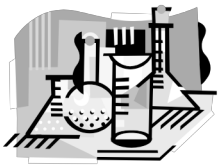
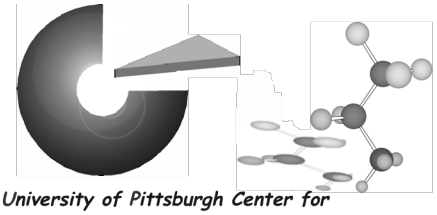
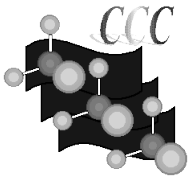
Pharm 5119 – Medicinal Chemistry & Drug Discovery



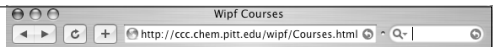
Dr. Peter Wipf
Department of Chemistry
University of Pittsburgh

http://ccc.chem.pitt.edu/wipf/index.html





University of Pittsburgh Center for Chemical Methodologies & Library Development



http://ccc.chem.pitt.edu/wipf/Courses.html

- ***Introduction***
- ***Survey of current drug targets***
- ***Drug discovery challenges***
- ***Methods to identify new leads***
- ***Case studies from industry***
- ***Structure-based design***

Course Links

Undergraduate Courses

Chem 0320; Fall 2005
Chem 1140; Spring 2005
Chem 0310; Spring 2004

Graduate Courses

Pharm 5119 Drug Development 1; Spring 2007
Advanced Organic Chemistry 2320; Spring 2007
Molecular Pharmacology 3360; October 2006
Pharm 5119 Drug Development 1; Spring 2006
Advanced Organic Chemistry 2320; Spring 2006

Medicinal Chemistry

The science that deals with the discovery or design of new therapeutic agents and their development into useful medicines.

It involves:

- Organic Synthesis
- Biological Target Identification & Assay Development
- Structure-Activity Relationships (SAR)
- Absorption, distribution, metabolism, and excretion (ADME)

Treatments

Cinchona has been used for a number of medical reasons such as:

- Treats malaria
- Kills parasites
- Reduces fever
- Regulates heartbeat
- Calms nerves
- Stimulates digestion
- Kills germs
- Reduces spasms
- Kills insects
- Relieves pain
- Kills bacteria and fungi
- Dries secretions

Malaria has been designated as "*the most significant disease for world civilization over the past three millennia".*

… and the quinine, the most celebrated cinchona alkaloid that was claimed as "*the drug to have relieved more human suffering than any other in history'.*

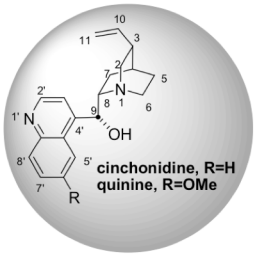
D. A. Casteel in Burgers Medicinal Chemistry and Drug Discovery, 5th Ed., Vol. 5 (Ed.: M. E. Wolff), Wiley, NewYork, 1997, Chap. 59, p. 16.

Timeline-Quinine

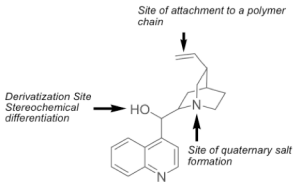
1820/Pelletier and Caventou
Two French chemists isolated quinine from the bark of cinchona tree which are found in the eastern slopes of the Andes mountains from Venezuela to Bolivia, and the natives called the cinchona tree "quina-quina" ("bark of barks', known as "fever stick").

Natives
Bark was dried, ground to a fine powder and mixed into a liquid (usually wine) before being served.
Effective muscle relaxant and antipyretic agents.

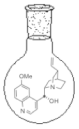
1600s/Europe
Quinine was first used to treat malaria in Rome in 1631, where malaria was epidemic and caused countless deaths in Europe.



1900s-present/Versatile Catalysts and Ligands in Asymmetric Synthesis



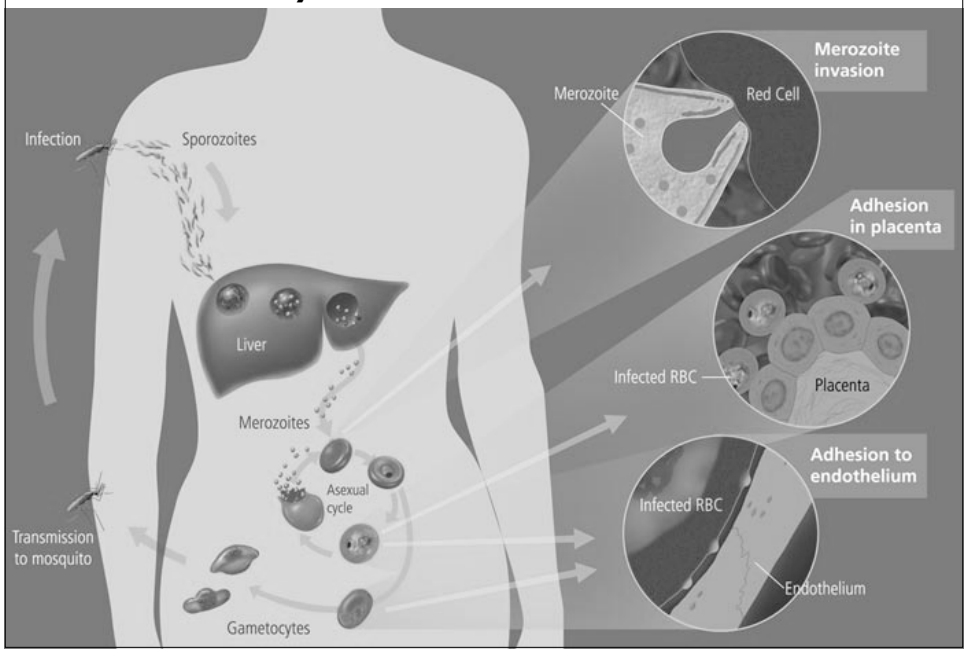
1940s-present/Total Syntheses
Woodward and Doering
Uskokovic
Stork
Jacobsen
Kobayashi
Williams



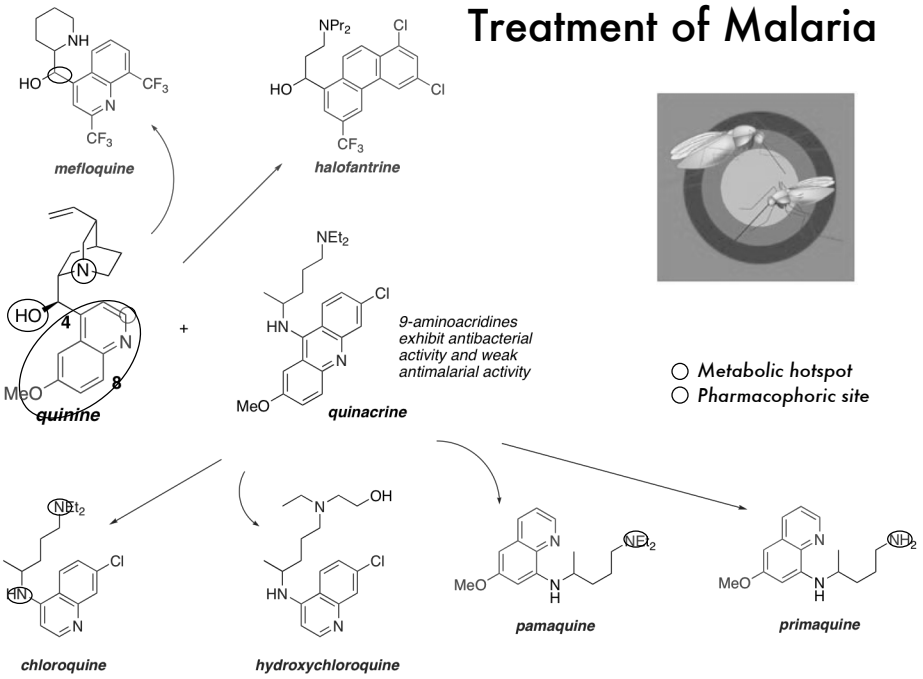
1820-1930s/Searching for Alternative Methods
Pelletier and Caventou, Hofmann, Rabe
Streaker (1854 Emperical formula $C_{20}H_{24}N_2O_2$)
Perkins, Prostenik and Prelog

D. A. Casteel in Burgers Medicinal Chemistry and Drug Discovery, 5th Ed., Vol. 5 (Ed.: M. E. Wolff), Wiley, NewYork, 1997, Chap. 59, p. 16.

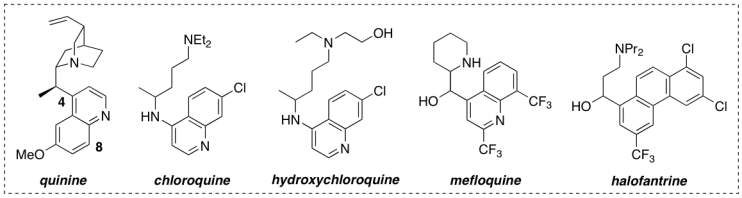
Lifecycle of Malaria Parasite

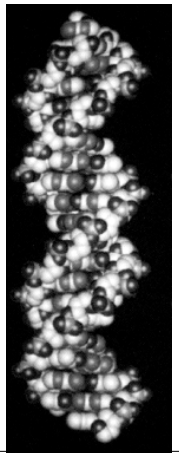


Treatment of Malaria



4-Substituted Quinolines - MOA

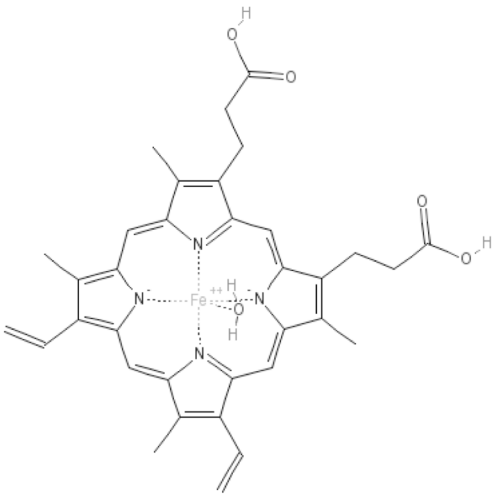




DNA Intercalation

- discounted since the concentration for inhibition of nucleic acid synthesis is significantly higher than what is necessary for inhibition of the plasmodium parasite

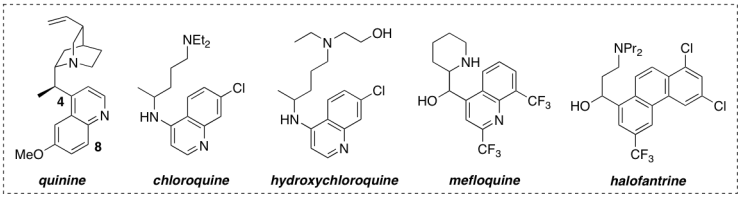
4-Substituted Quinolines - MOA



Ferriprotoporphyrin IX (FPIX)

-the plasmodium parasite utilizes host hemoglobin as a source of amino acids, producing a waste product containing FPIX. This compound is toxic to all cells, host and plasmodium cells, but usually bound to protein. In the presence of 4-substituted quinolines, FPIX is decomplexed from protein, regaining its toxicity toward erythrocytes and parasites.
-However, there is evidence against this mode of action

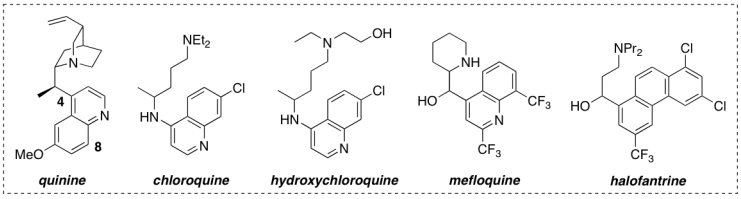
4-Substituted Quinolines - MOA



Weak Base Hypothesis:

-4-Substituted quinolines are weak bases and therefore sequestered to the more acidic locations (parasites lysosome pH is 4.8-5.2. The resulting buffering of the parasite's lysosomes reduces their ability to digest hemoglobin and access amino acid nutrients.
-Most likely, this is a minor MOA since it would require large amounts of drug to cause minor effects.

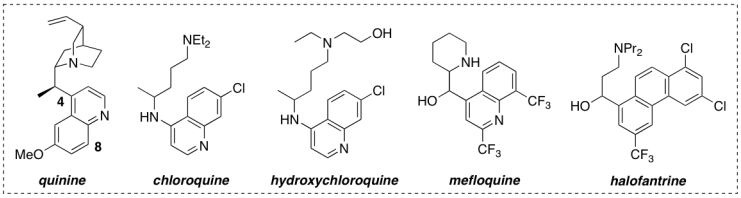
4-Substituted Quinolines - MOA



Conclusion:

-The actual target and mechanism of action is not really known.
-These drugs are toxic to the malaria parasite, specifically by interfering with the parasite's ability to break down and digest haemoglobin, thus starving the parasite and/or causing the build-up of toxic levels of partially degraded haemoglobin.

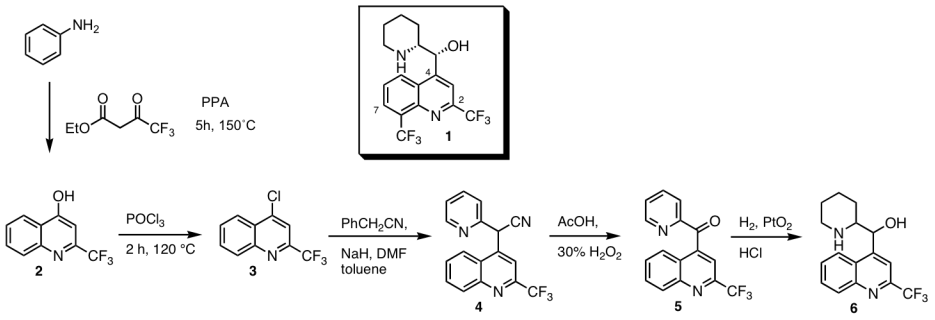
4-Substituted Quinolines - MOR



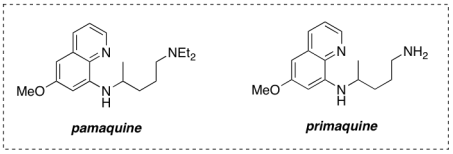
Mechanism of Resistance:

-The development of resistance is thought to be by spontaneous gene mutation.
-Most resistant plasmodium strain activate a drug efflux pump.
-Rapid metabolism of antimalaria drugs can also play a role. Cytochrome P-450 activity parallels increased resistance.

4-Substituted Quinolines - Synthesis

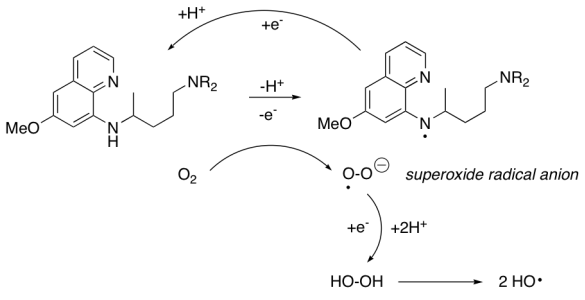


8-Substituted Quinolines - MOA

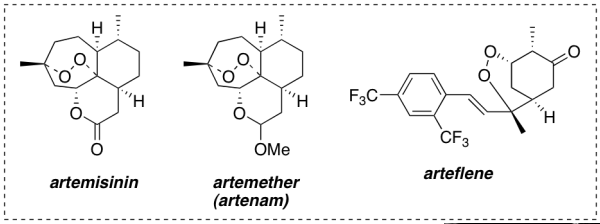


Generation of ROS

- ROS attack proteins and DNA and lead to cell damage



New Antimalaria Drug Therapies



-Isolated from Artemisia annua (qinghao; chinese herbal medicine)
-Kill parasites by a free radical mechanism (directly, not via ROS)

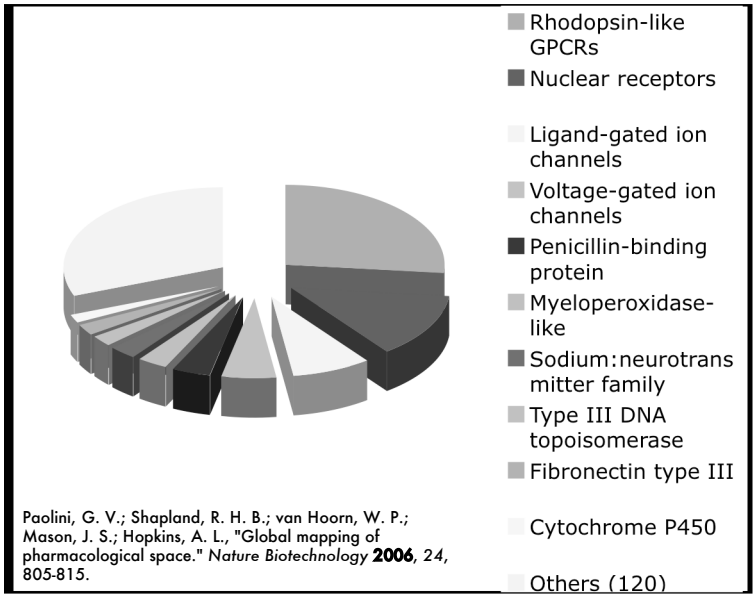
The State-of-the-Art in Drug Discovery:

Small, incremental advances in fundamental biology, chemistry, and clinical sciences

"The Most Fruitful Basis for the Discovery of a New Drug is to Start with an Old Drug"

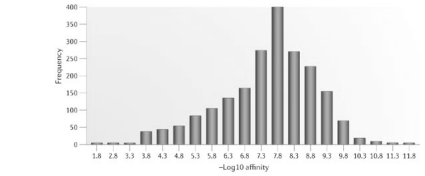
James Black - 1988 Nobel Prize in Physiology and Medicine

Gene-Family Distribution of Current Drugs

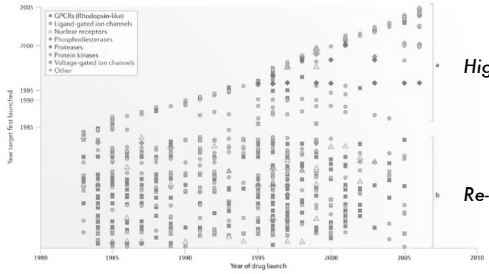


Paolini, G. V.; Shapland, R. H. B.; van Hoorn, W. P.; Mason, J. S.; Hopkins, A. L., "Global mapping of pharmacological space." *Nature Biotechnology* **2006**, *24*, 805-815.

Drug Potencies & Rate of Target Innovation



Median affinity = 20 nM



High Target Innovation

Re-Use of Established Mechanisms

- *Ca. 130 "privileged druggable domains" cover all current drug targets (vs. total number of protein families (16,000) and folds (10,000).*
- *Of ca. 1,620 distinct human protein sequences are linked to a genetic disease, only 105 are actual drug targets.*
- *Target innovation is slow - The average rate over the past 20 years has been quite constant at 5 new "drugged" targets per year.*
- *The first approved indications for drugs acting on new targets are usually orphan diseases.*

In Search of New Leads…..

A lead can be characterized as a compound that

• has some desirable biological activity,

• is not extremely polar or lipophilic,

• and does not contain toxic or reactive functional groups.

Often, Lipinski's rule of 5 (molecular weight (<500), lipophilicity (log P < 5, # of hydrogen bond donors <5, # of hydrogen bond acceptors <10), is used for evaluating the most obvious characteristics of a drug-like lead.

The lead should also have a series of congeners that modulate biological activity, indicating that further structural modification will improve selectivity and potency.

'Drug-like' & 'Lead-like' Properties

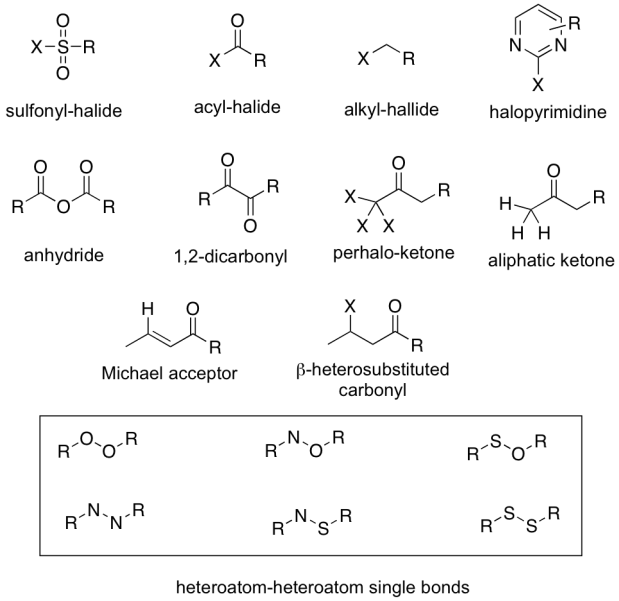
• Lipinski's rule-of-five deals with orally active compounds that achieved phase II, so it is not a method to distinguish between drugs and non-drugs but point to compounds that might have poor absorption or permeability.
• A more general approach is to use rules based on limits of physicochemical properties and on structural filtering, in a progressive scoring function.
• The evaluation for drug-like properties is based on:
  - Absence of atoms other than C, O, N, S, P, F, Cl, Br, I, Na, K, Mg, Ca, Li
  - 100 ≤ molecular weight ≤ 800 g mol^{-1}
  - log *P* ≤ 7
  - HBA ≤ 10
  - HBD ≤ 5
  - rotatable bonds ≤ 15
  - halogen atoms ≤ 7
  - alkyl chains ≤ -$(CH_2)_6CH_3$
  - no perfluorinated chains
  - smallest set of small rings ≤ 6
  - no medium or large rings (8 or higher)
  - at least one N or O atom
  - no reactive functions (Oprea, T. I. *J. Comp.-A. Mol. Des.* **2000**, *14*, 251).

'Drug-like' & 'Lead-like' Properties

- Optimization of a lead compound generally results in an increase of MW, log P, and complexity.
- A lead-like filter needs to select polar compounds with simple chemical structures.
- Hann, M. M.; Oprea, T. I. *Curr. Op. Chem. Biol.* **2004**, *8*, 255.
- The evaluation for lead-like properties is based on:
  - Absence of atoms other than C, O, N, S, P, F, Cl, Br, I, Na, K, Mg, Ca, Li
  - 100 ≤ molecular weight ≤ 460 g mol^{-1}
  - -4 ≤ log *P* ≤ 4.2
  - log Sw ≥ −5
  - HBA ≤ 9
  - HBD ≤ 5
  - rotatable bonds ≤ 10
  - halogen atoms ≤ 7
  - alkyl chains ≤ -$(CH_2)_6CH_3$
  - no perfluorinated chains
  - smallest set of small rings ≤ 4
  - no medium or large rings (8 or higher)
  - at least one N or O atom
  - no reactive functions

'Drug-like' & 'Lead-like' Properties

- Reactive functionalities should be filtered:



Where Did (Do) Our Drugs Come From?

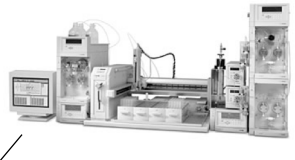
Past & current drug discovery strategies are based on

- **Folk medicine**
- **Screening of natural products**
- **Mimicry of biological metabolites & substrates**
- **Luck (also known as serendipity)**
- **"Me Too" approach**
- **Rational drug design**, often based on "hits" from (high-throughput) screening of large chemical libraries: "hit-to-lead"; use of SAR analyses and molecular modeling

Screening of natural product extracts

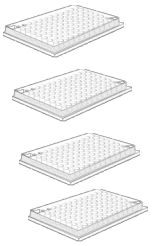
High-Pressure Accelerated Solvent Extractor System

DCM & MeOH Extracts



Parallel HPLC

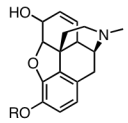
HT Solvent Concentrator



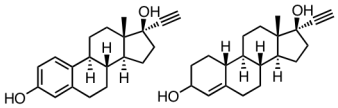
Multiwell Plates For HTS

Examples of Natural Products as Leads & Drugs

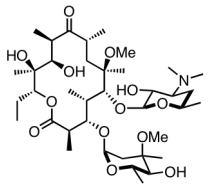
Cardiac glycosides, morphine, quinine, salicylic acid, taxol, camptothecin, penicillin, cyclosporin A, warfarin, artemisine….



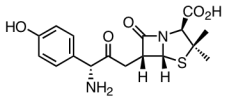
R = H: Morphine
R = Me: Codeine
(pain killer)



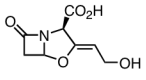
17-ethynylestradiol norethindrone
(the "Pill"; contraceptive)



Clarithromycin
(antibacterial)

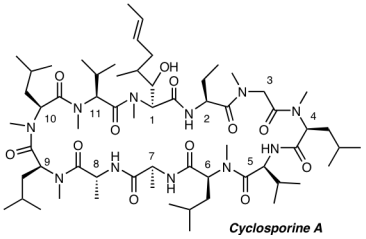


Ampicillin
(antibiotic)



Clavulanic acid
(β-lactamase inhibitor)

Augmentin
(antibiotic)



Cyclosporine A

Serendipitous Drug Discovery

• The use of nitrous oxide and ether as narcotic gases in surgery resulted from the observation that people who inhaled these chemicals [in parties] did not experience any pain after injury.

• The vasodilatory activity of amyl nitrite and nitroglycerin was discovered by chemists who developed strong headaches after inhaling or ingesting minor amounts.

• A wrong working hypothesis on chloral hydrate, which was supposed to degrade metabolically to narcotic chloroform, led to its application as a strong sedative (in reality, the metabolite trichloroethanol is the active form). Similarly, urethane was supposed to release ethanol but is a hypnotic by itself.

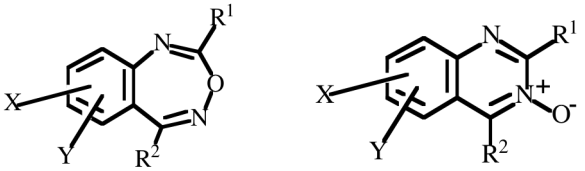
• Acetylsalicylic acid was thought to be just a better tolerable prodrug of salicylic acid, but turned out to have a unique mechanism.

• Phenolphthalein was considered as a useful dye for cheap wines; after a heroic self-experiment, a pharmacologist experienced its drastic diarrheic activity.

• Warfarin was used a rat poison.

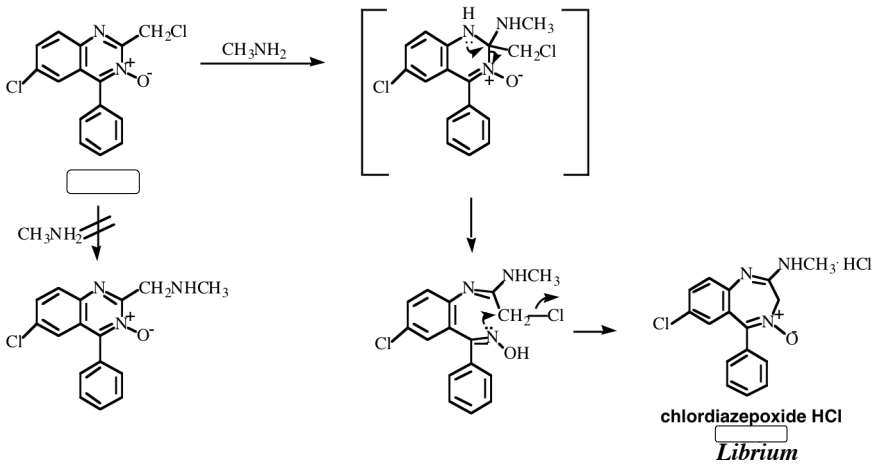
Serendipitous Discovery of Librium without a Lead

In 1955 Roche set out to prepare a series of benzheptoxadiazines as potential new tranquilizer drugs, but the actual structure was found to be that of a quinazoline 3-oxide.



In 1957, during a lab cleanup, a vial containing what was thought to be the latter compound (X = 7-Cl, $R^1 = CH_2NHCH_3$, $R^2 = C_6H_5$) was sent for testing, and it was highly active.

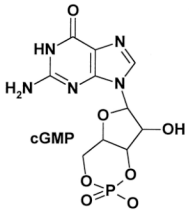
Further analysis showed that the actual structure of the compound was the benzodiazepine 4-oxide, **Librium**, presumably produced in an unexpected reaction of the corresponding chloromethyl quinazoline 3-oxide with methylamine.

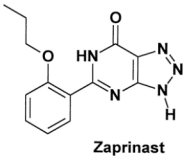


"Me Too" Compounds

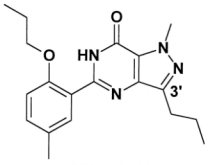
Copying existing drugs with only minor chemical variations is usually referred to as "me too" research. Interestingly, sometimes these close analogs demonstrate major (usually unexpected) advantages, like the bioavailable, broad-spectrum lactamase-resistant penicillins, polar H1 antihistamines without sedative side effects, statins, or PDE5 inhibitors.

Turko, I. V.; Ballard, S. A.; Francis, S. H.; Corbin, J. D., "Inhibition of cyclic GMP-binding cyclic GMP-specific phosphodiesterase (type 5) by sildenafil and related compounds." *Molec. Pharmacol.* **1999**, *56*, 124-130.

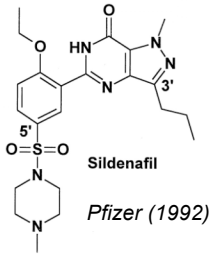




May & Baker Ltd. (1972)



Pfizer (1999)



Pfizer (1992)

Rational Drug Discovery

• Nearly every modification of neurotransmitters dopamine, serotonin, histamine, or acetylcholine by classical medicinal chemistry led to a compound with modified activity and selectivity.

• Steroid hormone modifications led to similar success stories.

• Many enzyme inhibitors were developed from leads that mimic the transition state of the corresponding enzyme. Protease inhibitors started from cleavage-site peptides by converting the critical amide bond into another functionality. For example, aspartyl protease inhibitors should contain the amino acids at both sides of the cleavable peptide bond, and the latter bond needs to be replaced by a stable isostere that resembles the transition state.

• In the 1980's and 1990's, computer modeling of enzyme-substrate complexes became a major driving force for rational drug discovery and the interpretation of SAR results.

Structure-Activity Relationships (SARs)

1868 - Crum-Brown and Fraser

Examined neuromuscular blocking effects of a variety of simple quaternary ammonium salts to determine if the quaternary amine in curare was the cause for its muscle paralytic properties.

Conclusion: the physiological action is a function of chemical constitution

Structurally specific drugs (most drugs):

Act at specific sites (receptor or enzyme)

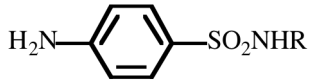
Activity/potency susceptible to small changes in structure

Structurally nonspecific drugs:

No specific site of action

Similar activities with varied structures (various gaseous anesthetics, sedatives, antiseptics)

Example of SAR



sulfa drugs

Lead: sulfanilamide (R = H)

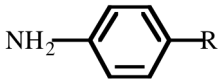
Thousands of analogs synthesized

From clinical trials, various analogs shown to possess three different activities:

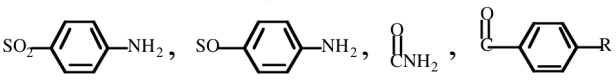
- Antimicrobial
- Diuretic
- Antidiabetic

SAR

General Structure of Antimicrobial Agents



R = SO_2NHR', SO_3H

- Groups must be *para*
- Must be NH_2 (or converted to NH_2 in vivo)
- Replacement of benzene ring or added substituents decreases or abolishes activity
- R can be 

  (but potency is reduced)
- R = $SO_2NR'_2$ gives inactive compounds

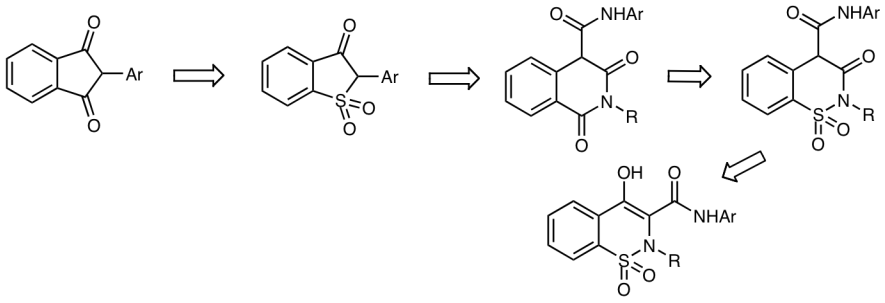
Rational Drug Discovery - Piroxicam

• It took Pfizer ~18 years to develop the anti-inflammatory drug piroxicam, which was launched in 1980 during the "golden age of rational drug discovery".

• The starting point for the development was chemistry-driven, I.e. to identify acidic, but not carboxylic acid-containing (salicylic acid) structurally novel compounds.

• Measurement of a physical property (pKa) as well as serum half-life in dogs was the guide for the synthesis program.

• Several generations of leads were refined and ultimately led to a successful structure with an acceptable safety and activity profile:



Bioisosterism

Bioisosteres - substituents or groups with chemical or physical similarities that produce similar biological properties. Can attenuate toxicity, modify activity of lead, and/or alter pharmacokinetics of lead.

Classical Isosteres

1. Univalent atoms and groups

a. CH_3 NH_2 OH F Cl
b. Cl PH_2 SH
c. Br *i*-Pr
d. I *t*-Bu

2. Bivalent atoms and groups

a. $-CH_2-$ $-NH-$ $-O-$ $-S-$ $-Se-$

b. $-COCH_2R$ $-CONHR$ $-CO_2R$ $-COSR$

3. Trivalent atoms and groups

a. $-CH=$ $-N=$

b. $-P=$ $-As=$

4. Tetravalent atoms

a. $-C(-)(-)-$ $-Si(-)(-)-$

b. $=C=$ $=\overset{+}{N}=$ $=\overset{+}{P}=$

5. Ring equivalents

a. $-CH=CH-$ $-S-$ (e.g., benzene, thiophene)

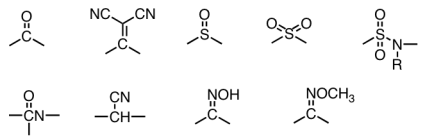
b. $-CH=$ $-N=$ (e.g., benzene, pyridine)

c. $-O-$ $-S-$ $-CH_2-$ $-NH-$ (e.g., tetrahydrofuran, tetrahydrothiophene, cyclopentane, pyrrolidine)

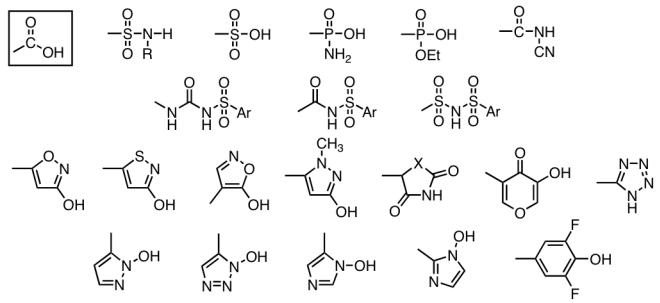
Non-Classical Isosteres

Do not have the same number of atoms and do not fit steric and electronic rules of classical isosteres, but have similar biological activity.

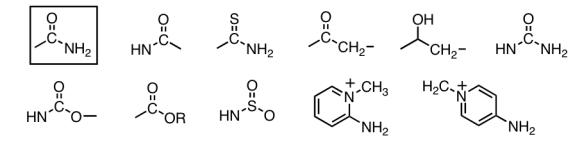
1. Carbonyl group

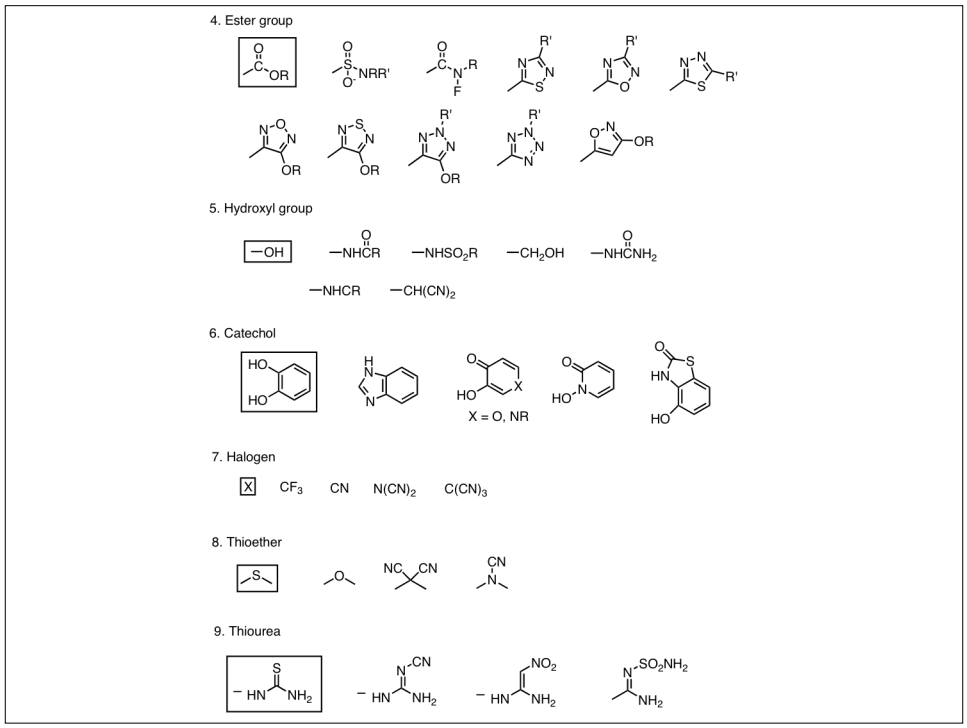


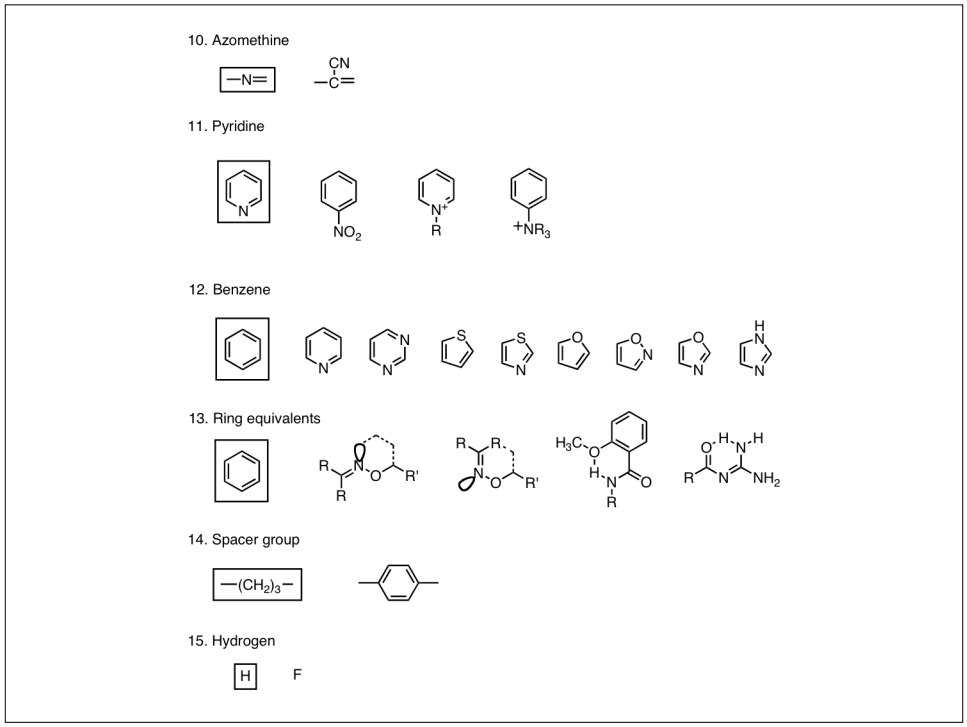
2. Carboxylic acid group



3. Amide group

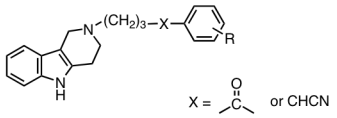



4. Ester group
5. Hydroxyl group
—OH
—NHCR
—NHSO2R
—CH2OH
—NHCNH2
—NHCR
—CH(CN)2
6. Catechol
X = O, NR
7. Halogen
X
CF3
CN
N(CN)2
C(CN)3
8. Thioether
9. Thiourea

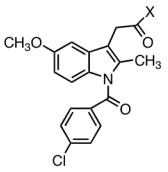

10. Azomethine
—N=
11. Pyridine
12. Benzene
13. Ring equivalents
14. Spacer group
—(CH2)3—
15. Hydrogen
H
F

Examples of Bioisosteric Analogues

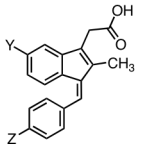
Neuroleptics (antipsychotics)



Anti-inflammatory agents



X = OH (indomethacin)
= NHOH



Y = CH_3O Z = Cl

Y = F Z = SCH_3 (sulindac)

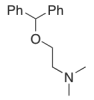
Antihistamines

$R–X–(CH_2)_n–Y$ X = NH, O, CH_2

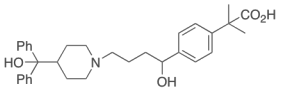
Y = $N(CH_3)_2$ (n = 2)

(n = 1)

(n = 1, 2)



Diphenhydramine
(Benadryl)



Fexofenadine
(Allegra)

Changes resulting from bioisosteric replacements:

Size, shape, electronic distribution, lipid solubility, water solubility, pK_a, chemical reactivity, hydrogen bonding

Effects of bioisosteric replacement:

1. **Structural** (size, shape, H-bonding are important)

2. **Receptor interactions** (all but lipid/H_2O solubility are important)

3. **Pharmacokinetics** (lipophilicity, hydrophilicity, pK_a, H-bonding are important)

4. **Metabolism** (chemical reactivity is important)

Bioisosteric replacements allow you to tinker with whichever parameters are necessary to increase potency or reduce toxicity.

Bioisosterism allows modification of physicochemical parameters

Multiple alterations may be necessary:

If a bioisosteric modification for receptor binding decreases lipophilicity, you may have to modify a different part of the molecule with a lipophilic group.

Where on the molecule do you go to make the modification? The auxophoric groups that do not interfere with binding.

Rational Drug Discovery - From Hit to Lead

Case Study: Use of a combined rational design - combinatorial chemistry strategy

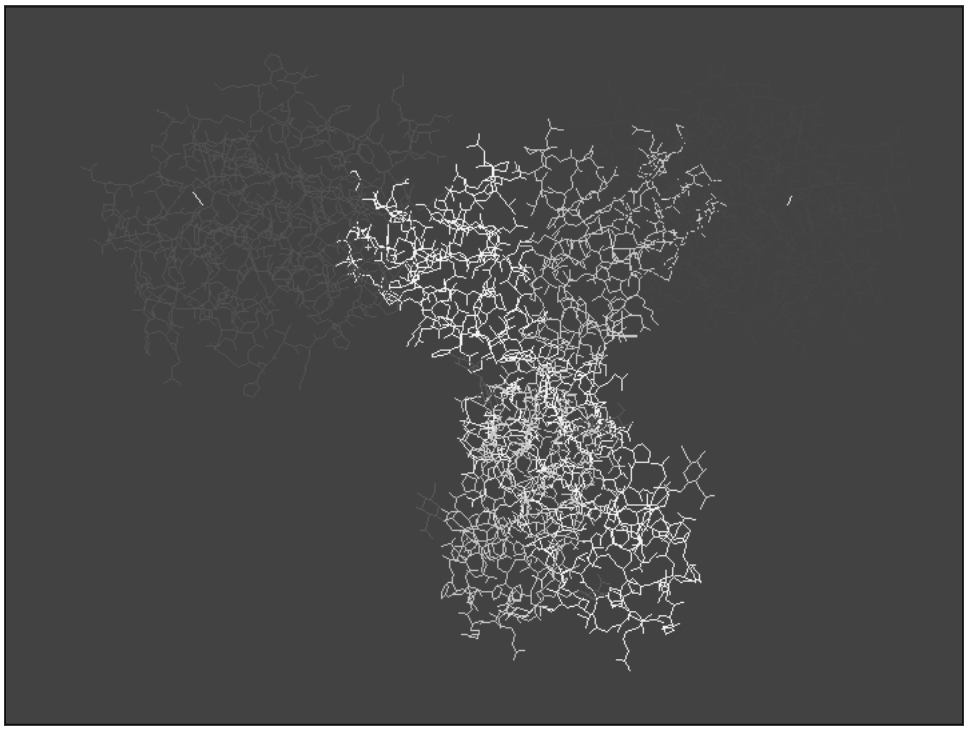
Gadek, T. R.; Burdick, D. J.; McDowell, R. S.; Stanley, M. S.; Marsters Jr., J. C.; Paris, K. J.; Oare, D. A.; Reynolds, M. E.; Ladner, C.; Zioncheck, K. A.; Lee, W. P.; Gribling, P.; Dennis, M. S.; Skelton, N. J.; Tumas, D. B.; Clark, K. R.; Keating, S. M.; Beresini, M. H.; Tilley, J. W.; Presta, L. G.; Bodary, S. C., "Generation of an **LFA-1** (leukocyte functional antigen–1) antagonist by the transfer of the **ICAM-1** (intercellular adhesion molecule–1) immunoregulatory epitope to a small molecule." *Science* **2002**, *295*, 1086-1089.

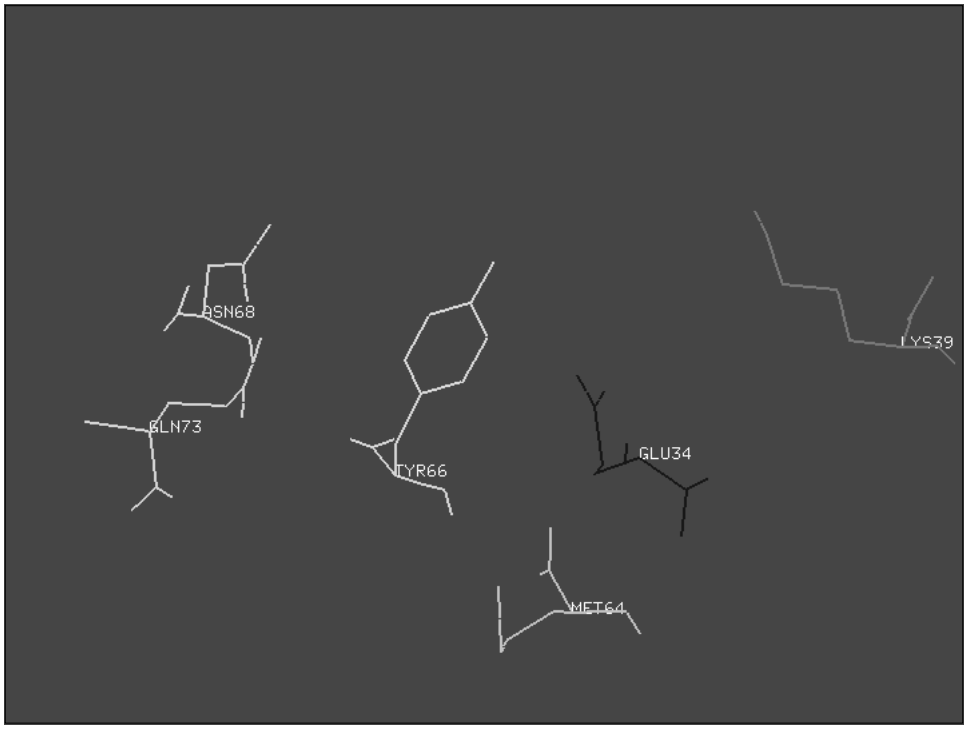
The interaction of LFA-1 with the ICAM proteins 1, 2, and 3 is critical to the adhesion, migration, and proliferation of lymphocytes.

A disruption of these protein-protein interactions could lead to agents for the treatment of psoriasis and transplant rejection.

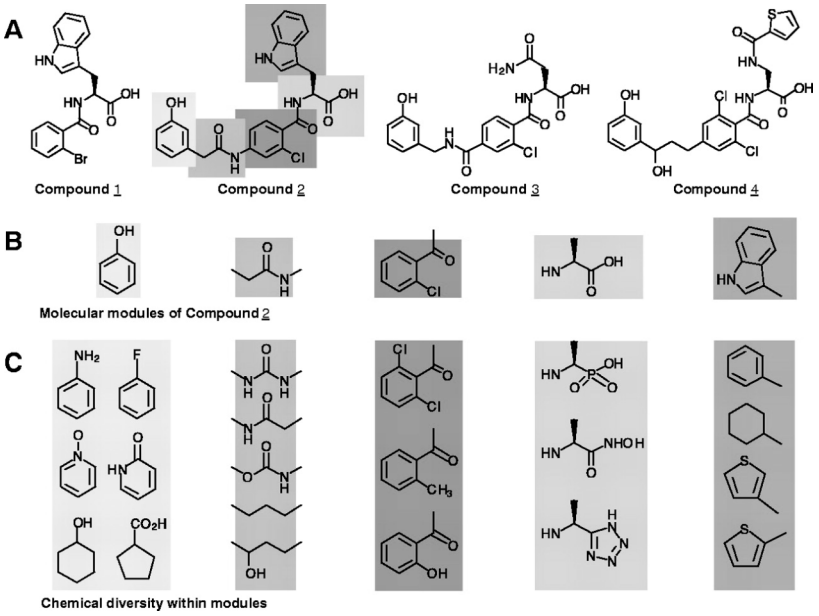
An epitope comprising residues E34, K39, M64, Y66, N68, and Q73 within ICAM-1's first domain was identified as essential for its interaction with LFA-1. The function of this epitope is embedded in the **carboxylic acid, amine, sulfide, phenol, and carboxamide** chemical functionalities of the amino acid side chains of these six residues and their display in three dimensions along one face of the protein.

Molecules which mimic this epitope could capture the LFA-1 binding specificity and safety inherent in ICAM-1's function as a regulator of the immune system.





More or less serendipitously, compound **1** was found to be an inhibitor of LFA-1.

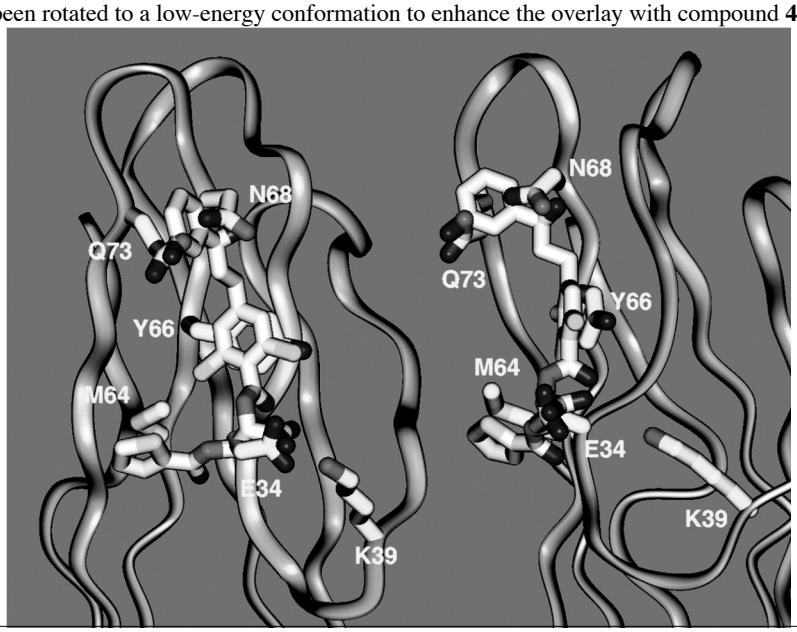


Comparison of the inhibition of ICAM-1/LFA-1 binding and the inhibition of mixed lymphocyte reaction (MLR). IC_{50} values were determined from a 4P fit of data from titrations over concentrations of 10^{-3} to 10^{-10} M. Values reported are the mean±standard deviation for $n>2$ of experiments run in triplicate. ND, not determined. NA, not applicable.

Substance	LFA-1 ELISA IC_{50} (μM)	MLR IC_{50} (μM)
Kistrin	0.70 ± 0.21	40*
H_2N-CRGDMPC-COOH	207 ± 69	ND
H_2N-CGFDMPC-COOH	13 ± 3.2	ND
H_2N-CGY$^{(m)}$DMPC-COOH†	1.6 ± 0.1	ND
Compound **1**	1.4 ± 0.7	ND
Compound **2**	0.047 ± 0.014	10.3 ± 6.3
Compound **3**	0.0037 ± 0.0015	1.33 ± 1.1
Compound **4**	0.0014 ± 0.00014	0.003 ± 0.002
Cyclosporine A	NA‡	0.061 ± 0.034
MHM 24 Fab§	0.0023 ± 0.0001	0.020 ± 0.008

*Incomplete titration, value estimated at 50% inhibition. †Y$^{(m)}$ = *meta*-tyrosine. ‡The immunosuppressive activity of cyclosporine does not involve its direct binding to LFA-1 or ICAM-1. §MHM 24 Fab is the Fab fragment of the murine anti–human antibody recognizing LFA-1's CD11a subunit (*7*).

Two orthogonal views of the superimposition of compound **4** on the crystal structure of the first domain of ICAM-1 indicating that compound **4** mimics the ICAM-1 epitope. Residues highlighted in blue contribute significantly to LFA-1 binding. The E34 side chain of ICAM-1 has been rotated to a low-energy conformation to enhance the overlay with compound **4**.



Conclusions:

Compounds 2 through 4 appear to be mimics of ICAM-1 resulting from the transfer of the ICAM epitope to a small molecule.
Compound 4 is a potent LFA-1 antagonist, which binds LFA-1, blocks the binding of ICAM-1, and inhibits LFA-1 mediated lymphocyte proliferation and adhesion in vitro.

This work represents the first reduction of a nonlinear, discontinuous but contiguous protein epitope (encompassing five residues spanning three different b-strands across the face of a protein surface) from a protein to a small molecule.

Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

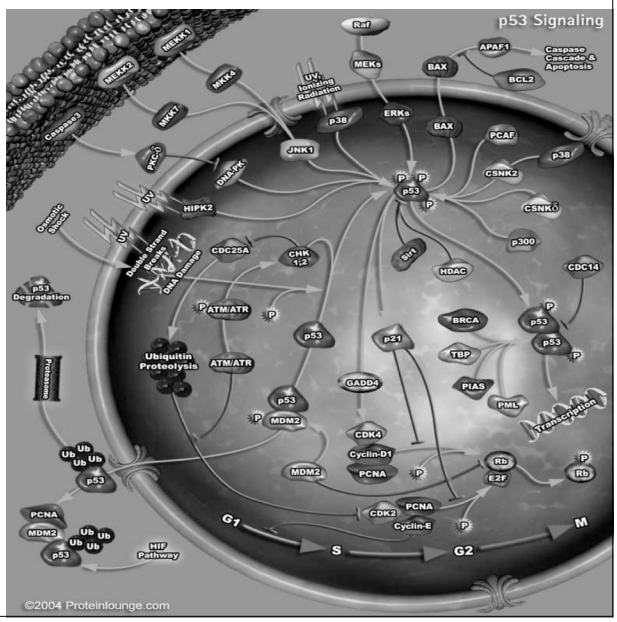
The pharmacological hypothesis:

The p53 tumor suppressor plays a central role in controlling cell cycle progression and apoptosis, and it is an attractive cancer therapeutic target because its stimulation kills tumor cells.

Its low intracellular concentration is maintained by MDM2-mediated ubiquitination and resulting proteolysis.

An approach toward stimulation of p53 activity would be to block its interaction with the MDM2 oncoprotein.

Ding et al. JACS 2005, 127, 10130.



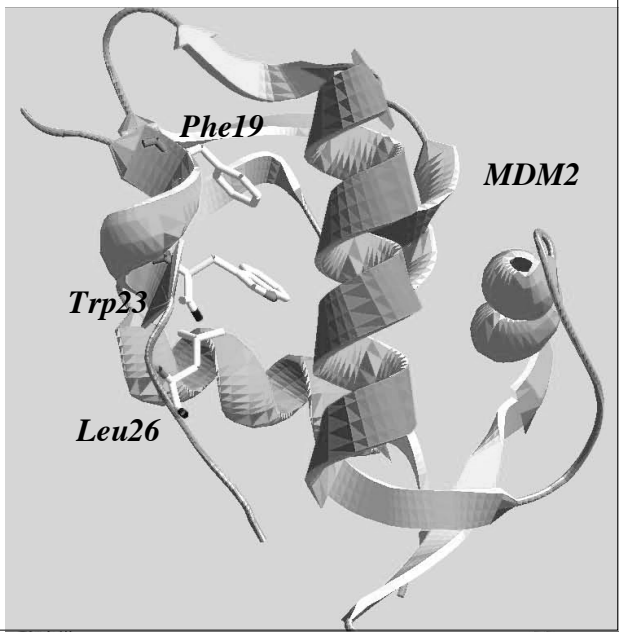
Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

Structure-Based Design:

The p53-MDM2 interaction is primarily mediated by three hydrophobic residues of p53 and a small but deep hydrophobic cleft in MDM2. This cleft is ideal for the design of agents that block the p53-MDM2 interaction.

Trp23 appears to be buried most deeply in the hydrophobic cavity, and its NH group forms a hydrogen bond with a backbone carbonyl in MDM2. Indeed, imidazolines were previously reported to inhibit MDM2 ("Nutlins").

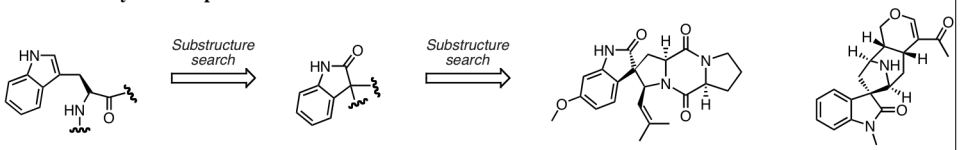
What other chemical moieties can mimic the indole ring?



Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

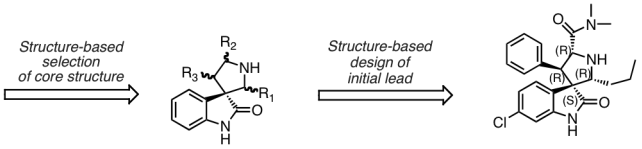
Structure-Based Strategy:

1. **The oxindole is a bioisostere of the indole.**
2. **Identify natural products that contain an oxindole substructure.**



Trp23 in p53 | Oxindole core structure | Spirotryprostatin A | Alstonisine

3. **Although spirotryprostatin and alstonisine fit poorly into the MDM2 cavity, the spiro-oxindole-pyrrolidine core structure fit well.**
4. **Two additional hydrophobic groups are needed to mimic the side chains of Phe19 and Leu26. Candidates were evaluated by molecular modeling & docking.**

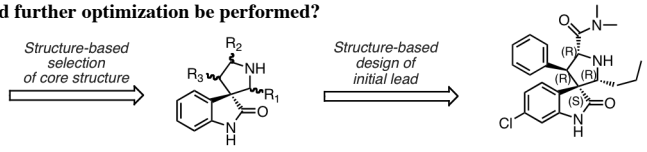


Spiro-oxindole core structure | Initial lead compound

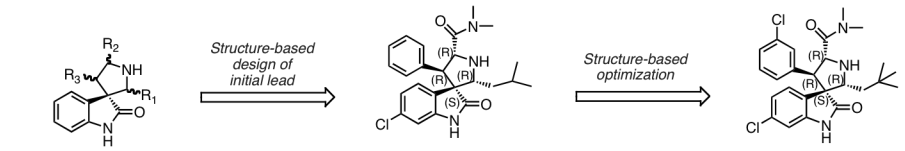
Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

Structure-Based Strategy:

1. **The initial lead compound was synthesized by an asymmetric 1,3-dipolar cycloaddition.**
2. **Biological analyses vs a fluorescent-labeled p53-based peptide (K_d 1 nM) provided a K_d of 9 uM for the lead compound.**
3. **How could further optimization be performed?**

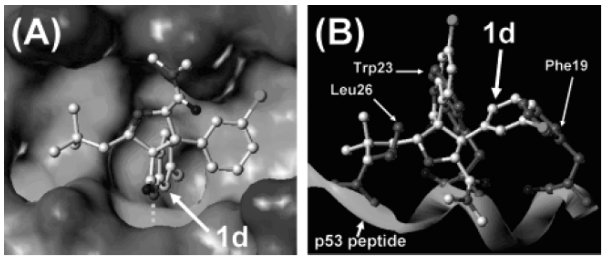


4. **Additional room in the MDM2 cavity could be exploited by larger hydrophobic groups (supported by modeling studies).**
5. **After several rounds of SAR, where the modeling was tested both by the synthesis of supposedly improved as well as inferior molecules, a new compound with K_d 86 nM was identified.**

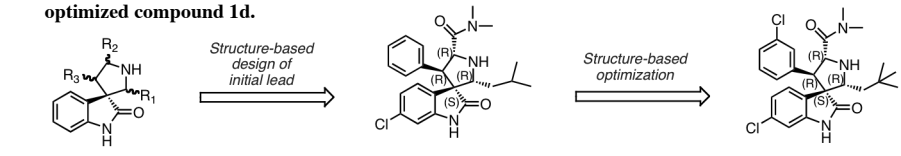


Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

Structure-Based Strategy:

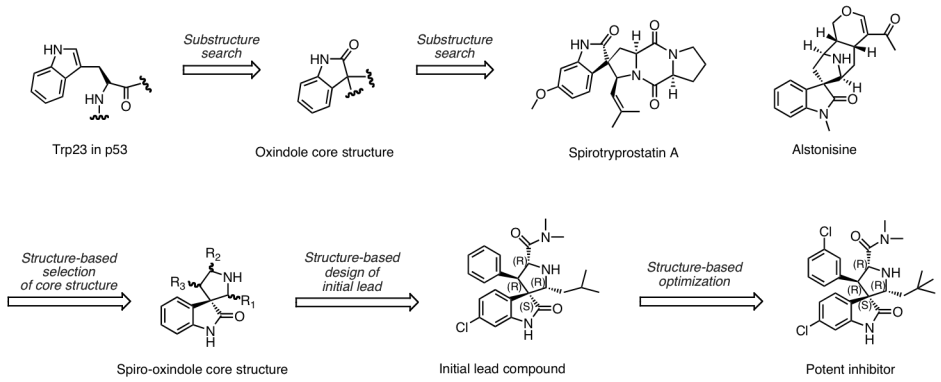


1. **Predicted binding model using computational docking for initial lead compound and for the optimized compound 1d.**



2. **What are the potential issues with MDM2 inhibitors?**

Structure-Based Design of Potent Non-Peptide MDM2 inhibitors



Further reading:

Lombardino, J. G.; Lowe, J. A., "A guide to drug discovery: The role of the medicinal chemist in drug discovery - then and now." *Nat. Rev. Drug Disc.* **2004**, *3*, 853-862.
Jorgensen, W. L., "The many roles of computation in drug discovery." *Science* **2004**, *303*, 1813-1818.
Gustafsson, D.; Bylund, R.; Antonsson, T.; Nilsson, I.; Nystroem, J.-E.; Eriksson, U.; Bredberg, U.; Teger-Nilsson, A.-C., "Case history: A new oral anticoagulant: The 50-year challenge." *Nat. Rev. Drug Disc.* **2004**, *3*, 649-659.