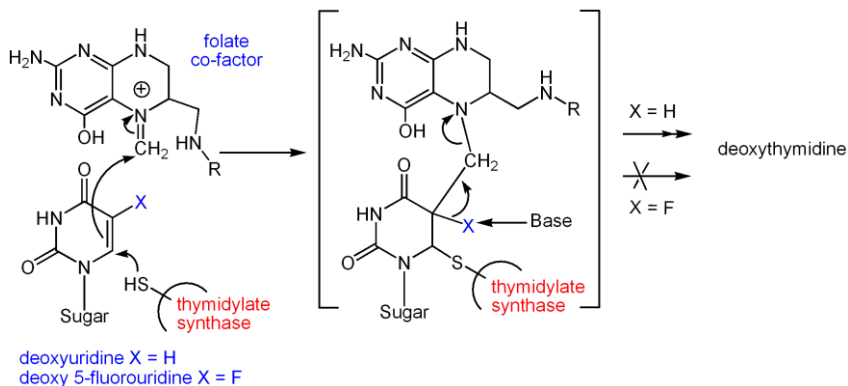


5-FC is an analogue of the naturally occurring cytosine, but is converted into 5-fluorouracil (5-FU) in the fungal cell by a de-aminase enzyme.

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5-FU: Mode of Action

5-Fluorouridine monophosphate stops deoxythymidine synthesis via irreversible inhibition of thymidylate synthase. Due to the presence of a key fluorine the covalent bond to the enzyme cofactor cannot be broken. This means that replication of DNA is stopped due to a lack of the right nucleoside.



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Rational drug design

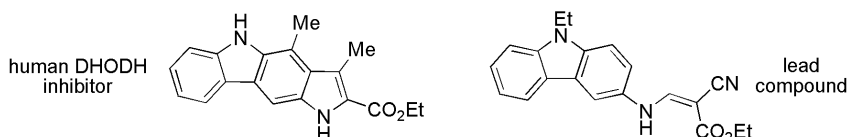
- Advances in molecular biology techniques means **making and isolating “large” amounts of proteins** much easier nowadays.
- X-ray crystallography has developed so that the determination of the **3-D crystal structures of proteins** and receptors is becoming easier.
- The Protein Data Bank (see <http://pdb.ccdc.cam.ac.uk/pdb/>) has data for hundreds of published structures which are all freely available on the WWW.
- Coupled with advances in computing power and molecular modelling the so-called **rational or structure-based drug design** has been advanced as “the way forward” in the search for new drugs.

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Design of DHODH inhibitors

DHODH is an enzyme involved in making the heterocyclic portion of **uridine monophosphate** (see before). DHODH is a new antimalarial drug target.

A screen of **inhibitors of human DHODH** against the **parasite DHODH** came up with a “hit” which is being developed into a potential lead compound.



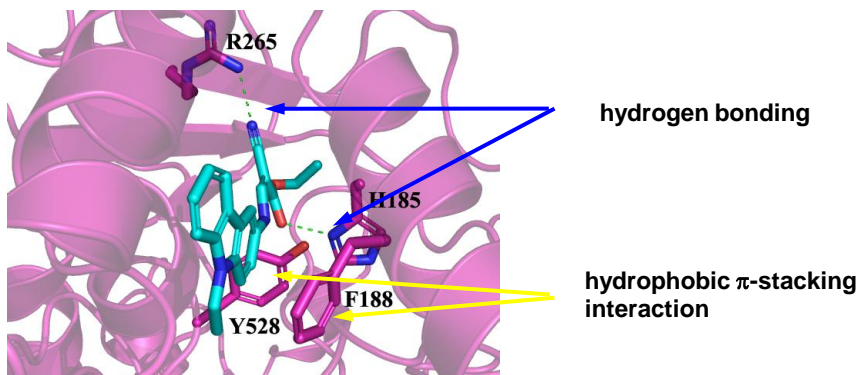
The **X-ray crystal structure** of the parasite enzyme is known so a computational study has allowed the “**docking**” (superposition) of the lead structure **into the active site of the enzyme**.

This study is directing optimization of the inhibitor structure through determination of the intermolecular forces between enzyme and inhibitor.

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Design of DHODH inhibitors

H-bonding, electrostatic and hydrophobic interactions can be identified and, hopefully, optimised by “*in silico*” design.



Using a protein X-ray structure for “*in silico*” *de novo* design of lead compounds is also of current academic interest.

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Analogue

Analogue design is an alternative to high-throughput screening (and the other methods discussed) for discovering a new lead, especially against a known drug target.

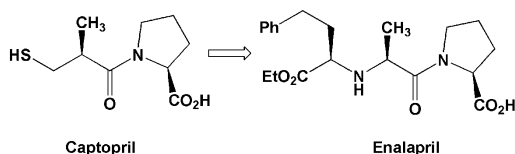
*Analogue*s can be compounds that either:

- A. exhibit chemical **and** pharmacological similarities, and these may be referred to as “*direct analogues*”;
- B. have chemical similarity, but which show unexpected pharmacological profiles - “*structural analogues*”;
- C. exhibit pharmacological similarity but have distinct chemical structures - “*functional analogues*”.

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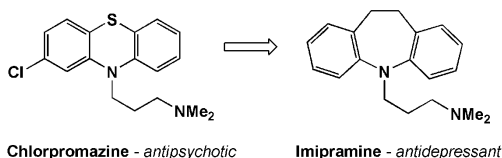
Direct and structural analogues

For “**direct analogues**”, a new lead must normally promise improvements in properties over an existing drug to be pursued. They are sometimes known as “*me-too compounds*”. For example ACE inhibitors:



For saturated markets the chances of finding patentable structures in this area may be more limited than for a follow up to a pioneer drug.

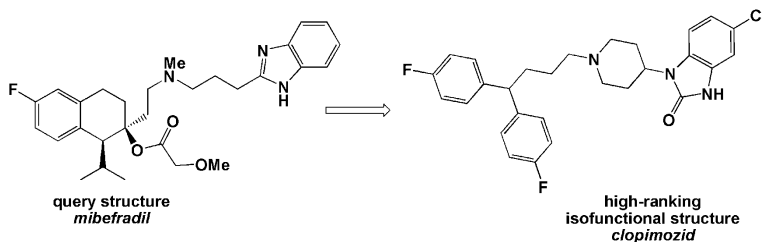
For “**structural analogues**” compounds are of no use unless they have an **alternative biological activity**. For example:



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Functional Analogues

How can **functional analogues be designed** (discovered in a non-random fashion)? The central core of a drug molecule, **the scaffold**, may be modified quite drastically to produce a functional analogue *if the essential activity-determining groups of the drug are retained*.



“**Scaffold hopping**” (or “scaffold morphing”) is a technique that use computational algorithms (i.e. a computer programme) to identify scaffolds from a “virtual library” of molecules or molecular fragments. This process can be assisted by manual intervention and structure ranking.

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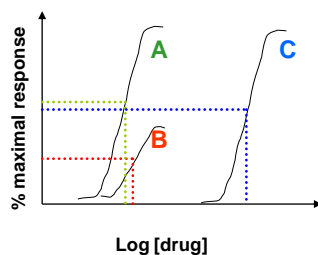
Assessing the quality of the lead

The **potency** of a substance (*the concentration required to achieve a defined biological effect*) must be significant in order to identify assay “hits” worth pursuing:

- For a hit <100 $\mu\text{mol/mL}$
- For a candidate drug < 10 nmol/mL

Selectivity, the effect of a lead on a desired target *versus* an undesired target, is also an issue - e.g. in the case of salbutamol and isoprenaline.

It is also important not to confuse **potency** with **efficacy**. Here **A** and **C** are equally **efficacious** (produce same maximal response), but **B** is less so. However the **potency** of **A** and **B** are similar, and both greater than **C**.



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Assessing the quality of the lead

Assuming your compound has the desired potency and selectivity from your screening assay you should consider the following:

Is it free from **structural elements** that are likely to engender **toxicity**?

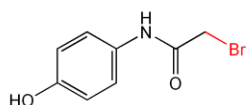
Is the molecule **easy to synthesise** and is it amenable to chemical modification?

How **soluble is it**? (see next lecture). Could “formulation” (i.e. converting the drug into the suitable pill, gel, cream suitable for treating the condition) be a problem?

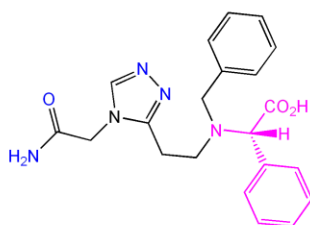
Does it resemble competitors' compounds? Will there be **patent problems**?

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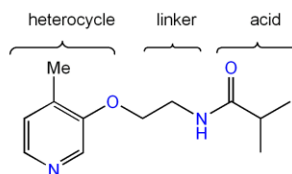
Good and bad leads



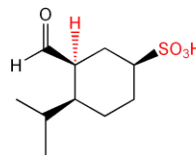
alkylating agent > toxicity problems!
(also Michael acceptors, C=C-C=O unit)



Chirality easily available from amino acid
H-bonding possibilities > water solubility



simple structure
obvious synthesis: > easy to make analogues
H-bonding possibilities > water solubility



no obvious synthesis / source of chiral starting materials
may readily epimerise α - to aldehyde
sulfonic acid bad for oral absorption

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Finding lead compounds - summary

A lead compound is a **first foothold on the drug discovery ladder**. Possible routes to identifying a lead compound are:

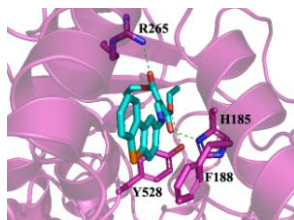
- Chance observations
- Selective optimisation of side effects (SOSA approach)
- Herbal / folk remedies
- Screening of natural product metabolites
- (high-throughput) Screening of compound libraries
- "Rational" drug design
 - Natural substrate-based drug design
 - Physiopathological hypotheses
 - (*in silico* design ?)

It takes much more effort to make a lead compound into a drug candidate.

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Drug Discovery

Lecture 5



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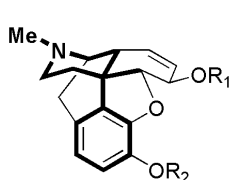
Structure Activity Relationship (SAR)

- The fundamental aim in taking a lead compound through to a drug candidate is to **improve the desired** and **reduce or eliminate the undesired** properties of a molecule.
- Determination of a **structure-activity relationship** (SAR) is the process by which chemical structure is correlated with biological activity (expressed as the **concentration** of a substance required to give a certain **biological response**).
- **Subtle modification of a structure may radically alter a molecule's potency**, e.g. by simple addition of a methyl group or changing an aryl ring substituent from an electron-withdrawing to an electron-donating group.
-*BUT* these changes may not only alter potency but may also effect the compound's physicochemical properties, i.e. solubility, pKa, as well as metabolic stability..... etc.

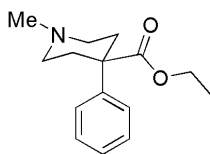
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Identification of a pharmacophore

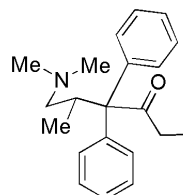
We have defined a lead compound as “a compound from a series of related compounds.....”. The question is therefore posed what are the essential structural elements for biological activity?



$R_1 = R_2 = \text{H}$, morphine
 $R_1 = R_2 = \text{OAc}$, heroin
 $R_1 = \text{H}$, $R_2 = \text{CH}_3$, codeine



meperidine



methadone

A **pharmacophore** is “a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity”. A pharmacophore **may not be identified** until **many analogues** have been made and tested. **But which ones to make?**

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Quantitative Structure Activity Relationships (QSARs)

Determining a quantitative structure-activity relationship (QSAR) is a process by which **biological activity is correlated with physicochemical properties** as expressed in the form of a mathematical relationship. This mathematical expression(s) can then be used to **predict** the biological response of other chemical structures.

In reality it is difficult to correlate biological activity with a single physicochemical property as the **problem is multivariate**, and the **Hansch equation** attempts to express this mathematically.

$$\log(1 / C) = k_1 \log P + k_2 \sigma + k_3 E_s + k_4 \pi$$

The physicochemical parameters in this equation are:

- **The overall hydrophobicity**, as measured by the partition coefficient P
 P is the **water-octanol partition coefficient** and is a measure of the equilibrium concentration of solute in octanol divided by the concentration of the same species in water. $\log P$ is a measure of hydrophilicity / phobicity of a compound. $c\log P$ and $M\log P$ values are calculated, as opposed to experimentally determined, values.

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QSAR - physicochemical parameters

- **Electronic effects**, as measured by the Hammett substituent constant σ “electronic effects” are the electron-withdrawing or donating effects (mesomeric or inductive) of a substituent on an aromatic ring. σ values are determined through comparison of reaction rates in closely related compounds. The ortho, meta or para positioning of a substituent can play a big role in the value and sign of σ .
- **Taft's steric factor**, E_s
Taft made an attempt to quantify the steric effect of substituents by looking the rate of hydrolysis of a series of similar esters.
- **The substituent hydrophobicity**, π
Analysis of $\text{Log}P$ values for a series of closely related molecules, e.g. simple substituted benzenes, can lead to an estimate of the **hydrophobic contribution**, π , of a given substituent to the overall hydrophobicity. A **positive value** for π means it is **more hydrophobic** than hydrogen, a **negative value** for π means it is **more hydrophilic**.)

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Topliss schemes and Craig plots

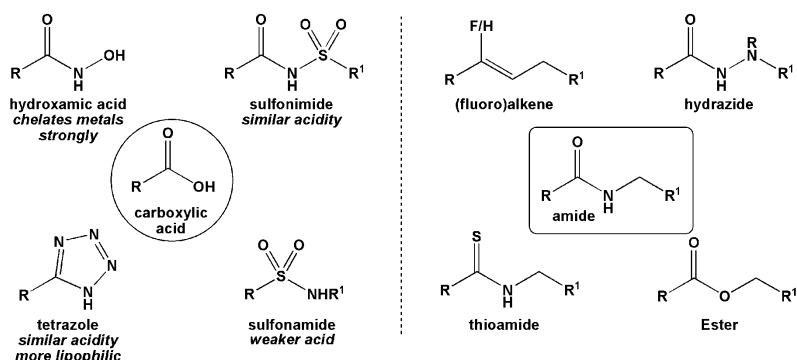
- **Topliss** suggested a **stepwise selection approach** towards analogue identification, designed to maximize the chances of synthesizing the most potent compounds in a series **as early as possible**. The Topliss schemes are simple **decision making tree charts** and were proposed for aromatic ring substituents and alkyl side chains.
 - The schemes are based on a fundamental assumption that the Hansch method is valid and that a particular substituent may modify activity relative to the parent compound by virtue of resulting changes in physicochemical properties.
- **Craig plots** are **visually accessible** correlations of substituent parameters. Two common Craig plots are π vs. σ and π vs. E_s .
- Considering these plots / decision marking charts **assist in analogue design and selection**.
- QSAR can be taken much further by incorporating **multidimensional analysis** (3D QSAR) into the process. Here biological activity is correlated with the three dimensional shape (conformation) of a molecule.

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Bioisosteric replacements

Bioisosteric groups (bioisosteres) are substituents or functional groups with **related physical (or chemical) properties** that give rise to **similar biological properties** in a compound.

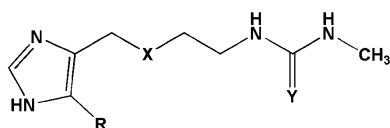
The purpose of making an isosteric replacement is to find enhanced biological (i.e. greater potency, less toxicity etc.) and / or physical properties in a compound, but **without making significant changes** to the chemical structure. Isosteric replacements may modulate molecular size, conformation, H-bonding, pKa, solubility and stability etc.



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Bioisosteres - examples

Making isosteric replacements were key steps in the development of the H₃-(histamine) receptor antagonist **cimetidine (7, Tagamet)** from the initial starting point of **burimamide (1)**.



Examples of conservative changes are simple replacements of H by F, Cl, Br or CH₃, or replacing SMe for OMe or NHMe etc.

E.g.	R	X	Y	ID ₅₀
1	H	CH ₂	S	6.1
2	H	S	S	5.0
3	Me	S	S	1.6
4	Me	S	O	27.0
5	Me	S	NH ₂	12.0
6	Me	S	N-NO ₂	2.1
7	Me	S	N-CN	1.4

ID₅₀ is the intravenous dose (μmol/kg) needed to reduce gastric acid secretion in anaesthetized rats by 50%.

Burimamide would itself have made a good drug on potency criteria, but could not be developed due to the fact that it is very poorly absorbed when given orally.

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Lipinski's "Rule of Five"

Christopher Lipinski proposed four parameters that define the "drug-likeness" of potential drug candidates based on analysis of existing drug molecules. "The Rule of Five" got its name from the cut-off values for each of these parameters of which all have values of five or a multiple of five.

The "rule" states that poor absorption or permeation is more likely when :

- A compound has more than 5 H-bond donors (sum of OHs and NHs);
- There are more than 10 H-bond acceptors (sum of Ns and Os);
- The MW is over 500;
- The LogP is over 5 (or MLogP is over 4.15).

The "rule" is used by many as a useful guide in drug design.

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Veber's rules

- Daniel Veber (and co-workers) proposed that the number of rotatable bonds in a drug candidate influences its oral absorption as shown by measurements in rats for over 1100 drug candidates (*J. Med. Chem.*, 2002, **45** (12), 2615).
- The predictors of oral bioavailability were found to be
 - i) molecular flexibility (measured by the number of rotatable bonds),
 - ii) polar surface area,
 - iii) total hydrogen bond count (sum of H-bond donors and acceptors).
- The study suggested that good absorption was independent of molecular weight. The supposed contradiction with Lipinski's MW "rule" was explained as both the number of rotatable bonds and hydrogen bond count *tend to increase with MW*.
- Veber's observations suggested that compounds which meet two criteria will have a high probability of good oral bioavailability (in the rat model)
 - (i) 10 or fewer rotatable bonds
 - (ii) polar surface area equal to or less than 140 Å² or twelve or fewer H-bond donors and acceptors.

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Prodrugs

A prodrug is drug which is given (taken) in an inactive form. Once administered, the **prodrug is metabolized by the body into the biologically active compound**. Prodrug strategies are used to overcome a variety of problems by:

1. altering solubility

Making a compound either more or less soluble may assist in achieving the desired formulation

2. improving membrane permeability

Absorption into a cell means crossing a hydrophobic cell membrane. If a drug is too polar drugs it may not pass the membrane, but too non-polar and it may not come back out!

3. Slow release of the active agent

If a drug is eliminated from the body quickly then an effective dosage cannot be sustained. Slow release of the active agent by controlled release from a prodrug allows a more controlled dosage of the active being released into the body.

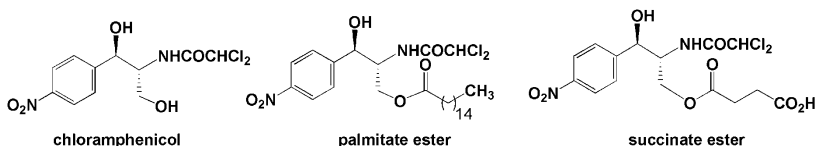
4. Masking drug toxicity or side effects

Many anticancer agents are cytotoxic, but it is the cancerous cells only which we want to kill. Masking toxicity can be achieved by prodrugging and the active agent is accumulates preferentially in the tumour (due to leaky vasculature).

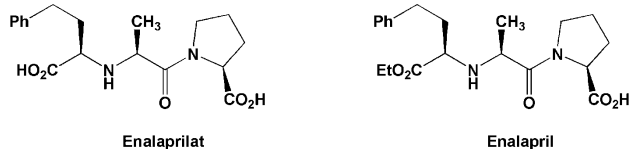
75

Prodrugs - examples

1. The antibiotic chloramphenicol is very **bitter**, but the **palmitate ester** does not get absorbed by the tongue so much when taken orally and so is more palatable. The **succinate ester** on the other hand makes it more soluble making intravenous formulation more effective. Once absorbed the esters are quickly hydrolysed.



2. The ACE inhibitor **enalaprilat** is potent *in vitro*, but is **poorly absorbed** and so not very effective *in vivo*. The ethyl ester **enalapril**, however, is **absorbed much better** but is a weak ACE inhibitor. It is hydrolyzed to the carboxylic acid by esterase enzymes in the blood, which is where ACE is found.



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Drug development phases

Clinical trials are designed to:

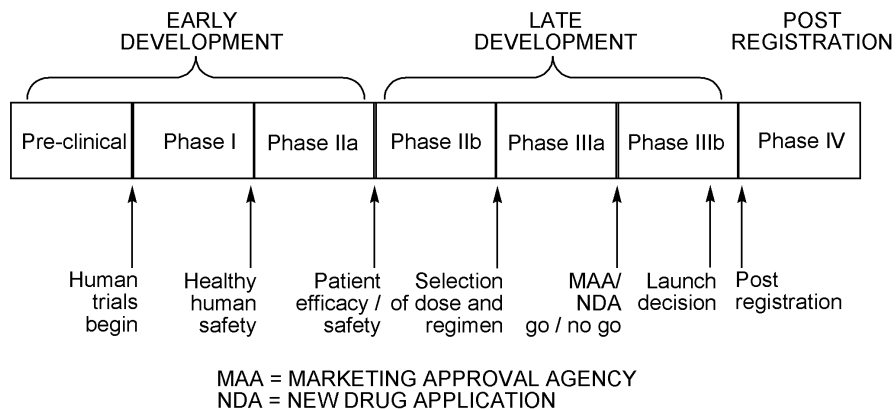
- determine safety and tolerance in man;
- pharmacokinetics;
- bioavailability for a range of doses;
- determine the pharmacological profile.

The main phases of pre-clinical and clinical trials are:

Pre-clinical	Animal studies. Submission of "Investigational New Drug" application to government bodies such as US FDA.
Phase I	Normal, healthy human volunteers.
Phase II	To evaluate safety and efficacy of drug in patients.
Phase III	Large patient number study to establish efficacy <i>versus</i> a placebo or comparator compound.
Phase IV	Long-term surveillance / monitoring of adverse reactions.

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Drug Development Phases and Decision Checkpoints



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This document is available, along with selected links to relevant journal articles on-line, from <http://www.hull.ac.uk/php/chsanb/teaching>

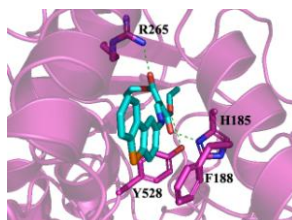
Library resources - Section RS 4xx of the library

1. '*Medicinal Chemistry, Principles and Practice*' Ed. F.D. King, Royal Society of Chemistry, 1994, **RS 403 M4**
2. '*Medicinal Chemistry: A Biochemical Approach*' T. Nogrady, OUP, 1988, **RS 403 N7**
3. '*Introductory Medicinal Chemistry*' J.B. Taylor and P.D. Kennewell, Ellis Horwood, 1981, **RS 403 T2**
4. '*Combinatorial Chemistry*' N.K. Terrett, OUP Oxford Chemistry Masters Series, 1998, **RS 419 T3**
5. '*An Introduction to Medicinal Chemistry*' G.L. Patrick, 2nd Ed., Oxford University Press, 2001, **RS 419 P3**
6. '*Burger's Medicinal Chemistry and Drug Discovery*' (comprehensive but advanced) 4th Ed., Ed. M.E. Wolff, Wiley, 1995, **RS 403 B9** (also 5th Ed. in Ferens' library)
7. '*Medicinal chemistry: an introduction*' G. Thomas, Wiley, 2000, **RS 403 T4**
8. '*Drug discovery : a history*' W. Sneader, Wiley, 2005, **RS 61 S6**
9. '*New drug discovery and development*, D Lednicer, Wiley, 2007, **RS 403 L4**

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Development of ACE inhibitors for controlling hypertension

Lecture 6



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The discovery of renin



Robert Tigerstedt (1853 - 1923)

Tigerstedt and Bergman (1898) found that a renal extract increased blood pressure in dogs. They called the extract renin. They also made a prophetic suggestion that renin could explain the known link between kidney disease and heart hypertrophy (an increase in the thickness of the heart muscle).



Harry Goldblatt (1891 - 1977)

Loesch (1933) and Goldblatt (1934) showed that ischemia (restriction in blood supply) to the kidneys lead to hypertension.

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The discovery of angiotensin



Irvine Page (1901 - 1991)

Braun-Menendez (Argentina) and Page and Helmer (USA) found in the 1940s the actual substance that caused hypertension - called either "hypertensin" or "angiotonin". Both semi-purified renin and showed it was not hypertensive unless in the presence of blood plasma.

Page and Helmer wrote "It is suggested that renin is an enzyme contained in the kidneys without pressor properties which interacts with renin-activator contained in the blood to form angiotonin, a highly active pressor substance from which several crystalline derivatives have been prepared".

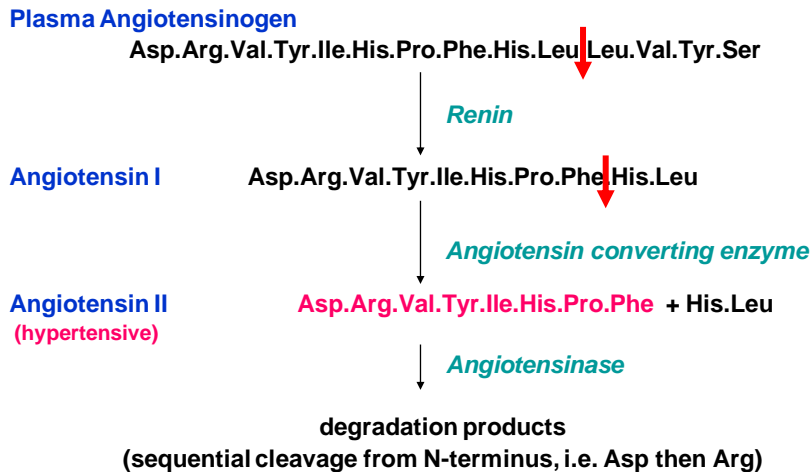


Leonard Skeggs (1918 -)

Skeggs and co-workers (1956) showed that angiotensin came in two forms, angiotensin I and angiotensin II, and the latter was produced from the former by action of an enzyme *angiotensin converting enzyme*, *thought to be a zinc metalloprotein*.

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Renin and the angiotensins



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The renin-angiotensin pathway

The protein **angiotensinogen**, produced in the liver, is cleaved by the action of the enzyme **renin**, produced in the kidneys, to give **angiotensin I**, a decapeptide.

Angiotensin I is converted to **angiotensin II**, an octapeptide, by the action of the enzyme **angiotensin converting enzyme (ACE)** in the blood. **ACE** cleaves a specific bond between the Phe-His residue so removing a dipeptide unit from the C-terminus of **angiotensin I** producing **angiotensin II**.

Angiotensin II interacts with receptors on the surface of blood vessel cells mediating vasoconstriction to increase the blood flow. It also causes fluid retention by stimulating the production of aldosterone, and together these can result in high blood pressure (hypertension).

Fine regulation of this system is controlled by another peptide, bradykinin (Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg), which antagonizes the activity of **angiotensin II**. Bradykinin, which is also hydrolyzed by ACE, is said to be hypotensive.

The action of **angiotensin II** is also moderated by **angiotensinase** an enzyme that degrades **angiotensin II** in to smaller inactive fragments.

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Control of hypertension

Alternatives for the control of hypertension were considered:

– Finding an inhibitor of renin

- efforts resulted in the finding of inhibitors with powerful *in vitro* potency. In addition much was learned on how to convert peptides into more drug-like molecules, however no orally active agent was found.

– Potentiating the formation of bradykinin

- the enhancement of enzyme action (i.e. stimulation of the production of more bradykinin) was known to be far more difficult than inhibition of an enzyme and so this route was rejected.

– Finding an inhibitor of ACE

- Early work suggested that bradykinin was inactivated by an enzyme similar to ACE. When this enzyme was proved to *be* ACE the inhibition of ACE became *the* target for a new anti-hypertensive agent.

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Bothrops jararaca and teprotide



Sir John Vane (1927 - 2004)

Working at the Institute of Basic Medical Sciences at the Royal College of Surgeons, Vane found that an **extract from the venom** of the Brazilian viper, *Bothrops jararaca*, blocked the production of angiotensin II by inhibition of ACE.



David Cushman

A collaboration with Ondetti and Cushman at the Squibb Institute for Medical Research in New Jersey led to the isolation of peptides with ACE inhibitory activity (in some early papers these were referred to as *Bradykinin-potentiating peptides*, BPPs). One of the most potent of these peptides was called **teprotide**.



Miguel Ondetti

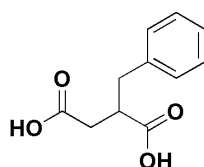
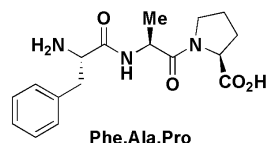
Teprotide
(pyro)Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH

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Peptides, benzy succinic acid and breakthrough

Teprotide was shown to be a potent anti-hypertensive agent when administered intravenously, but was **inactive by mouth** (enzymic degradation of the peptide in the stomach).

The Squibb research group screened over 2000 peptides and non-peptides, but **no orally active ACE inhibitor** was found. The SAR work on peptide analogues helped, however, the **development of a hypothetical model** of the active site in ACE, and provided **a simple *in vitro* assay**. The C-terminal tripeptide sequences Trp.Ala.Pro, and more stable Phe.Ala.Pro, were found to be optimal for the binding to the active site.



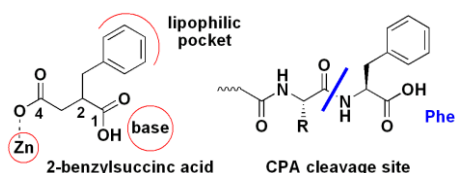
The **breakthrough** came in 1974 when Cushman and Ondetti discussed the significance of a research paper by Wolfenden and Byers describing the inhibition of another zinc metalloprotease, carboxypeptidase A (CPA), by **2-benzylsuccinic acid** (left).

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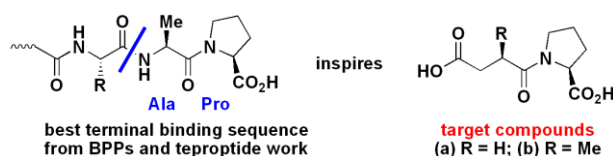
An early active site model of ACE

As a result of this paper Cushman and Ondetti **hypothesized** that:

The **4-CO₂H** bound to a **zinc atom** in the active site of CPA, and the 2-benzyl-1-CO₂H group **mimicked a phenylalanine** C-terminal amino acid residue peptides that are cleaved by CPA.



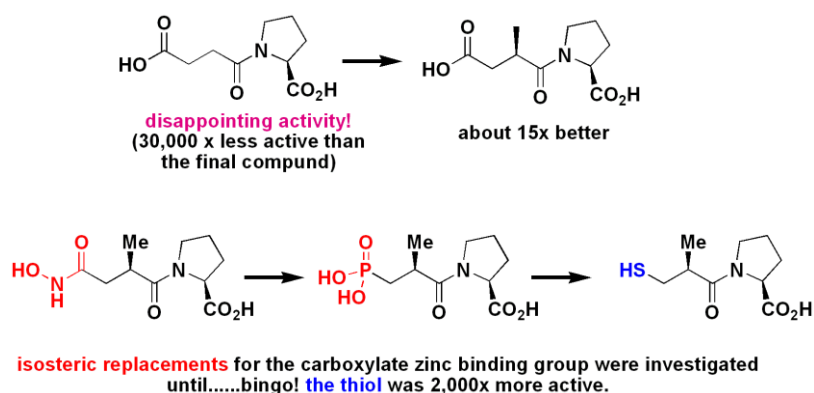
Carboxypeptidases **cleave** sequentially a **single amino acid** from the C-terminus of a peptide, whereas **ACE** **cleaves a dipeptide** from angiotensin I. So the distance between the zinc binding group and the C-terminus binding group should therefore be greater in an ACE inhibitor than a CPA inhibitor.



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The final breakthrough

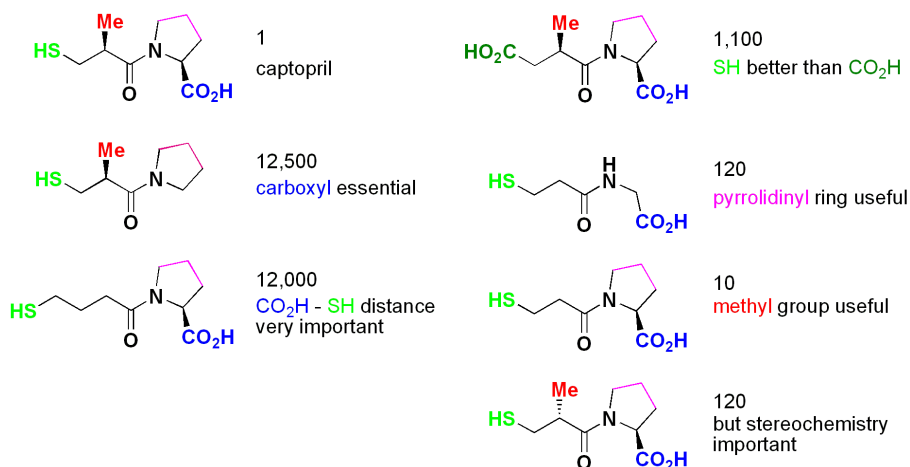
With hindsight captopril was only two steps away from the initial target compound succinyl-Pro, but in all about 60 compounds were made and tested to make a full structure activity relationship.



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SARs in the captopril series

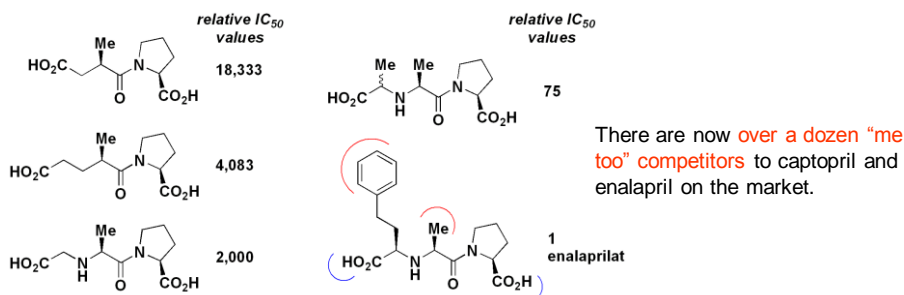
The numbers are relative k_i values against ACE.



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Success inspires competition

Since the discovery of captopril many new ACE inhibitors have been discovered. The [active site model of ACE was significantly improved](#), and the development of enalaprilat (enalapril) showed that carboxylates could be used as the zinc-binding motif if the structure benefited from additional hydrophobic binding.



Ref (1): D.W. Cushman and M.A. Ondetti, History of the design of captopril and related inhibitors of angiotensin converting enzyme, *Hypertension* **1991**,17, 589-592.

Ref (2): L.H. Opie and H. Kowolik, The discovery of captopril: from large animals to small molecules, *Cardiovascular Research* 1995, **30**, 18-25.

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