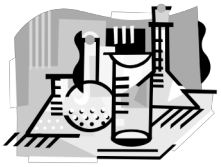
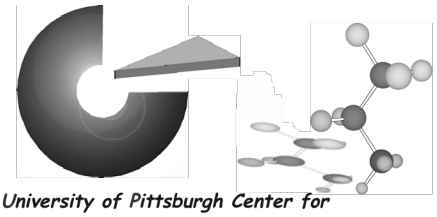
Pharm 5119 – Medicinal Chemistry & Drug Discovery

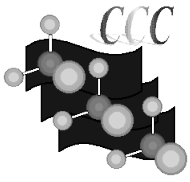


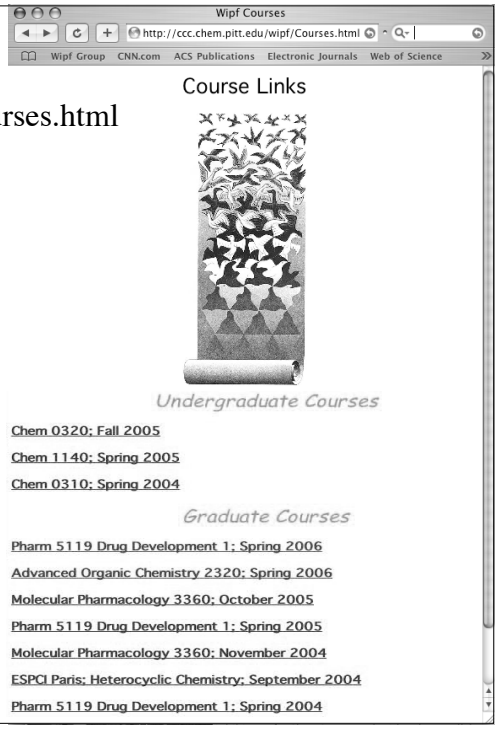
Dr. Peter Wipf
Department of Chemistry
University of Pittsburgh

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

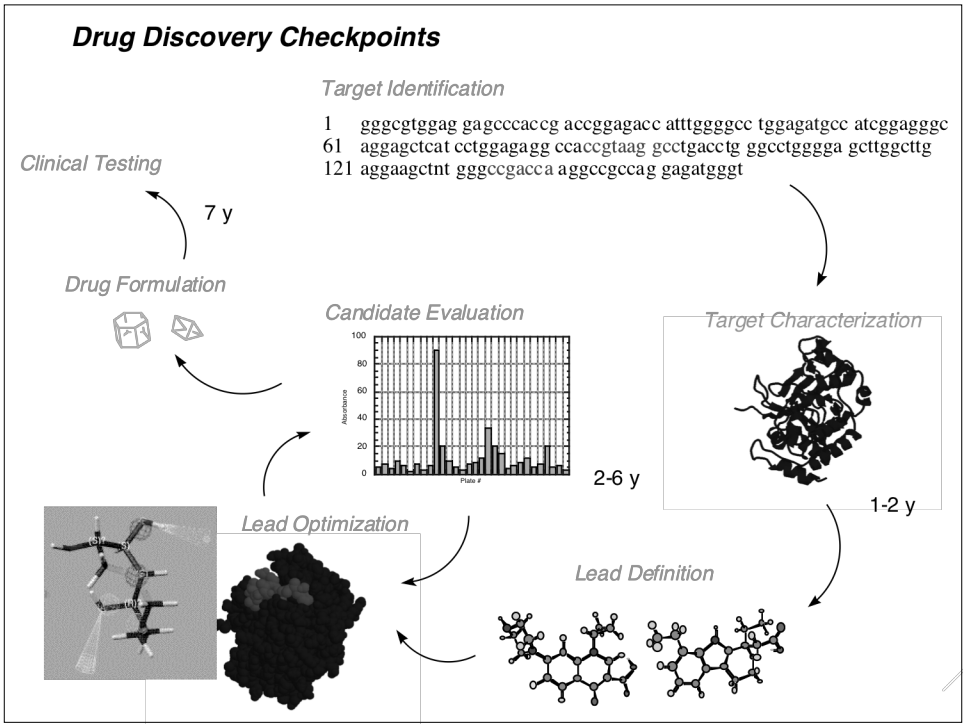
- ***Survey of current drugs***
- ***Drug discovery challenges***
- ***Methods to identify new leads***
- ***Case studies from industry***
- ***Structure-based design***

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)

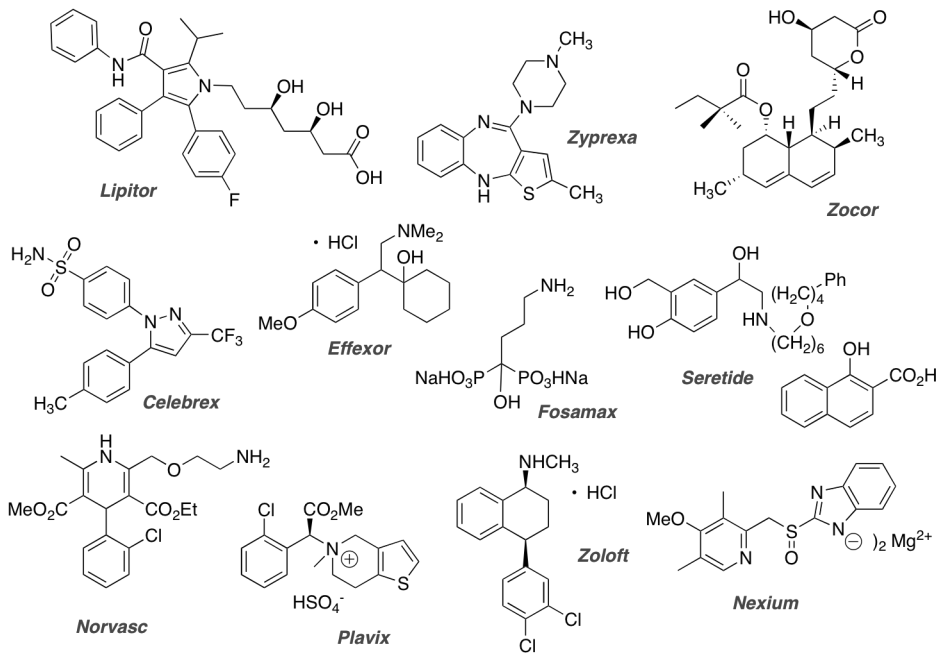


2004 Blockbusters at the Drugstore
(Worldwide ~$550b (+8.6%); US ~$250b)

National Restaurant Association (NRA) estimated industry sales: $476 billion/y (US only)

Lipitor (Pfizer) *cholesterol* $11 billion (+14% over 2003)
Zocor (Merck) *cholesterol* $5.2 billion (-6%)
Seretide/Advair (GSK) *asthma* $4.5 billion (+23%)
Norvasc (Pfizer) *blood pressure* $4.5 billion (+1%)
Zyprexa (Eli Lilly) *antipsychotic* $4.4 billion (-4%)
Nexium (AstraZeneca) *ulcers* $3.9 billion (+25%)
Erypo /Eprex/Procrit (J&J) *anemia* $3.6 billion (-4%)
Zoloft (Pfizer) *depression* $3.4 billion (0%)
Effexor (Wyeth) *depression* 3.4 billion (+20%)
Plavix (BMS-Sanofi) *blood thinner* $3.3 billion (+31%)
Celebrex (Pfizer) *arthritis* $3.3 billion (?%)
Fosamex (Merck) *osteoporosis* $3.2 billion (?%)

Source: http://wistechnology.com/article.php?id=1885, June 2005

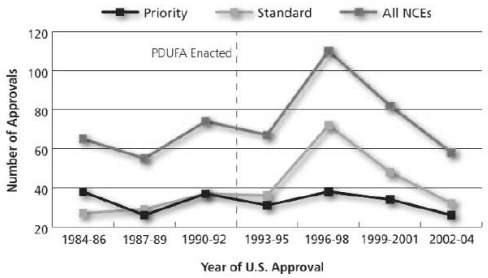


"Big Pharma" Drug Discovery in the 21st Century

***The Problem**:* The pharmaceutical industry is short of new drugs. In the 2nd part of the 20th century, about 50-60 new drugs (NCEs) were approved by the FDA every year. In contrast, in 2002, **a historical low of 18 NCEs were approved** (2001: 24; 2000: 27; 2003: 21 NCEs; 2004: 36; 2005: 20 NCEs). Conversely, research costs for a new drug are estimated to be in the $1-1.5 Bi. range. Considering all high-profile failures in recent drug discovery, this figure is likely to increase even further.

The challenge: increase new product approvals; focus on therapeutic value

U.S. Approvals of Priority and Standard, and All NCEs 1984-2004



As drug development becomes more complex and expensive, developers must concentrate available resources on fewer projects. Fewer development projects, in turn, lead to fewer new drug approvals. Key challenges for the industry, as well as for regulators, are to enhance development of more complex drugs, improve assessments of product safety and effectiveness, and focus on medicines that offer high therapeutic value.

Source: Tufts Center for the Study of Drug Development

From: http://csdd.tufts.edu/InfoServices/OutlookReports.asp

Current Drug Discovery Challenges

The decline in the number of new drugs is based, among other reasons, on:

- ..
- ..
- ..
- ..
- ..

In Search of New Leads…..

A lead can be characterized as a compound that

- ..,
- ..,
- ...

..

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

Medicinal Chemistry Folklore

Earliest medicines ~ 5100 years ago

Chinese emperor Shen Nung - book of herbs, Pen Ts'ao

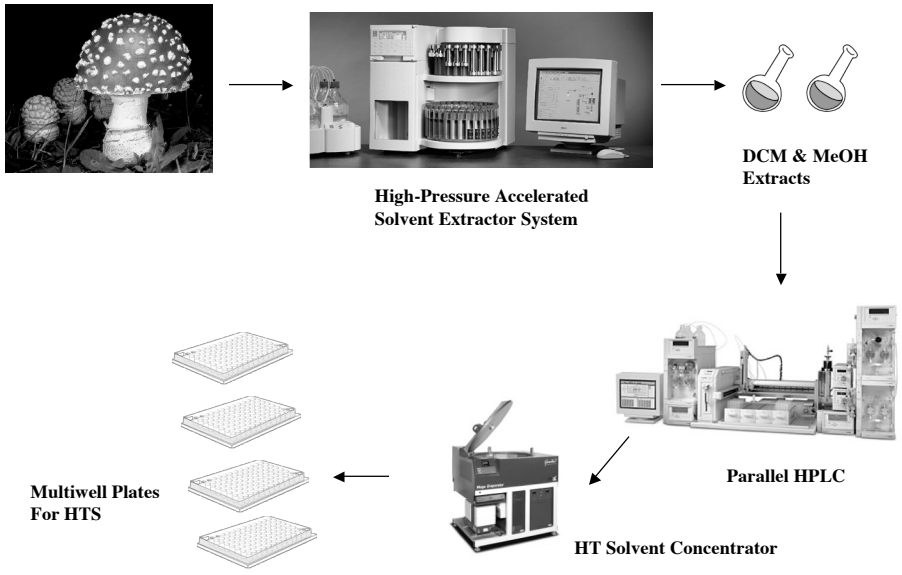
Ch'ang Shan - contains alkaloids; used today in the treatment of malaria and for fevers

Ma Huang - contains ephedrine; used as a heart stimulant and for asthma. Now used by body builders and endurance athletes because it quickly converts fat into energy and increases strength of muscle fibers.

Modern therapeutics:

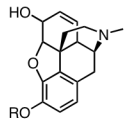
Extract of foxglove plant, cited by Welsh physicians in 1250.
Used to treat dropsy (congestive heart failure) in 1785
Contains digitoxin and digoxin; today called digitalis

Screening of natural product extracts

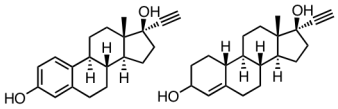


Examples of Natural Products as Leads & Drugs

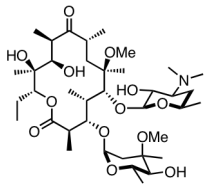
Cardiac glycosides, morphine, quinine, salicylic acid, taxol, camptothecin, penicillin, cyclosporin A, warfarin, artemisine….



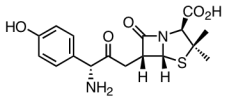
R = H: Morphine
R = Me: Codeine
(pain killer)



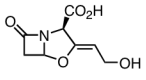
17-ethynylestradiol norethindrone
(the "Pill"; contraceptive)



Clarithromycin
(antibacterial)

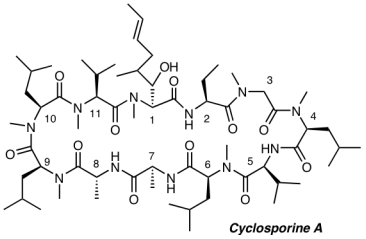


Ampicillin
(antibiotic)



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.

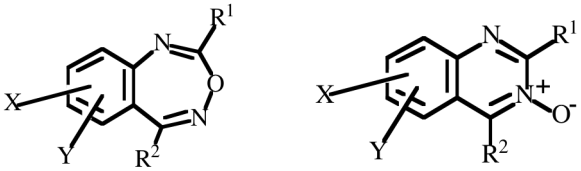
• Acetylsalicylic acid was thought to be just a better tolerable prodrug of salicylic acid, but turned out to have a unique mechanism.

• 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

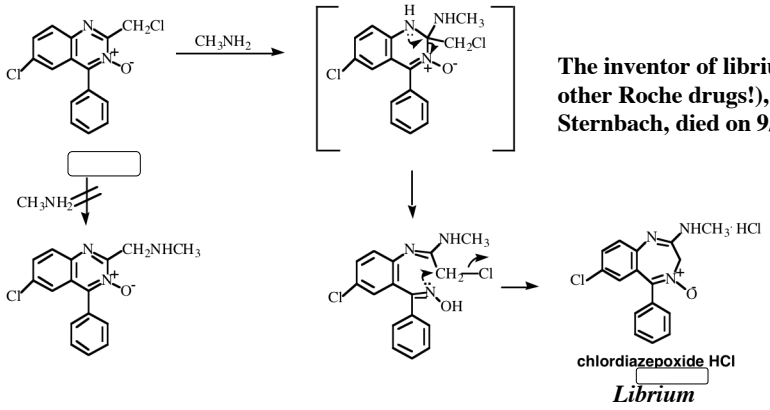
In 1955 Roche set out to prepare a series of benzheptoxadiazines as potential new tranquilizer drugs, but the actual structure was found to be that of a quinazoline 3-oxide.



No active compounds were found, so the project was abandoned

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.

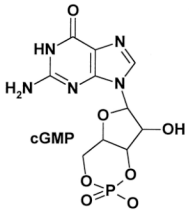


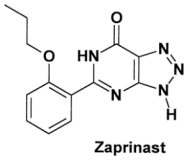
The inventor of librium (and 12 other Roche drugs!), Leo Sternbach, died on 9/30/05.

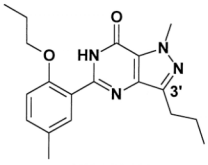
"Me Too" Compounds

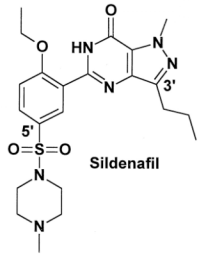
Copying existing drugs with only minor chemical variations is usually referred to as "me too" research. Interestingly, sometimes these close analogs demonstrate major (usually unexpected) advantages, like the bioavailable, broad-spectrum lactamase-resistant penicillins, polar H1 antihistamines without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









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)

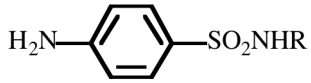
Activity/potency susceptible to small changes in structure

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)

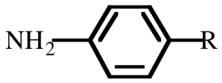
Thousands of analogs synthesized

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

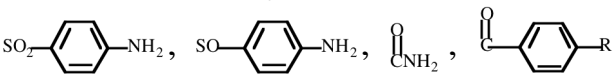
- Antimicrobial
- Diuretic
- Antidiabetic

SAR

General Structure of Antimicrobial Agents



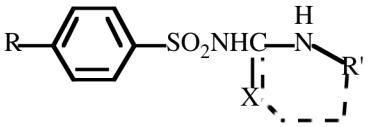
R = SO_2NHR', SO_3H

- 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

SAR

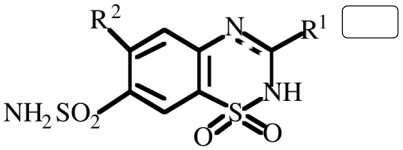
Antidiabetic Agents

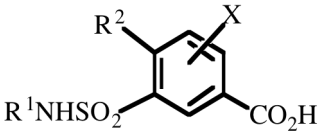


X = O, S, or N

SAR

Diuretics (2 types)





hydrochlorothiazides high ceiling type

R^2 is an electrophilic group

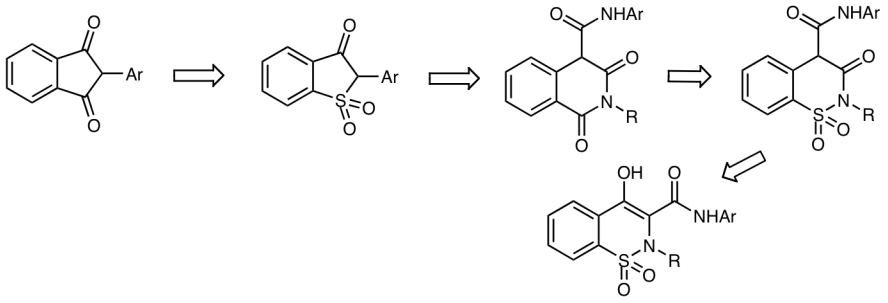
Rational Drug Discovery - Piroxicam

• 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.

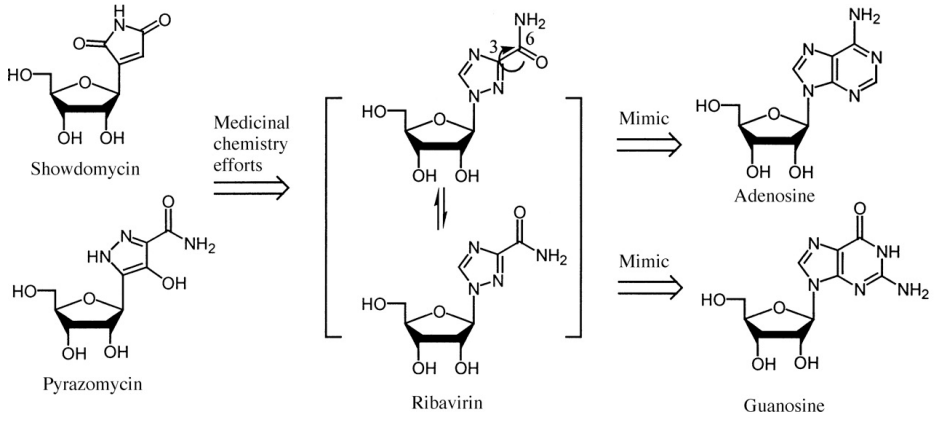
• Measurement of a physical property (pKa) as well as serum half-life in dogs was the guide for the synthesis program.

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

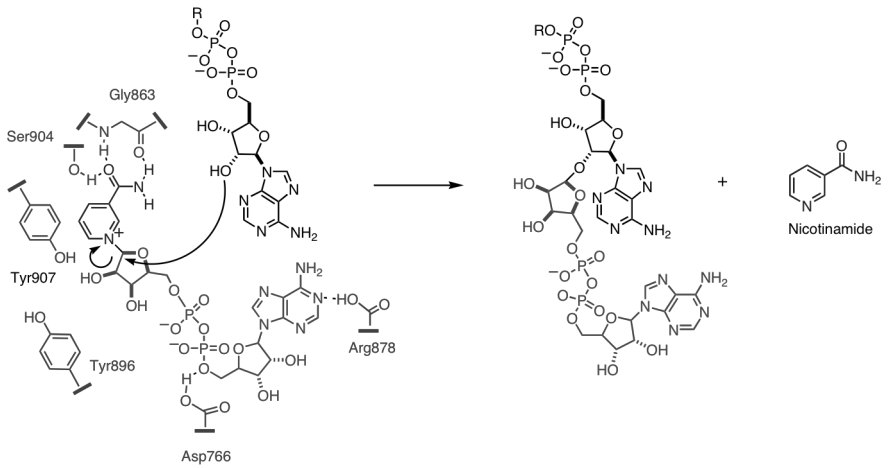


Rational Drug Discovery - BioMimicry

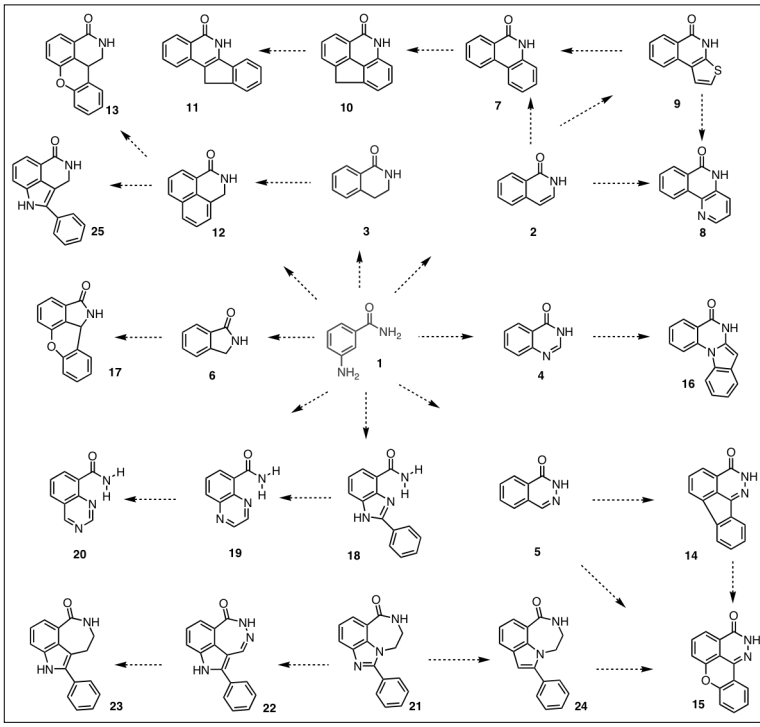
Ribavirin was conceived from the two natural ribonucleosides, showdomycin and pyrazomycin, both of which show significant broad-spectrum antiviral activity. Ribavirin is capable of adopting multiple conformations by rotating the C3–C6 bond to mimic both adenosine and guanine ribonucleosides.



Schematic representation of the binding of NAD+ to poly(ADP-ribose) polymerase (PARP) protein and the catalytic mechanism of PARP1.



Jagtap, P.; Szabo, C. *Nature Reviews Drug Discovery* **2005**, *4*, 421-440.



Structures of representative classes of poly(ADP-ribose) polymerase (PARP) inhibitors derived from the classical PARP scaffolds (benzamide or cyclic lactams)

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. $-\overset{|}{\underset{|}{C}}-$ $-\overset{|}{\underset{|}{Si}}-$

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

5. Ring equivalents

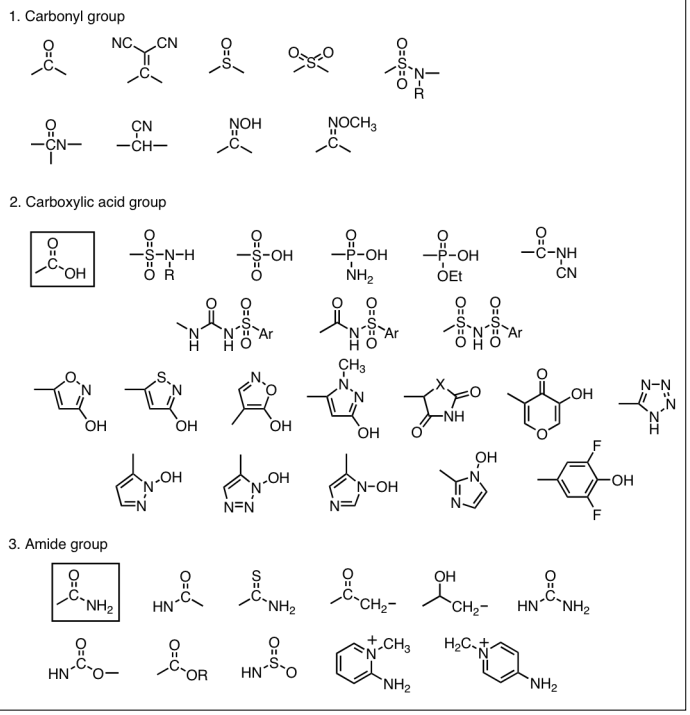
   a. $-CH=CH-$ $-S-$ (e.g., benzene, thiophene)

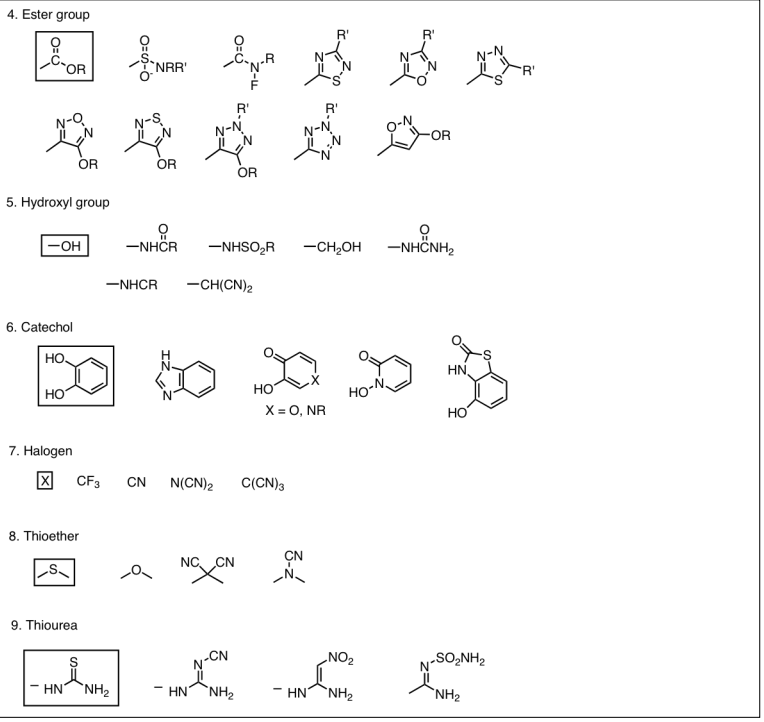
   b. $-CH=$ $-N=$ (e.g., benzene, pyridine)

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

Non-Classical Isosteres

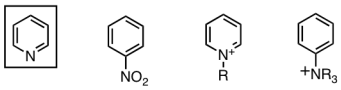
Do not have the same number of atoms and do not fit steric and electronic rules of classical isosteres, but have similar biological activity.



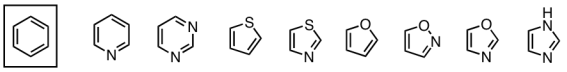


10. Azomethine

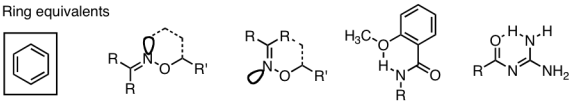
11. Pyridine



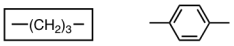
12. Benzene



13. Ring equivalents



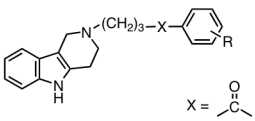
14. Spacer group



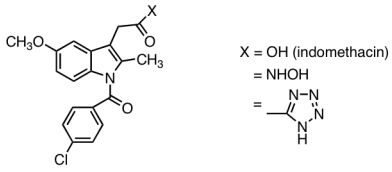
15. Hydrogen

Examples of Bioisosteric Analogues

Neuroleptics (antipsychotics)



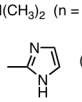
Anti-inflammatory agents



Antihistamines

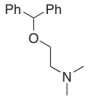
$R-X-(CH_2)_n-Y$ X = NH, O, CH_2

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

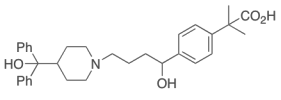


(n = 1)

(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?

Rational Drug Discovery - From Hit to Lead

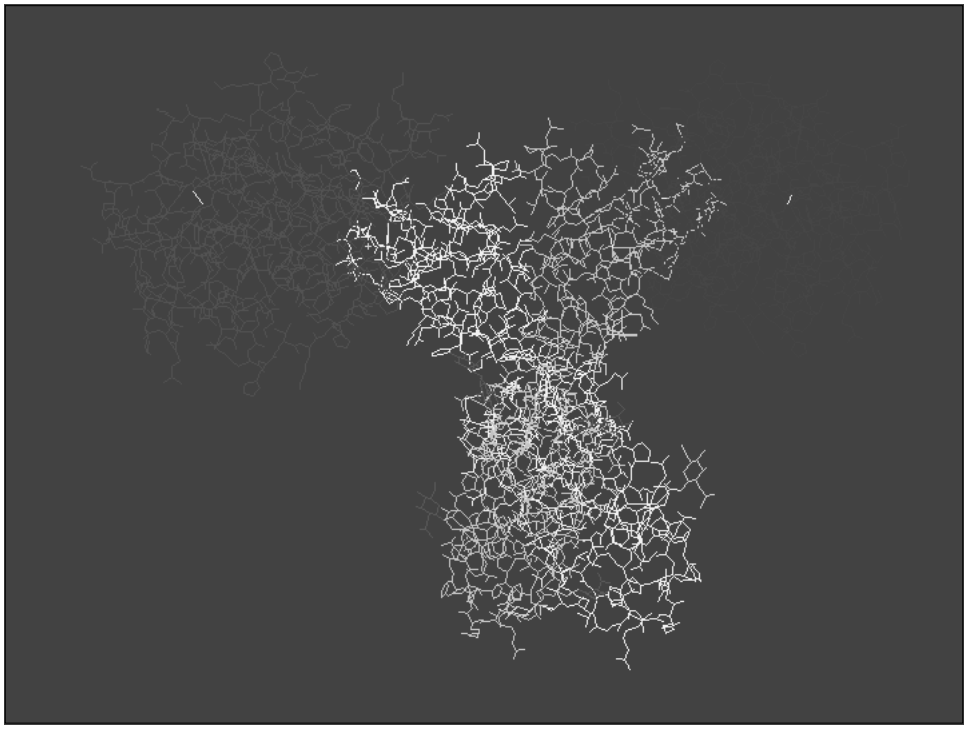
Case Study: Use of a combined rational design - combinatorial chemistry strategy

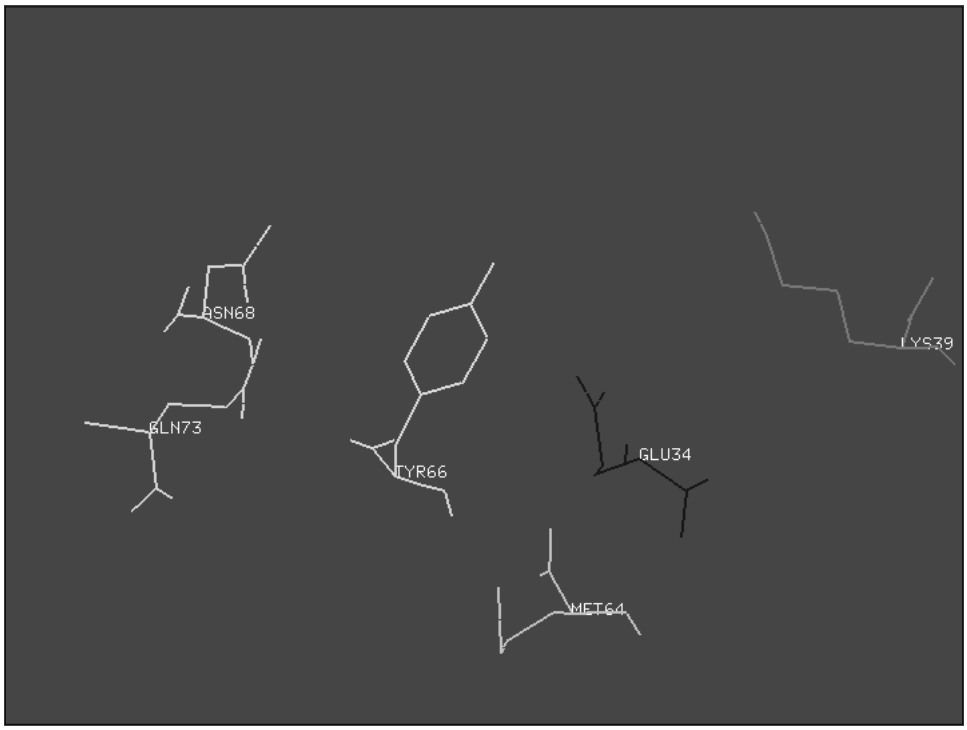
Gadek, T. R.; Burdick, D. J.; McDowell, R. S.; Stanley, M. S.; Marsters Jr., J. C.; Paris, K. J.; Oare, D. A.; Reynolds, M. E.; Ladner, C.; Zioncheck, K. A.; Lee, W. P.; Gribling, P.; Dennis, M. S.; Skelton, N. J.; Tumas, D. B.; Clark, K. R.; Keating, S. M.; Beresini, M. H.; Tilley, J. W.; Presta, L. 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.

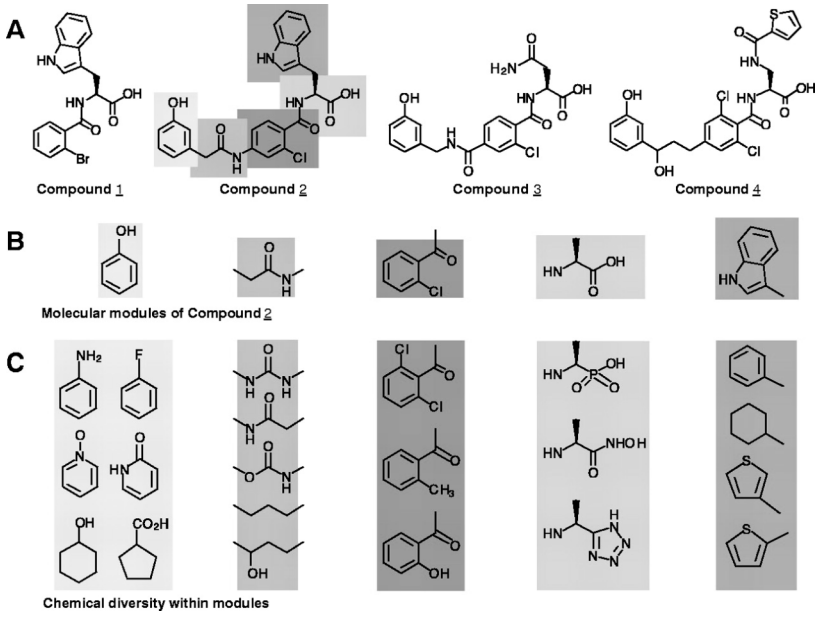
An epitope comprising residues E34, K39, M64, Y66, N68, and Q73 within ICAM-1's first domain has been identified as essential for its interaction with LFA-1. The function of this epitope is embedded in the **carboxylic acid, amine, sulfide, phenol, and carboxamide** chemical functionalities of the amino 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.




ASN68
GLN73
TYR66
GLU34
LYS39
MET64

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

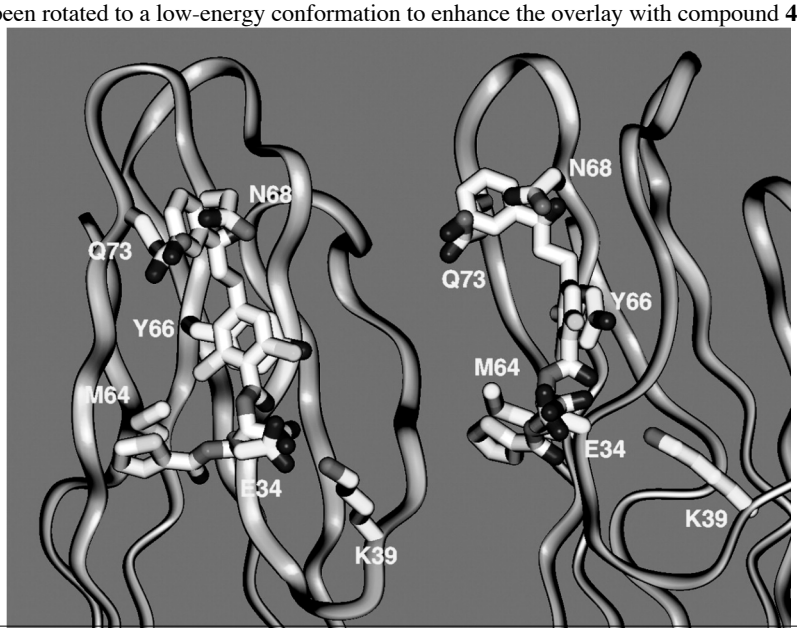


Comparison of the inhibition of ICAM-1/LFA-1 binding and the inhibition of mixed lymphocyte reaction (MLR). IC_{50} values were determined from a 4P fit of data from titrations over concentrations of 10^{-3} to 10^{-10} M. Values reported are the mean±standard deviation for $n>2$ of experiments run in 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 β-strands across the face of a protein surface) from a protein to a small molecule.

Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

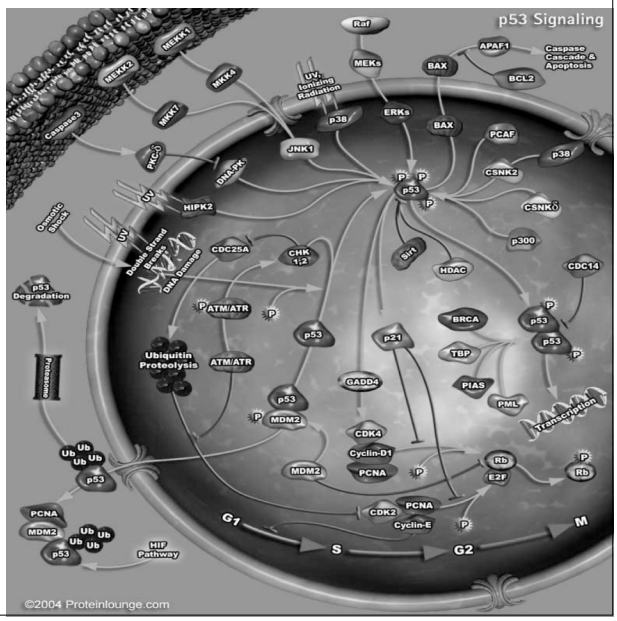
The pharmacological hypothesis:

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.



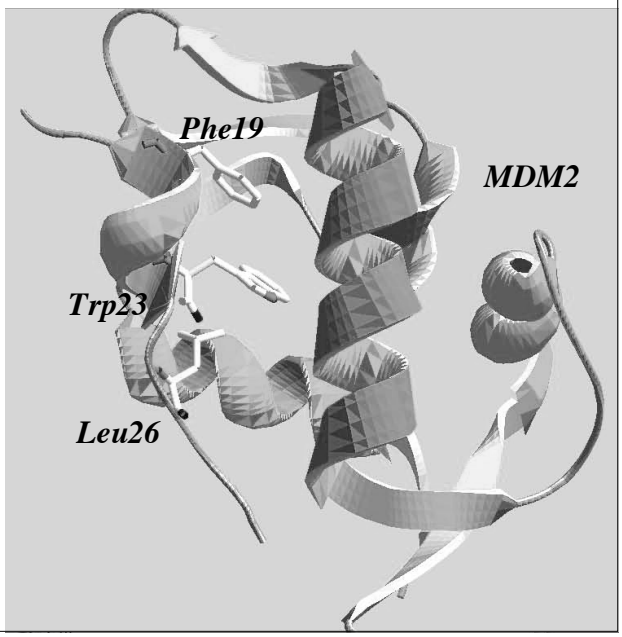
Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

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").

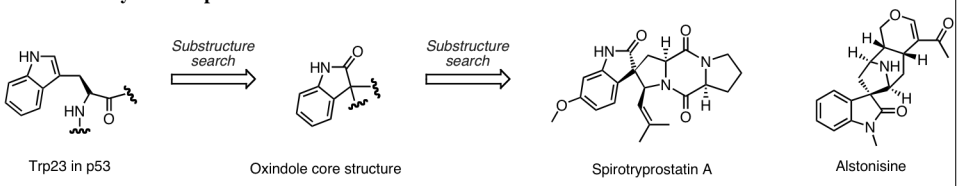
What other chemical moieties can mimic the indole ring?



Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

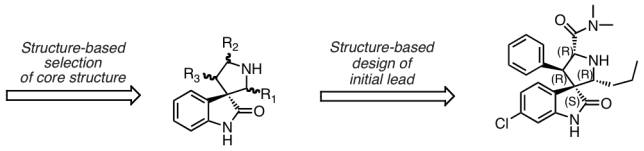
Structure-Based Strategy:

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



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

3. **Although spirotryprostatin and alstonisine fit poorly into the MDM2 cavity, the …… 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.**



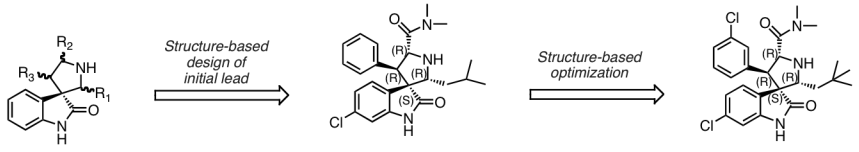
Spiro-oxindole core structure | Initial lead compound

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?**

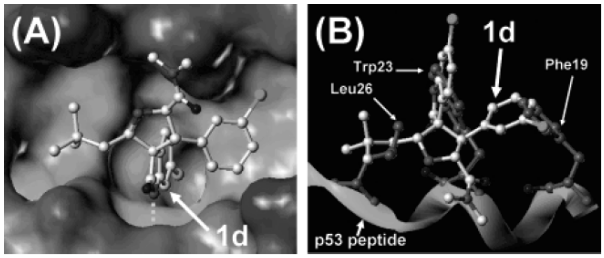
4. **..**
5. **After several rounds of SAR, where the modeling was tested both by the synthesis of supposedly improved as well as inferior molecules, a new compound with K_d 86 nM was identified.**



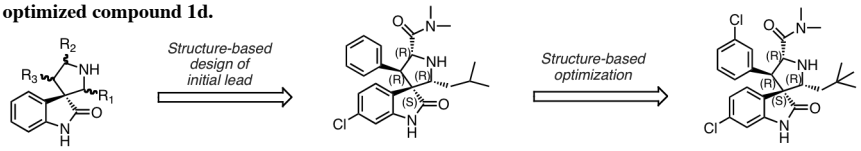
Spiro-oxindole core structure | Initial lead compound | Potent inhibitor

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.**



Spiro-oxindole core structure | Initial lead compound | Potent inhibitor

2. **What are the potential issues with 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.