Medicinal Chemistry & Drug Discovery

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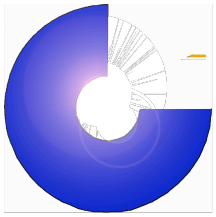
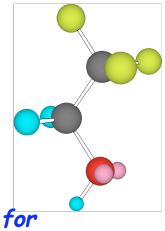
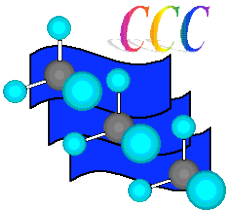


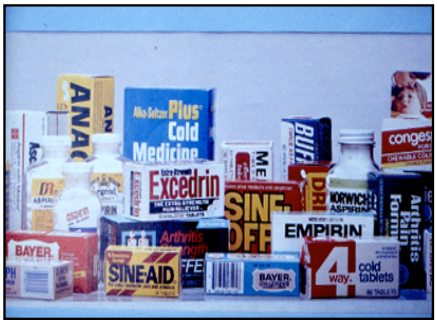




University of Pittsburgh Center for Chemical Methodologies & Library Development

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

- *Survey of current drugs*
- *Drug discovery challenges*
- *Methods to identify new leads*
- *Case studies from industry*
- *Forward chemical genetics - reverse pharmacology*



Medicinal Chemistry

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

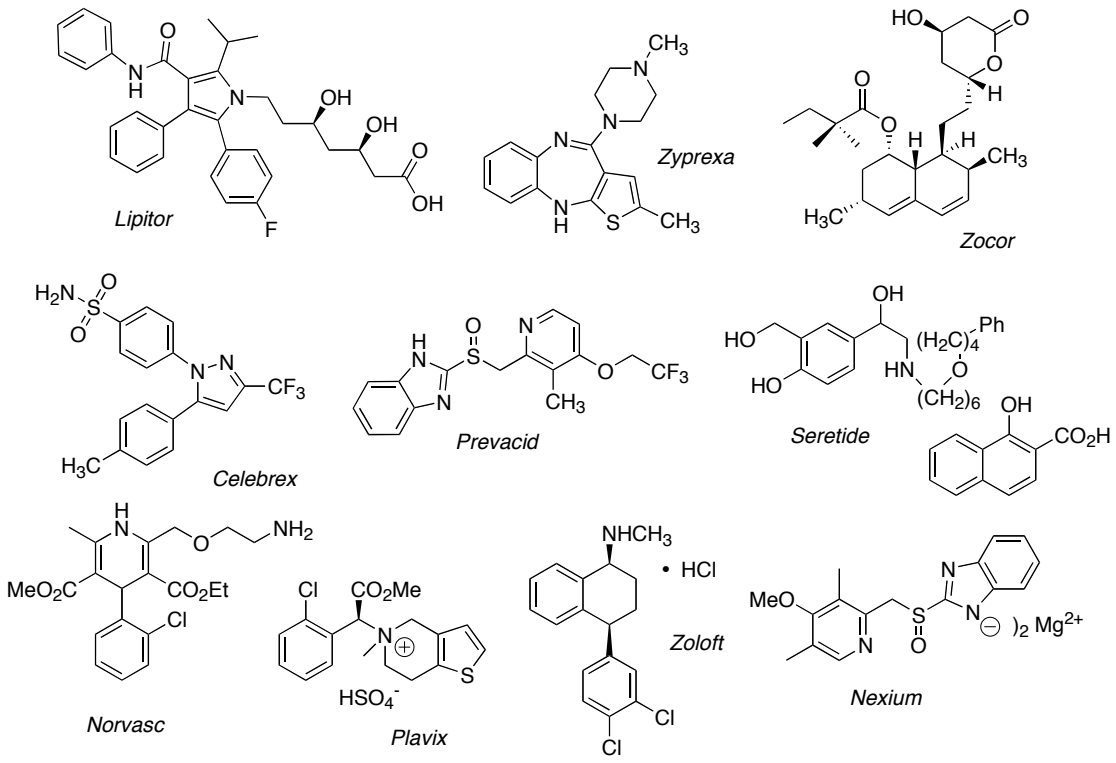
It involves:

- Synthesis
- Structure-Activity Relationships (SAR)
- Receptor interactions
- Absorption, distribution, metabolism, and excretion (ADME)

2003 Blockbusters at the Drugstore (US/Worldwide; out of ~$500b)

Lipitor (Pfizer) *cholesterol* $6.8/10.3 billion (66%)
Zocor (Merck) *cholesterol* $4.4/6.1 billion (72%)
Zyprexa (Eli Lilly) *antipsychotic* $3.2/4.8 billion (72%)
Norvasc (Pfizer) *blood pressure* $2.2/4.5 billion (40%)
Procrit (J&J) *anemia* $3.3/4.0 billion (83%)
Prevacid (TAP) *ulcers* $4.0/4.0 billion (100%)
Nexium (AstraZeneca) *ulcers* $3.1/3.8 billion (82%)
Plavix (BMS-Sanofi) *blood thinner* $2.2/3.7 billion (59%)
Seretide (GSK) *asthma* $2.3/3.7 billion (62%)
Zoloft (Pfizer) *depression* $2.9/3.4 billion (85%)
Epogen (Amgen) *anemia* $3.1/? billion (?%)
Celebrex (Pfizer) *arthritis* $2.6/? billion (?%)

Source: IMS Health, March 2004



"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 the FDA every year. In contrast, in 2002, **a historical low of 18 NCEs were approved** (in 2001, 24 NCEs, in 2000, 27 NCEs, in 2003, 21 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 unlikely to drop substantially.

"…gloom around the pharmaceuticals company.."

Merck Cancels Its Development Of Diabetes Drug

By Peter Landers

Merck & Co. halted development of a diabetes drug that had been in late-stage testing, a surprise blow that adds to the deepening gloom around the pharmaceuticals company.

Merck said it identified a rare malignant tumor in mice that had been given the drug. It said the relevance of that discovery in humans isn't known, but the company has told doctors to stop testing the drug in humans. The drug, known by the development number MK-767, had been in large-scale Phase III clinical trials, the final step before seeking approval from the Food and Drug Administration to sell the drug.

The cancellation of the trials follows Merck's announcement earlier this month that it was halting development of a depression drug called aprepitant that it had been studying for more than a decade. Aprepitant failed to show significant results in a large clinical trial of depressed patients. Earlier this year Merck abandoned development of two other drugs that were at an earlier stage of human trials.

Merck licensed MK-767 from **Kyorin Pharmaceutical** Co. of Japan. The other three canceled drugs were discovered by Merck researchers.

"The termination of the MK-767 program is clearly disappointing," said Merck Chief Executive Raymond Gilmartin in a statement. "Drug discovery is a risky and complicated business with more disappointments than successes." Mr. Gilmartin said Merck is pursuing research into a separate diabetes drug and hopes to file for FDA approval of that drug by the end of 2006.

The failure of MK-767 comes as a surprise and may have a bigger impact on Merck shares than the failure of the depression drug, which many investors had anticipated because an earlier trial had also shown the drug to be no better than a placebo.

Merck was once renowned for its research prowess but its researchers have hit a dry spell in recent years. The company's lineup of drugs in large-scale testing is now among the weakest in the industry. Its main hope is a vaccine that could protect women from cervical cancer, but analysts don't expect that to be ready until 2007.

Source: Wall Street J. 11/21/2003

Wallstreet (and companies) are looking for a scapegoat.."

Drug Industry's Big Push Into Technology Falls Short. Testing Machines Were Built To Streamline Research -- But May Be Stifling It

"A decade ago, pharmaceutical companies announced a revolutionary new way of finding drugs. Instead of relying on scientists' hunches about what chemicals to experiment with, they brought in machines to create thousands of chemical combinations at once and tested them out with robots. The new technology was supposed to bring a flood of medicines to patients and profits to investors.

Today, some leading chemists are calling the effort an expensive fiasco.

Machines churned out chemical after chemical that didn't produce useful results. And chemicals that seemed promising often turned out to have big flaws that traditional testing might have caught earlier on. Some drugs couldn't dissolve in water or be turned into pills, for example.

Critics believe these problems help explain the pharmaceutical industry's drought of new products. "That's the secret of why they're spending billions of dollars and getting nothing," says James Hussey, a former Bristol-Myers Squibb Co. manager who now leads biotech company NeoPharm Inc.

….."

Source: Wall Street J. 2/24/2004

In Search of New Leads…..

The decline in the number of new drugs is based, among other reasons, on the current high therapeutic standard in many indications, focusing research on chronic diseases such as coronary heart, Alzheimer's, arthritis, cancer, and AIDS, as well as the enhanced regulatory requirements for efficacy and safety of new drugs.

A lead can be characterized as a compound that has some desirable biological activity, not extremely polar or lipophilic, and not contain toxic or reactive functional groups. Often, molecular weight (<350) and lipophilicity (log P < 3) are considered 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.

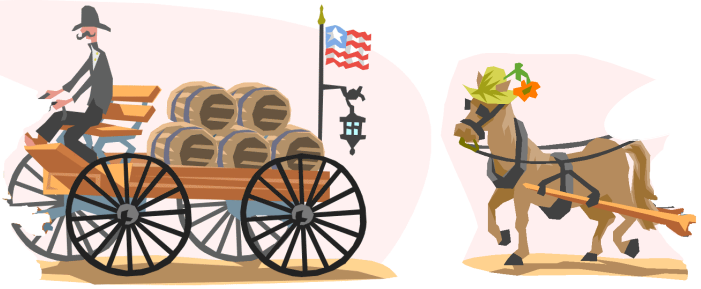
Why Is It So Difficult to Make Drugs?

Estimates of the number of possible drug molecules average 10^{40}. In contrast, the number of seconds since the Big Bang is only 10^{17}.

If 10,000 chemists were to prepare 1 compound each per second, it would take 10,000,000,000,000,000,000,000,000,000 years to finish the job.

Drug Discovery Paradigm

Currently: Biology-driven drug discovery



Chemistry-driven drug discovery - back to the future?

Where Did (Do) Our Drugs Come From?

New drugs from old poisons

The reductionist approach to medicine began with the isolation of opium alkaloids

Medicinal Chemistry Folklore

Earliest medicines ~ 5100 years ago

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

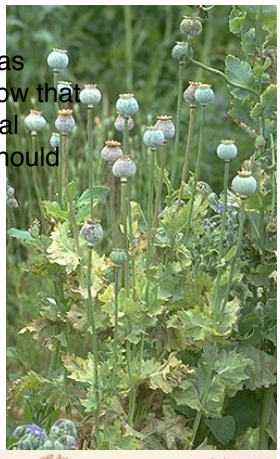
Ch'ang Shan - contains alkaloids; used today in the treatment of malaria and for fevers

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

At least a quarter of all prescriptions dispensed in the US and UK contain, as active compounds, molecules derived from flowering plants. Other data show that 12 out of the top 25 highest earning drugs in 1995 were derived from natural products. The importance of plants as medicines in the developing world should also be acknowledged. Here, they are estimated to comprise 80% of the medication used in primary healthcare (Source: Houghton, P. J., "Roots of remedies: Plants, people and pharmaceuticals." *Chem. Ind.* **1999**, 15).

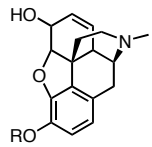


- **Ethnographies show that humans are great botanical experimentors**
- **Perhaps human brains, drugs and spices evolved together!**

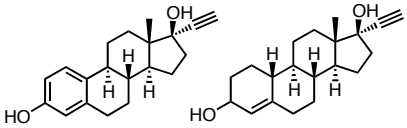

Pharmaceutical companies are tapping local knowledge in the search for new drugs

Examples of Natural Products as Leads & Drugs

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

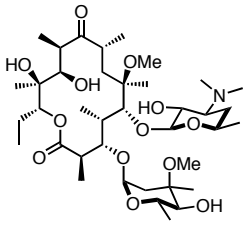


R = H: Morphine
R = Me: Codeine
(pain killer)

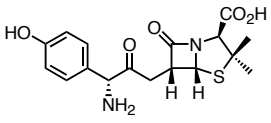


17-ethynylestradiol norethindrone

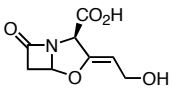
(the "Pill"; contraceptive)



Clarithromycin
(antibacterial)

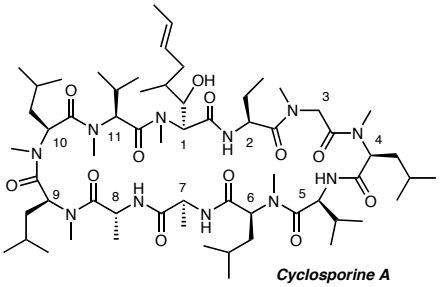


Ampicillin
(antibiotic)



Clavulanic acid
(β-lactamase inhibitor)

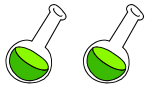
Augmentin
(antibiotic)



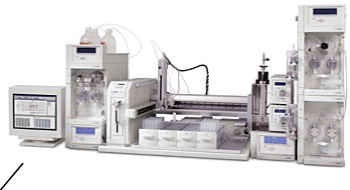
Cyclosporine A

Current use of natural product extracts

High-Pressure Accelerated
Solvent Extractor System

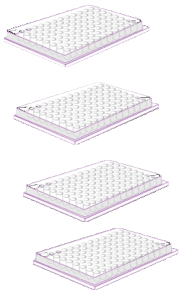


DCM & MeOH
Extracts



Parallel HPLC

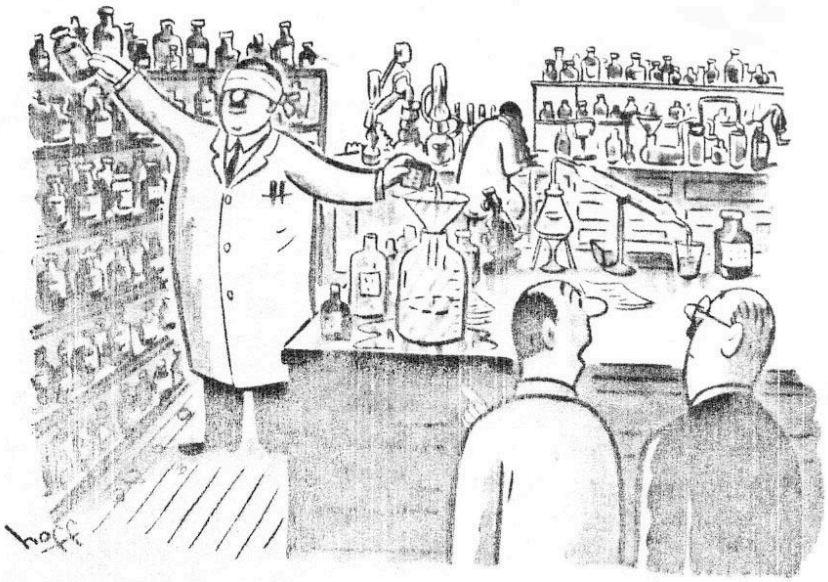
HT Solvent Concentrator



Multiwell Plates
For HTS

Drug Discovery

One way to "discover" drugs



'That's Dr Arnold Moore. He's conducting an experiment to test the theory that most great scientific discoveries were hit on by accident.'

Drawing by Hoff; © 1957
The New Yorker Magazine, Inc.

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.

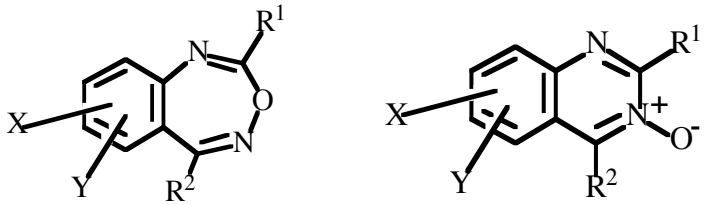
• Acetylsalicylic acid was thought to be just a better tolerable prodrug of salicylic acid, but turned out to have a unique mechanism.

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

• Warfarin was used a rat poison.

Serendipitous Discovery of Librium without a Lead

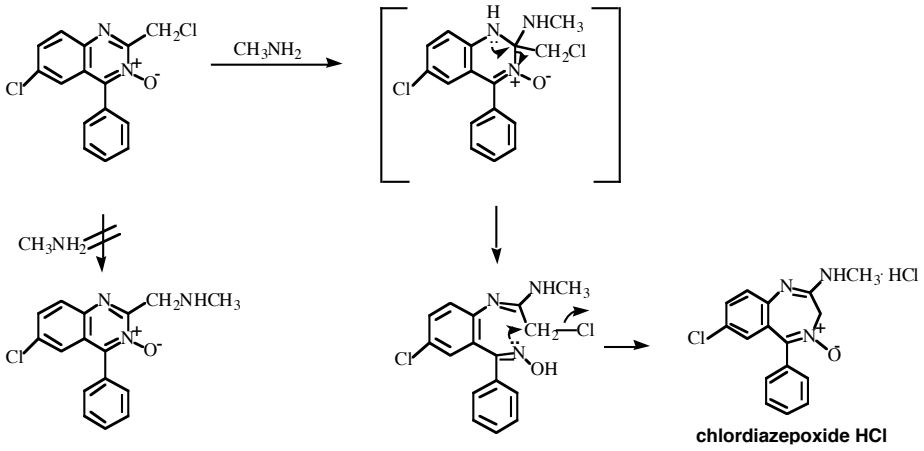
In 1955 Roche set out to prepare a series of benzheptoxdiazines as potential new tranquilizer drugs, but the actual structure was found to be that of a quinazoline 3-oxide.



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.



chlordiazepoxide HCl

Librium

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)

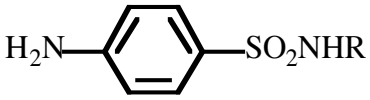
Activity/potency susceptible to small changes in structure

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)

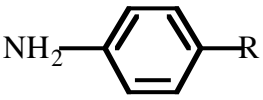
Thousands of analogs synthesized

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

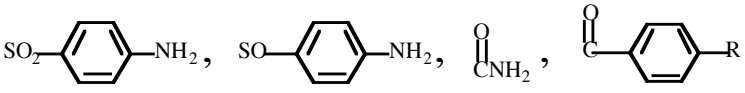
- Antimicrobial
- Diuretic
- Antidiabetic

SAR

General Structure of Antimicrobial Agents



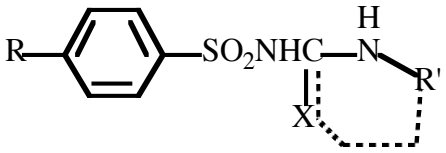
R = SO_2NHR', SO_3H

- 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

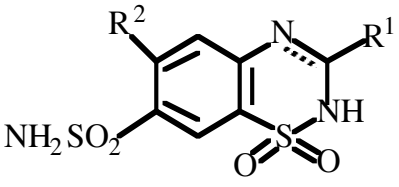
Antidiabetic Agents



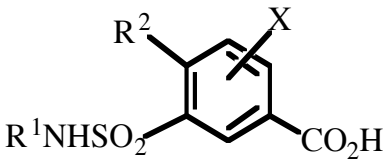
X = O, S, or N

SAR

Diuretics (2 types)



hydrochlorothiazides



high ceiling type

R^2 is an electrophilic group

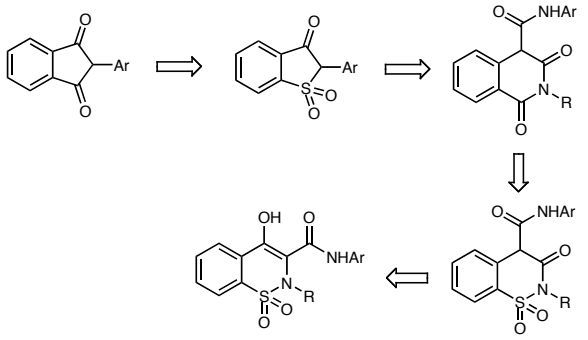
Rational Drug Discovery - Piroxicam

• It took Pfizer about 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, ie. 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= =$\overset{+}{N}$= =$\overset{+}{P}$=

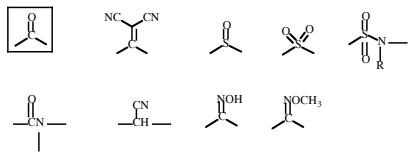
5. Ring equivalents

   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)

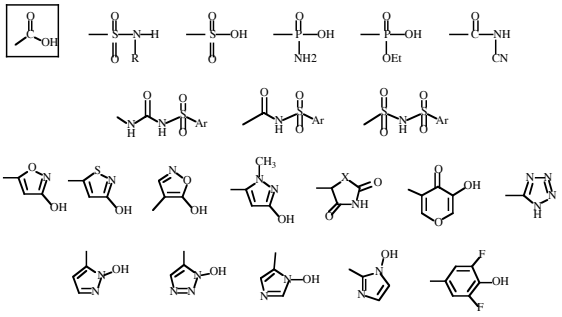
Non-Classical Isosteres

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

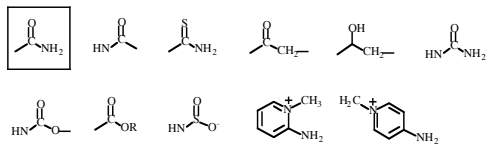
1. **Carbonyl group**



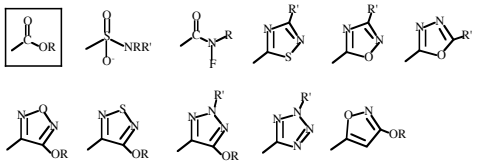
2. **Carboxylic acid group**



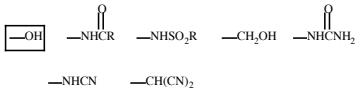
3. **Amide group**



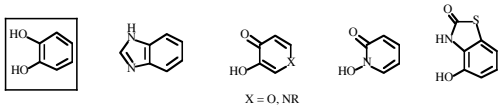
4. **Ester group**



5. **Hydroxyl group**

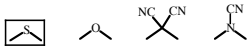


6. **Catechol**

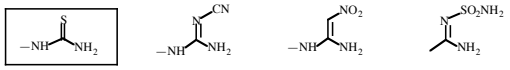


7. **Halogen**

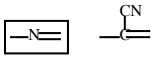
8. **Thioether**



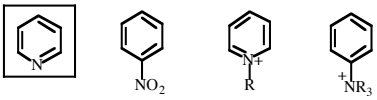
9. **Thiourea**



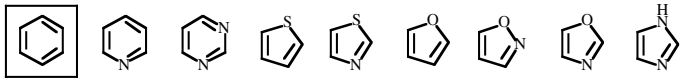
10. Azomethine



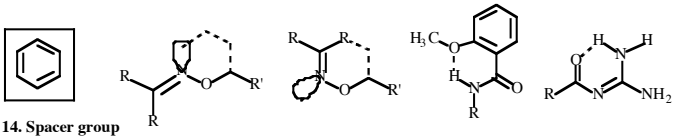
11. Pyridine



12. Benzene



13. Ring equivalents



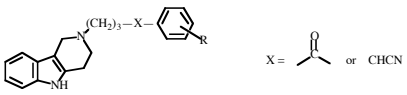
14. Spacer group

15. Hydrogen

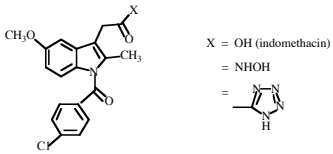
H F

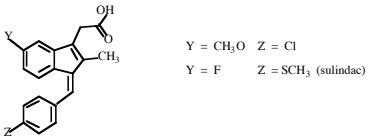
Examples of Bioisosteric Analogues

Neuroleptics (antipsychotics)

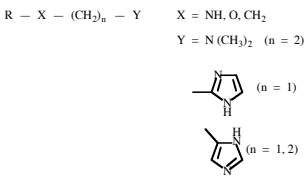


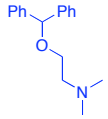
Anti-inflammatory agents



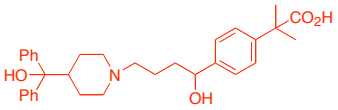


Antihistamines





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.

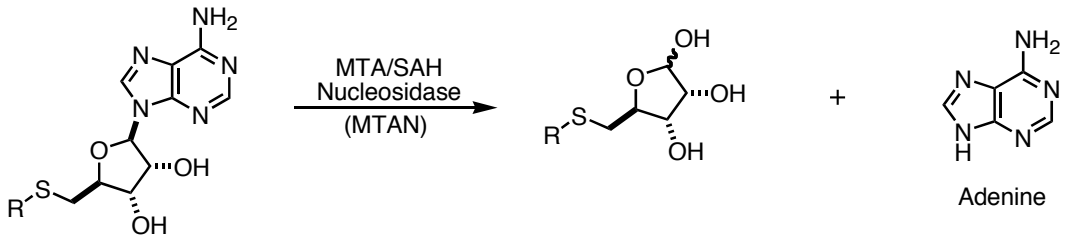
Where on the molecule do you go to make the modification? The auxophoric groups that do not interfere with binding.

Rational Drug Discovery - From Hit to Lead

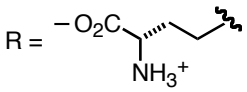
- Li, X.; Chu, S.; Feher, V. A.; Khalili, M.; Nie, Z.; Margosiak, S.; Nikulin, V.; Levin, J.; Sprankle, K. G.; Tedder, M. E.; Almassy, R.; Appelt, K.; Yager, K. M., "Structure-based design, synthesis, and antimicrobial activity of indazole-derived SAH/MTA nucleosidase inhibitors." *J. Med. Chem.* **2003**, *46*, 5663-5673.

-Background: "*S*-adenosyl homocysteine/methylthioadenosine (SAH/ MTA) nucleosidase presents a potentially useful target:
-First, its active site is similarly highly conserved across bacterial species, while differing from that of the related mammalian proteins.
-Second, SAH/MTA nucleosidase participates in the synthesis of the quorum sensing autoinducer AI-2, which in turn stimulates expression of virulence factors. Consequently, inhibiting SAH/MTA nucleosidase should attenuate bacterial virulence.
-Third, and most importantly, such inhibition should kill bacteria because accumulation of SAH and MTA inhibits certain essential methyltransferase reactions and thereby impedes recycling of adenine and methionine, which are necessary for DNA and protein synthesis, respectively.

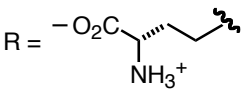
Rational Drug Discovery - From Hit to Lead



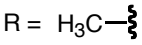
Adenine



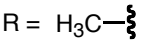
S-Adenosylhomocysteine (SAH)



S-Ribosylhomocysteine (SRH)



Methylthioadenosine (MTA)



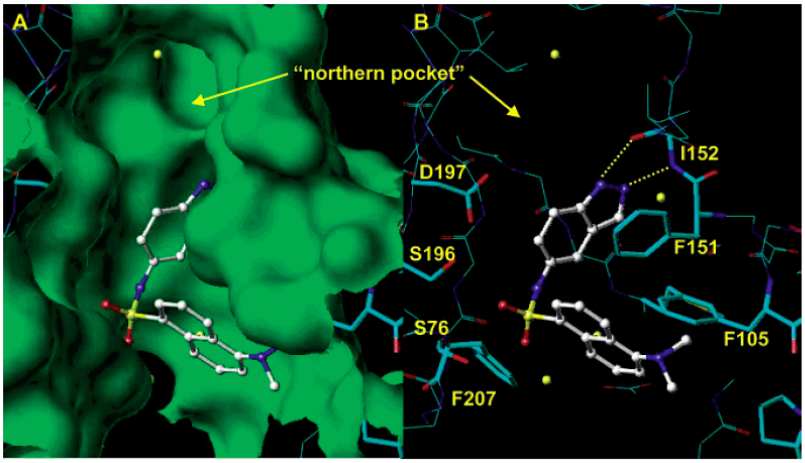
Methylthioribose (MTR)

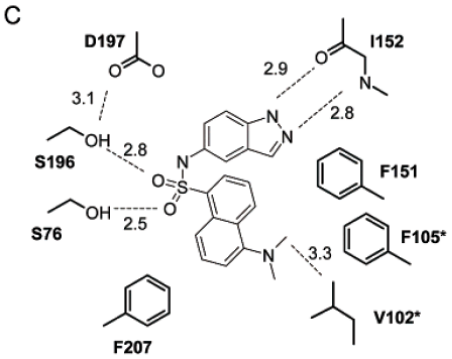
Rational Drug Discovery - From Hit to Lead

-SAR: **In Silico Lead Identification.** "Selection of compounds for screening in the SAH/MTA nucleosidase activity assay began by filtering a virtual library of 390 000 commercially available compounds to approximately 2000 satisfying the following criteria: (i) possessing three pharmacophoric features identified from inspection of homology model-substrate complexes, (ii) having molecular volumes less than that of the homology model binding pocket, and (iii) conforming to Lipinski guidelines (i.e., molecular weight < 500, -2.0 < ClogP < 5.0).

The resulting data set was clustered for 2D fingerprint diversity and a representative selection of 1288 compounds identified using SELECTOR (Tripos, Inc.).
X-ray structural determination of lead compounds cocrystallized with SAH/MTA nucleosidase derived from *Escherichia coli* and other pathogenic species revealed the mode of inhibitor binding within the active site. These co-structures provided the structural information for design of individual compounds and focused libraries."

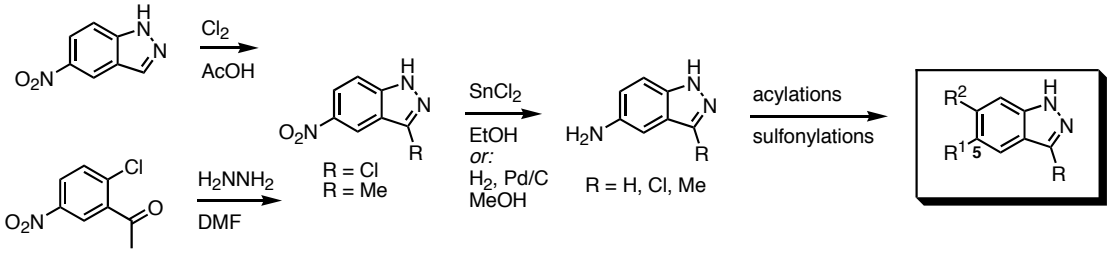
Use of 5-aminoindazole as the core scaffold provided a structure-guided series of low nanomolar inhibitors with broad-spectrum antimicrobial activity. The implementation of structure-based methodologies provided a 6000-fold increase in potency over a short timeline (several months) and an economy of synthesized compounds.

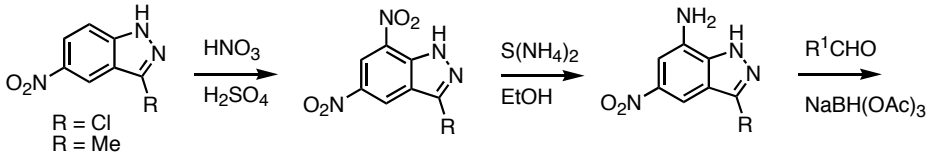


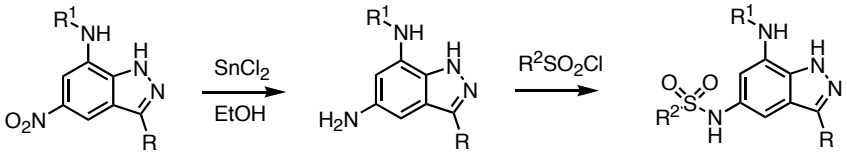


The lead indazole (*K*i=2.8 *u*M) bound in the active site of SAH/MTA nucleosidase as determined by X-ray crystallography. The solvent-accessible surface of the active site (A) illustrates the general shape of the pocket. The hydrogen bonding pattern comprised of protein backbone and side-chain interactions are illustrated in (B, C). Inhibitor hydrophobic interactions with a cluster of phenylalanines and V102 are also illustrated; residues from the associated monomer are indicated by an asterisk (C). Bound water molecules are indicated as yellow spheres (A, B).

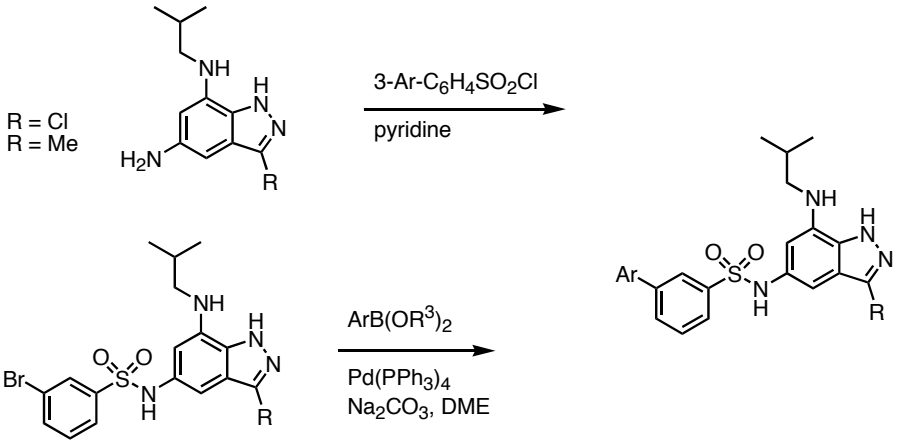
-Synthetic work:





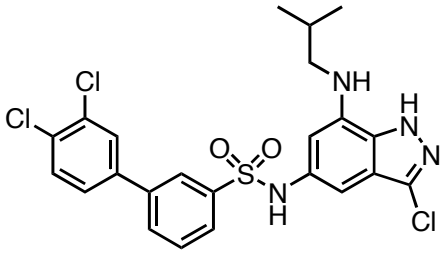


-Synthetic work:



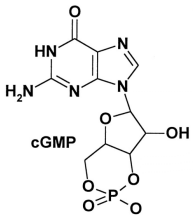
Rational Drug Discovery - From Hit to Lead

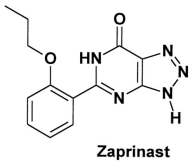
-Conclusions: Structure-based design of a series of indazole-based *S*-adenosyl homocysteine/methylthioadenosine (SAH/ MTA) nucleosidase inhibitors led to the discovery of a chlorinated indazole, a potent, low molecular weight inhibitor with nanomolar potency in an enzyme inhibition assay. In antimicrobial assays, this compound inhibits the growth of three important pathogenic species, showing MIC values of less than 10 *u*M.

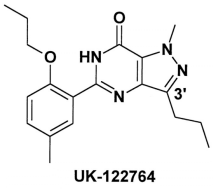


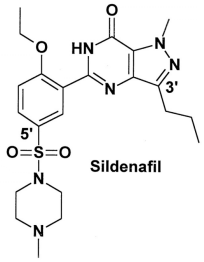
"Me Too" Compounds

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 antihistamins without sedative side effects, statins, or PDE5 inhibitors.









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

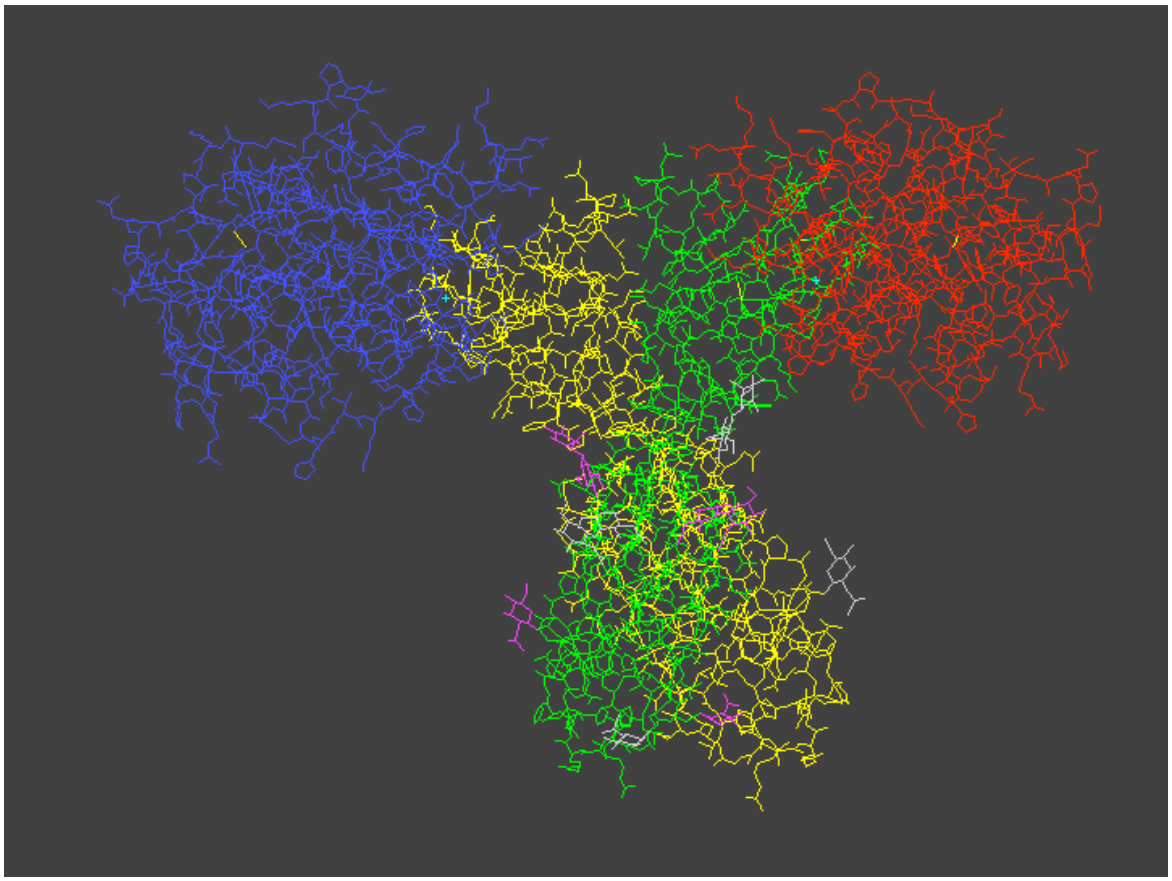
Gadek, T. R.; Burdick, D. J.; McDowell, R. S.; Stanley, M. S.; Marsters Jr., J. C.; Paris, K. J.; Oare, D. A.; Reynolds, M. E.; Ladner, C.; Zioncheck, K. A.; Lee, W. P.; Gribling, P.; Dennis, M. S.; Skelton, N. J.; Tumas, D. B.; Clark, K. R.; Keating, S. M.; Beresini, M. H.; Tilley, J. W.; Presta, L.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

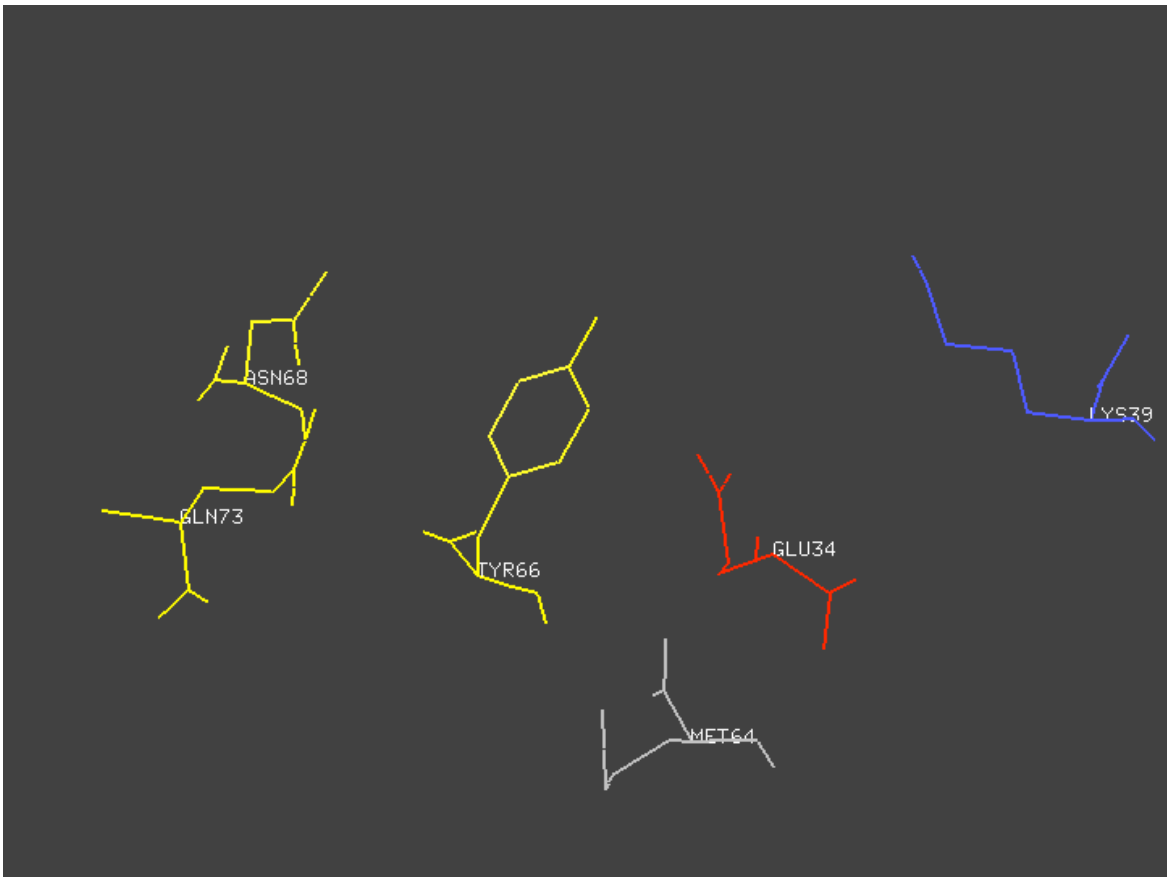
The interaction of LFA-1 with the ICAM proteins 1, 2, and 3 is critical to the adhesion, migration, and proliferation of lymphocytes.

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

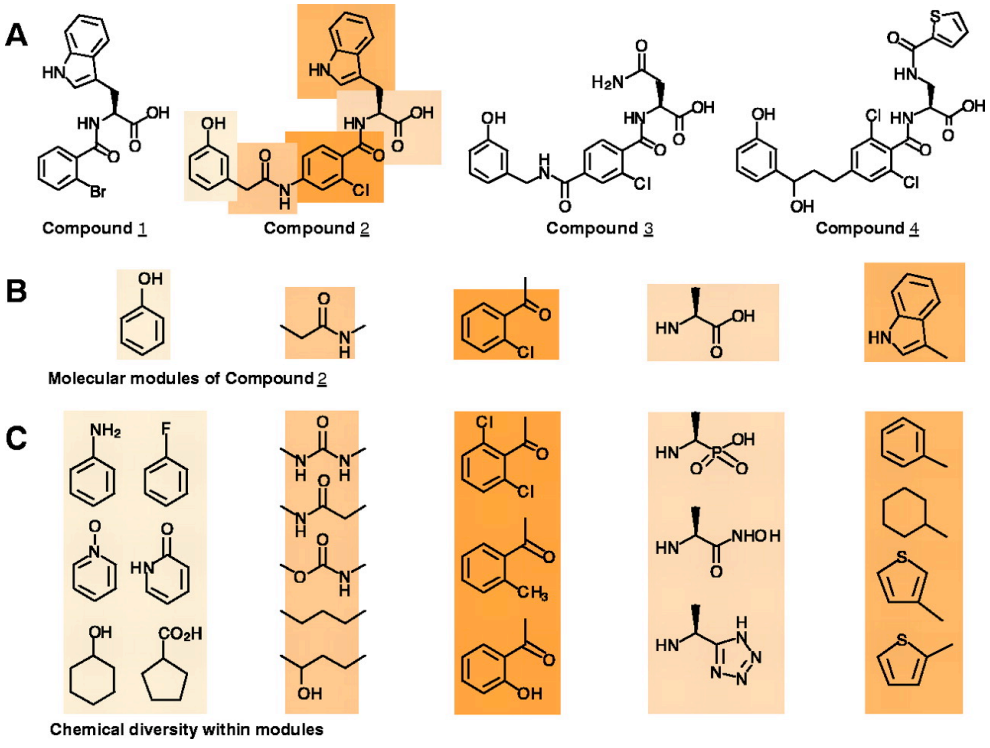
An epitope comprising residues E34, K39, M64, Y66, N68, and Q73 within ICAM-1's first domain has been identified as essential for its interaction with LFA-1. The function of this epitope is embedded in the **carboxylic acid, amine, sulfide, phenol, and carboxamide** chemical functionalities of the amino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





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

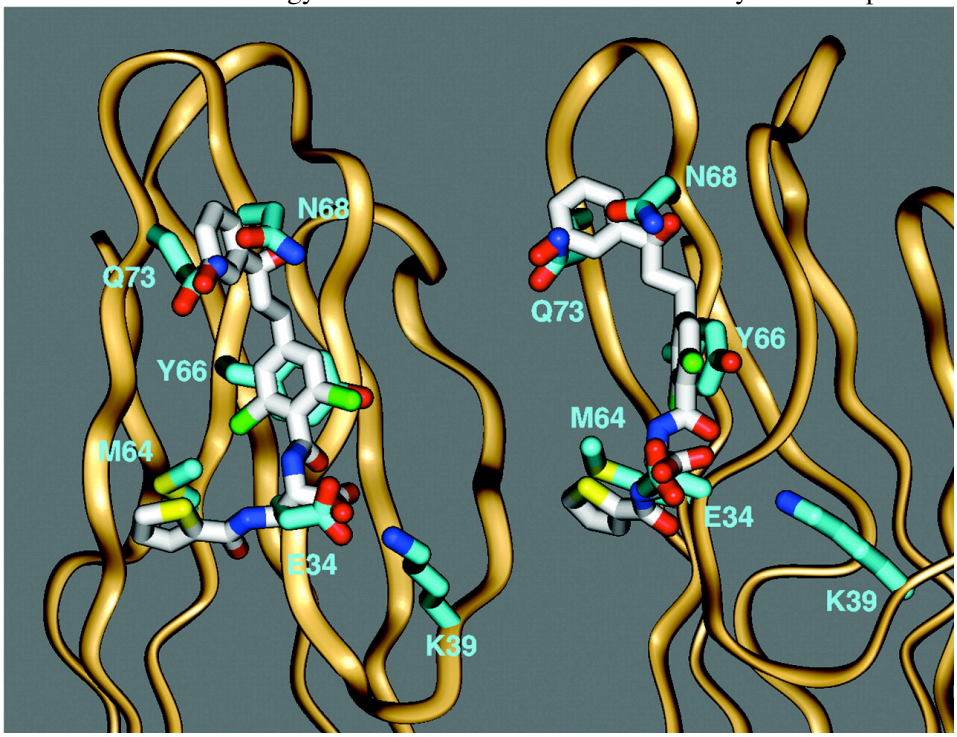


Comparison of the inhibition of ICAM-1/LFA-1 binding and the inhibition of mixed lymphocyte reaction (MLR). IC_{50} values were determined from a 4P fit of data from titrations over concentrations of 10^{-3} to 10^{-10} M. Values reported are the mean±standard deviation for $n>2$ of experiments run in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

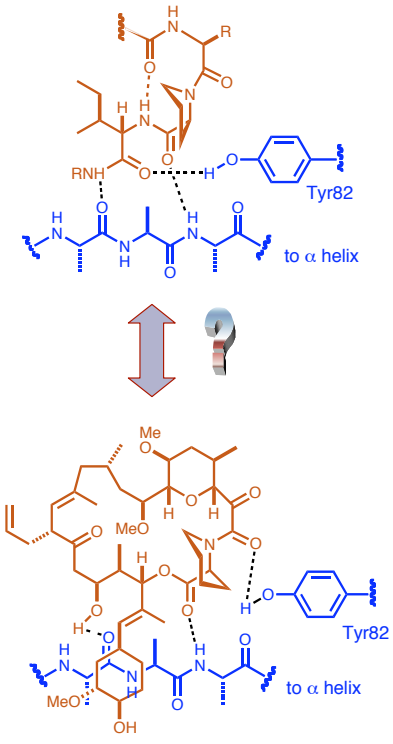


Conclusions:

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.

Peptides to Peptidomimetics



Evelyn Paul, "It Is Indeed the Cup" from: *Claire de Lune and Other Troubadour Romances*. London: G. G. Harrap & Co., Ltd., 1913

Peptides to Peptidomimetics

• Many substrates of enzymes, ie. angiotensinogen, fibrinogen, HIV protease, and receptors are either peptides or proteins, and the interaction of these ligands with their target is often mediated by only a few amino acid side chains; the rest of the protein stabilizes a certain 3D conformation. The RGD motif which interacts with different integrins in different conformations is a striking example.

• Peptides can be easily synthesized in large numbers, but the next step, the chemical conversion of such a peptide lead into a non-peptide mimetic is far from trivial. Achieving oral activity and bioavailability is even more challenging. The promiscuous benzodiazepines are examples of scaffold mimetics of peptide loops, but most other scaffolds described in the literature have not yet yielded similar successes.

• The comparison between enkephalins and morphine is often used as an example for peptide mimicry. However, this is obviously flawed because morphine was discovered first.

Peptide Mimetics - The Good News

Metabolic Stability of Biologically Active Peptides

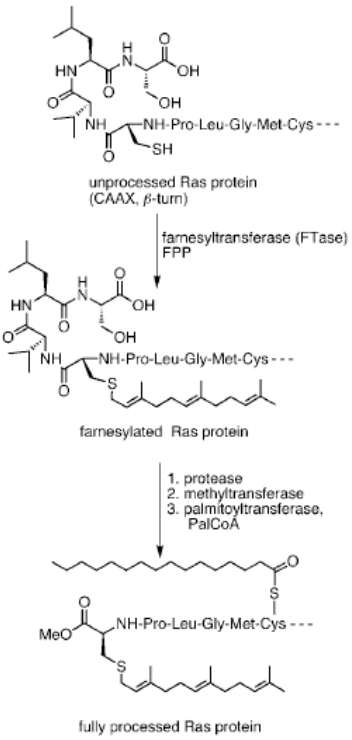
Enkephalin Analogs

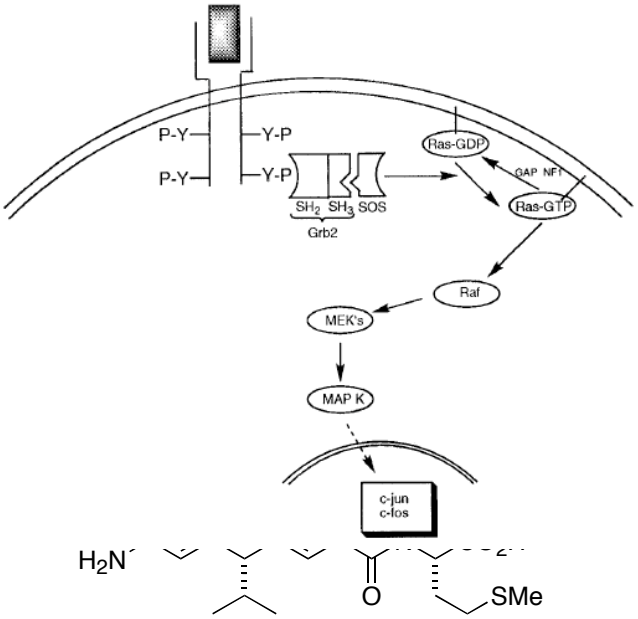
Sequence	Serum (Plasma) Half-live [min]
Tyr-Gly-Gly-Phe-Leu	7 (1.3)
Tyr-Gly-Gly-Phe-Met	(0.4, rat)
Tyr-D-Ala-Gly-Phe-Leu	63
Tyr-ψ[CH_2S]-Gly-Gly-Phe-Leu	47
Tyr-Gly-ψ[CH_2S]-Gly-Phe-Leu	35
Tyr-Gly-Gly-ψ[CH_2S]-Phe-Leu	67
Tyr-Gly-Gly-Phe-ψ[CH_2S]-Leu	157
Tyr-D-Ala-Gly-ψ[CH_2S]-Phe-Leu	>500

The primary degradation route of small peptides in serum is by exopeptidase activity, mostly aminopeptidase (AP) and dipeptidylaminopeptidase (DPAP)

Source: Powell, M. F. *Ann. Rep. Med. Chem.* **1993**, *28*, 285.

Preparation of a 27 nM Farnesyl Transferase Inhibitor based on Peptide Mimicry



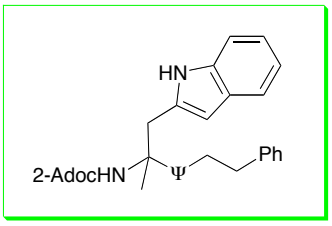


Yang, H.; Sheng, X. C.; Harrington, E. M.; Ackermann, K.; Garcia, A. M.; Lewis, M. D. *J. Org. Chem.* **1999**, *64*, 242.

Peptide Mimetics - The Bad News

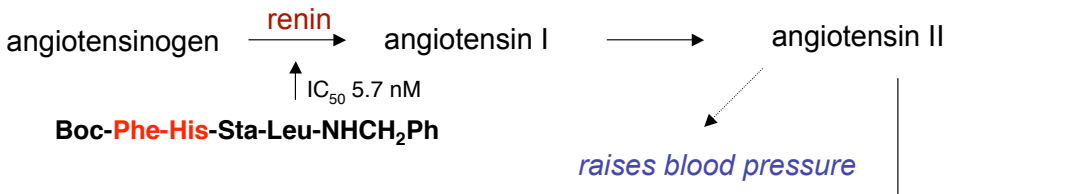
Amide Bond Replacements in Cholecystokinin Dipeptoids

Ψ	CCK-B Recepor Binding Affinity: IC_{50}[nM]
-CONH-	32
$-CH_2NH_2-$	1080
-CSNH-	808
-COO-	547
-COS-	932
-CON(Me)-	123
-HC=CH-	6340
$-CH_2CH_2-$	8690
$-CH(OH)CH_2-$	595



Source: Fincham, C. I.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; O'Toole, J. C.; Ratcliffe, G. S.; Rees, D. C.; Roberts, E. *J. Med. Chem.* **1992**, *35*, 1472-84.

Peptide Mimetics - The Ugly



"..We hoped that [replacing the P_3-P_2 amide bond with 13 different isosteres] would lead to enhanced stability to enzymatic hydrolysis and longer acting, possibly orally active, renin inhibitors." Kaltenbronn, J. S. et al. *J. Med. Chem.* **1990**, *33*, 838-845.

→ **The two most potent isosteres had IC_{50} values of 61 and 22 nM**

→ **Complete lack of response upon oral administration to high-renin monkeys!**

"..a rational mechanism for transforming [peptides] into non-peptide peptidomimetics has not been evident." Bursavich, M. G.; Rich, D. H. *J. Med. Chem.* **2002**, *45*, 541-558.

"Peptide mimicry by design used to be touted as a solution to [the limited bioavailability and oral activity of peptides] and was focused on impersonating secondary structure motifs, particularly β-turns, but this approach has yielded few pharmaceutical products." Beeley, N. R. A. *Drug Disc. Today* **2000**, *5*, 354-363.

What About Drug Discovery in Academia?

Forward Chemical Genetics

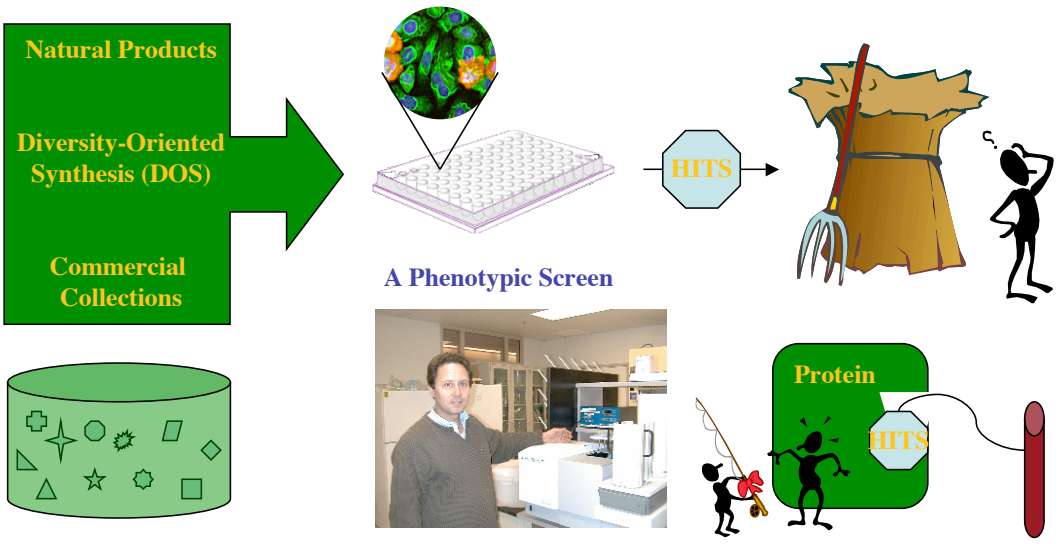
= screening small molecules for their ability to perturb cellular pathways, followed by identifying the specific targets of the active compounds. In general, whole cell assays are used.

This approach may lead to the identification of new regulatory mediators of biochemical pathways and the validation of molecular targets for therapeutic intervention. It is used to systematize the discovery and use of small molecules as tools for biological research.

"**Reverse Pharmacology**" enables identification of the molecular targets of efficacious agents with unknown mechanisms of action.

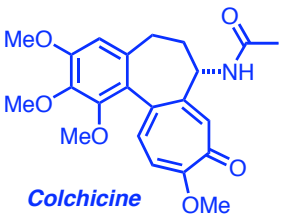
Ref: Lokey, R. S., "Forward chemical genetics: Progress and obstacles on the path to a new pharmacopoeia." *Current Opinion in Chemical Biology* **2003**, *7*, 91-96.

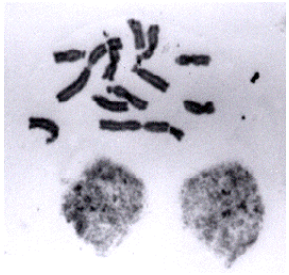
Forward chemical genetics involves 3 basic stages:
- a library of compounds
- an assay, usually a phenotypic assay
- a strategy to trace an active compound to its biological target

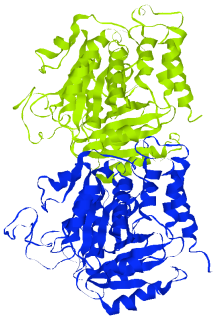


Success Stories of Forward Chemical Genetics and Phenotypic Screens:

• The study of the cellular effects of Colchicine was instrumental in the discovery of the cytoskeletal protein tubulin.







• Similarly, The Hsp90 inhibitor **geldanamycin**, the Golgi disruptor **brefeldin A** and the the G2 checkpoint inhibitor **isogranulatimide** were initially identified by their phenotypic effects, prior to the identification of their molecular targets. In some respects, Chemical Genetics is equivalent to "classical pharmacology on (technological) steroids".

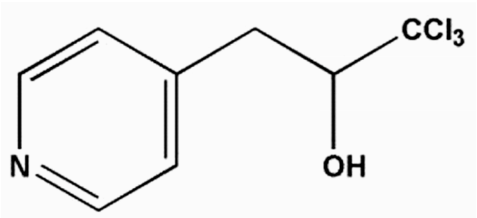
Success Stories of Forward Chemical Genetics and Phenotypic Assays from Chemical Library Screens:

• A 16,600-member library from Chembridge was used in a phenotypic assay for mitotic spindle disruptors, leading to the identification of the kinesin inhibitor monastrol (Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J., "Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen." *Science* **1999**, *286*, 971-974).

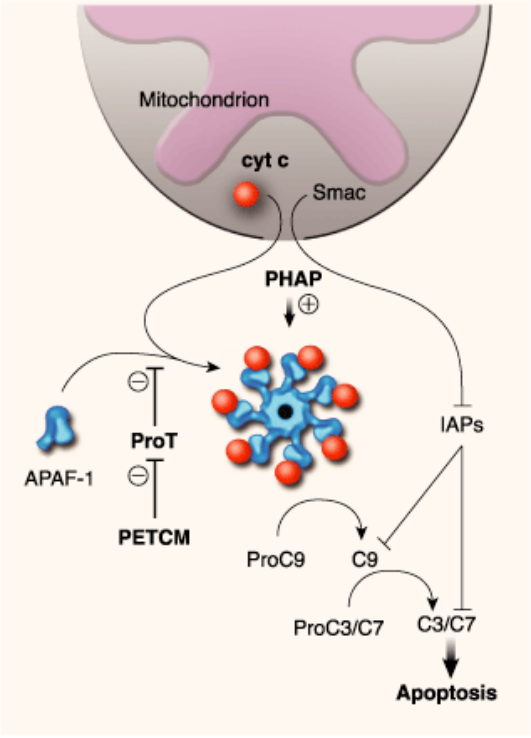
• Phenotypic screens of commercial collections led to compounds that inhibit the Ras pathway, Sir2-mediated transcriptional silencing in yeast, alter the developmental program of zebrafish, and modulate TGF-β signaling in mammalian cells.

•Jiang, X., H.-E. Kim, et al. (2003). "Distinctive roles of PHAP proteins and prothymosin-α in a death regulatory pathway." Science **299**(5604): 223-226. An example of an empirical forward chemical genetics search for pharmacological agents that induce apoptosis only in transformed cells.

HTS led to the identification of a small molecule, PETCM, an Abbot compound, that activates caspase-3 in cell extracts.



In elucidating the mechanism of action of PETCM, the oncoprotein prothymosin-α (Pro-T) and the tumor suppressor putative HLA-DR-associated proteins (PHAP) as important regulators of caspase-3 activation. These proteins appear to mediate distinct steps in the mitochondrial pathway: ProT blocks formation of the apoptosome, an event inhibited by PETCM. In contrast, PHAP appears to facilitate apoptosome-mediated caspase-9 activation.
The apoptosome is a macromolecular complex that is formed in response to the cellular commitment to apoptodic death. The apoptosome is crucial to activation of caspase-9.



Controlling the caspase cascade.
Formation of the heptameric "apoptosome" is a crucial step in the initiation of apoptosis. Selective mitochondrial proteins such as cytochrome c (cyt c) (red) are released from the mitochondrial intermembrane space in response to apoptotic stimuli. Cytochrome c induces the assembly of Apaf-1 monomers (blue) into the apoptosome, which recruits and activates caspase-9 (C9), which in turn activates caspase-3 (C3) and -7 (C7). IAP proteins inhibit rogue caspases, but their inhibitory actions are blocked by antagonists (such as Smac/Diablo and Omi/HtrA2) that are co-released with cytochrome c. Two additional tiers of apoptosome regulation have been identified: the oncoprotein proT, which retards assembly of the apoptosome complex, and tumor suppressor PHAP proteins, which stimulate the apoptosome's deadly activities

This work nicely illustrates the opportunity of forward chemical genetics to identify novel players in a complex biochemical pathway. It also illustrates some potential pitfalls:

• more work is needed to definitively establish the in vivo significance of the PHAP finding.
• High (0.2 mM) concentrations of PETCM are necessary to stimulate caspase-3 activity and drive apoptosome formation. Under these high, physiologially not relevant concentrations, many other signaling processes might be partially influenced.
• PETCM, identified by HTS, is not a "drug-like" molecule, and therefore is of questionable value as a lead structure for drug discovery.

Our Strategy for Integrating Organic Synthesis with Biology, Pharmacology and Drug Discovery

Diversity-Oriented Synthesis

Represents the synthesis of relatively small libraries of organic molecules that are structurally more complex, have a greater variety of core structures, and possess richer stereochemical variations than those produced by traditional combichem.

DOS Strategies include:

• Tandem or Multi-Component Reactions in which complex polycyclic products are synthesized in a minimum of steps

• Bi- and polyfurcating reaction pathways, giving access to different scaffolds depending on reaction conditions

• Biomimetic synthetic approaches for the preparation of complex polycyclic molecules

C&E News, October 4, 2004, Volume 82, Number 40 pp. 32-40

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.