## *Drug Discovery - The Origins of Drugs*

Molecular Pharmacology-Drug Discovery, October 17, 2006

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


- ***Global Mapping of Pharmacological Space***
- *Paolini, G. V.; Shapland, R. H. B.; Van Hoorn, W. P.; Mason, J. S.; Hopkins, A. L., Nature Biotechnology* ***2006****, 24, 805.*

- ***Medicinal Chemistry SAR***
- ***Chemical Structure - Biological Targets***
- ***Drug Like Properties***
- ***Drug Discovery Strategies***

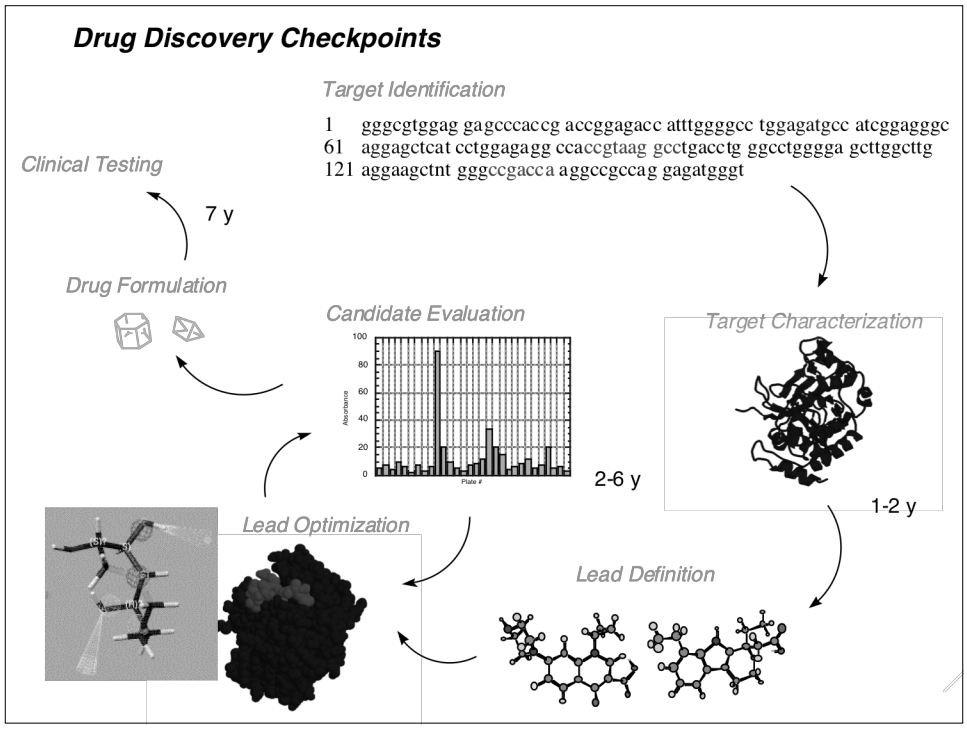
***For further reading, see***

Vieth, M.; Sutherland, J. J., "Dependence of molecular properties on proteomic family for marketed oral drugs." J. Med. Chem. 2006, 49, 3451-3453.

Brown, D.; Superti-Furga, G., "Rediscovering the sweet spot in drug discovery." Drug discovery today 2003, 8, 1067-1077.

Bleicher, K. H.; Boehm, H.-J.; Mueller, K.; Alanine, A. I., "A guide to drug discovery: Hit and lead generation: Beyond high-throughput screening." Nature Reviews Drug Discovery 2003, 2, 369-378.

Proudfoot, J. R., "The evolution of synthetic oral drug properties." Bioorg. Med. Chem. Lett. 2005, 15, 1087-1090.




# Pharmacological Target Space

| Gene taxonomy | All targets with <10 μM[a] binding affinity | Human targets with <10 μM binding affinity | Human targets with <1 μM binding affinity | Human targets with <10 μM binding affinity and rule-of-five[b] N > 1 | Human targets with <100 nM binding affinity | Human targets with <100 nM binding affinity and rule-of-five[b] n > 1 |
|---|---|---|---|---|---|---|
| Protein kinases | 131 | 105 | 99 | 98 | 89 | 83 |
| Peptide GPCRs | 110 | 63 | 59 | 59 | 55 | 42 |
| Transferases | 75 | 49 | 42 | 36 | 33 | 24 |
| Aminergic GPCRs | 72 | 35 | 35 | 35 | 35 | 35 |
| GPCRs (class A and others) | 68 | 44 | 44 | 40 | 38 | 32 |
| Oxidoreductases | 68 | 40 | 36 | 38 | 29 | 25 |
| Metalloproteases | 63 | 44 | 41 | 41 | 36 | 35 |
| Hydrolases | 56 | 36 | 29 | 30 | 25 | 21 |
| Ion channels (ligand-gated) | 55 | 29 | 28 | 24 | 25 | 22 |
| Nuclear hormone receptors | 47 | 24 | 24 | 22 | 23 | 19 |
| Serine proteases | 37 | 30 | 30 | 28 | 29 | 21 |
| Ion channels (others) | 24 | 18 | 16 | 16 | 13 | 11 |
| Phosphodiesterases | 23 | 19 | 19 | 19 | 18 | 18 |
| Cysteine proteases | 20 | 16 | 16 | 14 | 14 | 13 |
| GPCRs class C | 20 | 10 | 10 | 10 | 6 | 6 |
| Kinases (others) | 16 | 12 | 9 | 11 | 6 | 5 |
| GPCRs (class B) | 14 | 7 | 7 | 4 | 7 | 3 |
| Aspartyl proteases | 10 | 7 | 7 | 4 | 6 | 4 |
| Miscellaneous | 241 | 139 | 119 | 108 | 83 | 63 |
| Enzymes (others) | 156 | 109 | 97 | 90 | 69 | 47 |
| Total | 1,306 | 836 | 767 | 727 | 639 | 529 |

Lipinski's 'rule of 5' predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight (MW) is greater than 500 and the calculated Log P (CLogP) is greater than 5. *Adv. Drug Del. Rev.* **1997**, *23*, 3-25.

# Medicinal Chemistry

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

It involves:

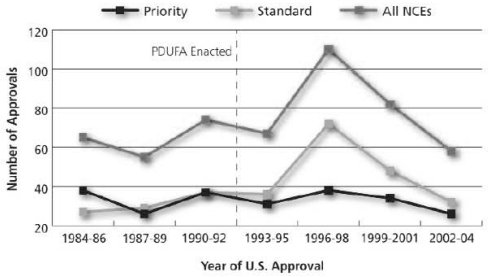
- Organic Synthesis
- Biological Target Identification & Assay Development
- Structure-Activity Relationships (SAR)
- Absorption, distribution, metabolism, and excretion (ADME)

## "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:

• the current high therapeutic standard in many indications

• research focus on chronic diseases such as coronary heart, Alzheimer's, arthritis, cancer, and AIDS, which require orally available drugs and extensive human trials

• the enhanced regulatory requirements for efficacy and safety of new drugs

• risk-adverseness in a mature field

• lack of novel chemical lead structures

## 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, molecular weight (<350) and lipophilicity (log P < 3) are considered the most obvious characteristics of a drug-like lead.

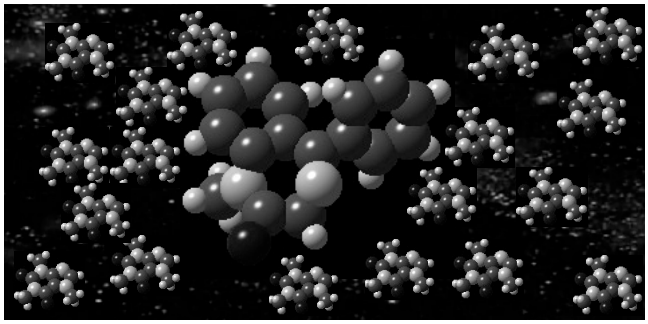
The lead should also have a series of congeners that modulate biological activity, indicating that further structural modification will improve selectivity and potency.

In order to maximize the success in finding new leads, it is important to have access to structurally diverse libraries of small organic molecules.

## Why Is Structural Diversity An Issue?

Even when molecules are limited to 11 atoms, MW<160, chemical stability & synthetic feasibility, 13.9 Mio compounds result [*Reymond et al. ACIE* ***2005****, 44, 1504*].

Extending the range to drug-like molecules with MW<500 provides estimates of $10^{24}$ compounds [*Leeson et al. DDT* ***2004****, 1, 89*].



If 1,000,000 chemists (robots?) were to prepare 1,000 compounds each per second, it would still take 35,000,000 years to synthesize the Universal Library*!!!*

## 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 ~ 5,100 years ago

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

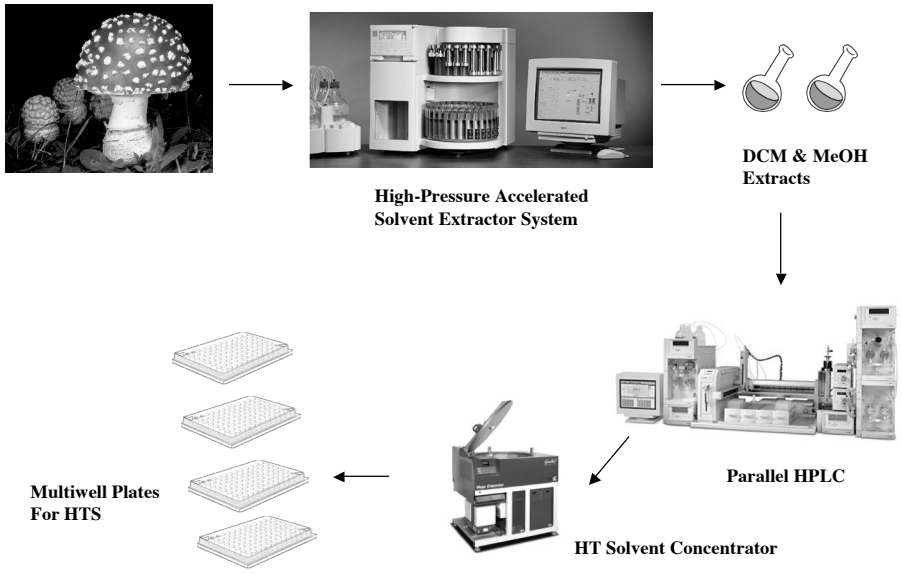
*Ch'ang Shan* - contains alkaloids; used today in the treatment of malaria and for fevers

*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:

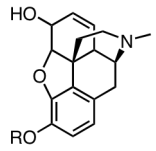
Extract of foxglove plant, cited by Welsh physicians in 1250.
Used to treat dropsy (congestive heart failure) in 1785
Contains digitoxin and digoxin; today called digitalis

**Modern investigation of natural product extracts**

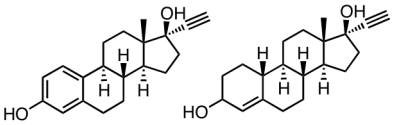


## Examples of Natural Products as Leads & Drugs

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

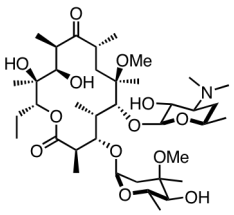


R = H: Morphine
R = Me: Codeine
(pain killer)

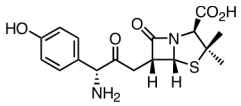


17-ethynylestradiol — norethindrone

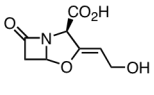
(the "Pill"; contraceptive)



Clarithromycin
(antibacterial)

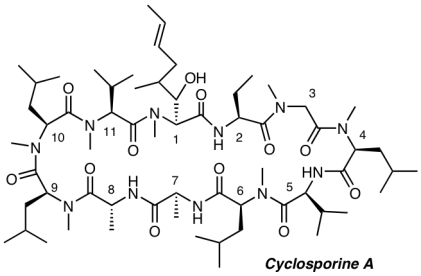


Ampicillin
(antibiotic)



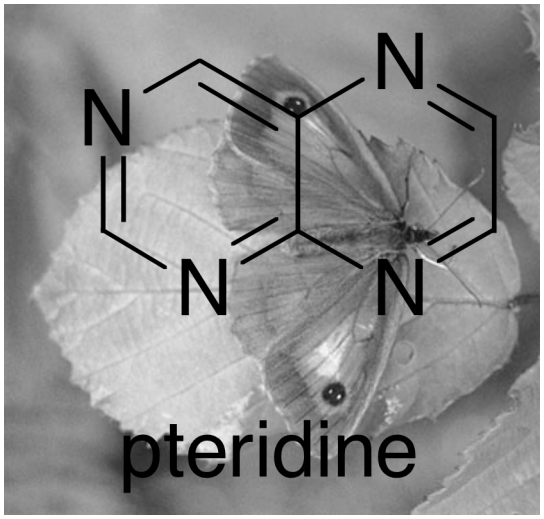
Clavulanic acid
(β-lactamase inhibitor)

Augmentin
(antibiotic)



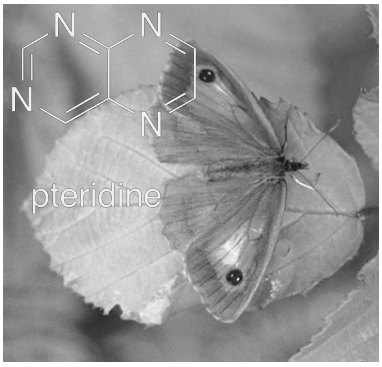
***Cyclosporine A***

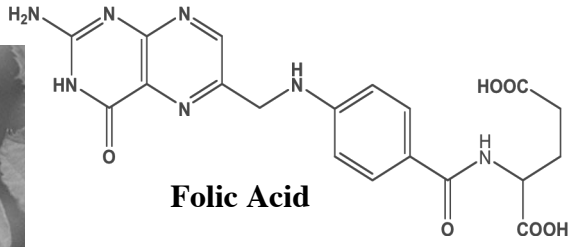
## Pyrazino[2,3-*d*]pyrimidines (Pteridines)



***Mimicry of Biological Metabolites & Enzyme Substrates***

The pteridine scaffold is a building block of the B vitamin **Folic Acid**. Folic acid is essential for several one-carbon transfer reactions in the body, including the conversion of uracil to thymine. Since dTMP is an essential precursor of DNA, any agent that lowered dTMP levels drastically affects cell division. Because rapidly dividing cells are particularly dependent on the activities of thymidylate synthase and dihydrofolate reductase, these enzymes have been major targets for ***antibacterial, antimalaria*** *and* ***anticancer drugs***. The inhibition of either or both of these enzymes blocks the synthesis of dTMP and therefore the synthesis of DNA.



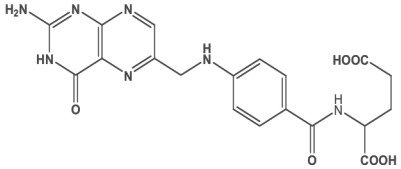



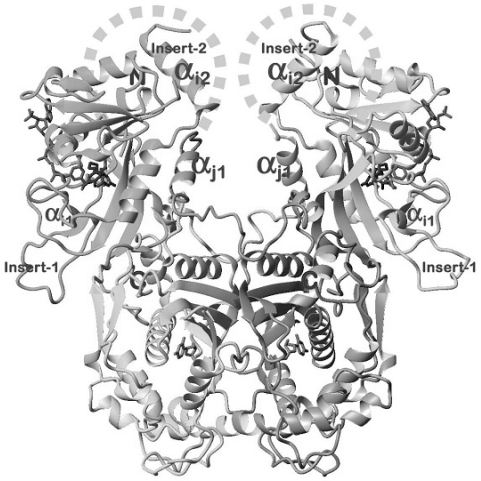

**Folic Acid**

Kompis, I. M.; Islam, K.; Then, R. L. "DNA and RNA synthesis: Antifolates." *Chem. Rev.* **2005**, *105*, 593-620.

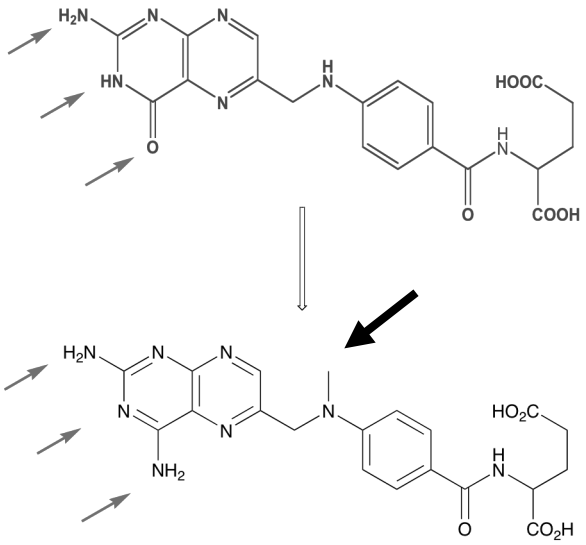
# Dihydrofolate Reductase

- DHFR is a small enzyme that plays an essential role in DNA synthesis
- Catalyzes the conversion of dihydrofolate to tetrahydrofolate, a cofactor required for the biosynthesis of thymidylate, pyrimidine nucleotides, methionine, and glycine
- **Target molecules for inhibiting this enzyme must be structurally similar to Folic Acid**



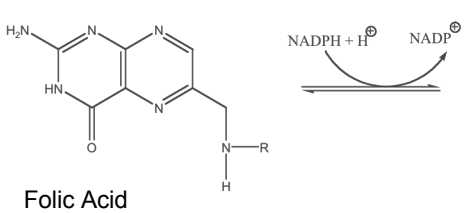
# Development of Antifolates



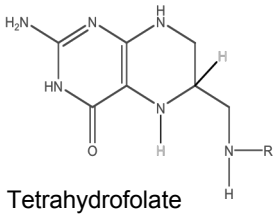
**Methotrexate (MTX)**

- **clinically used since 1953 as a potent chemotherapeutic agent**
- **converted to its polyglutamate form, which is required for intracellular retention, and represents the preferred substrate for most folate-dependent enzymes**

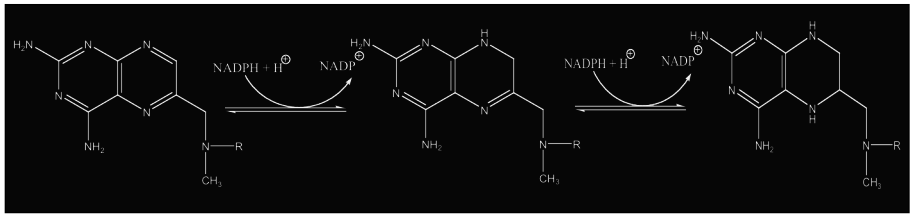
## Mechanism of MTX inhibition



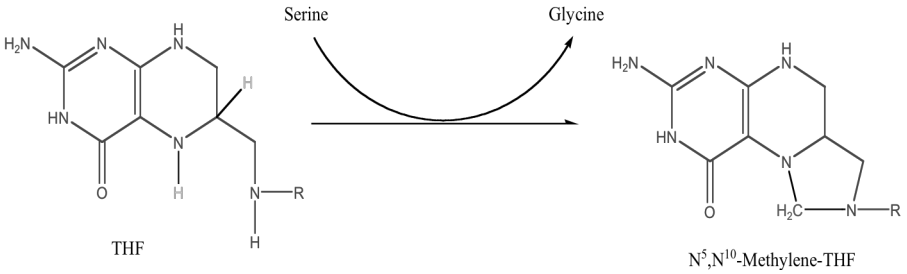
Folic Acid

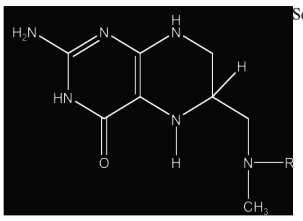


Tetrahydrofolate



Methotrexate



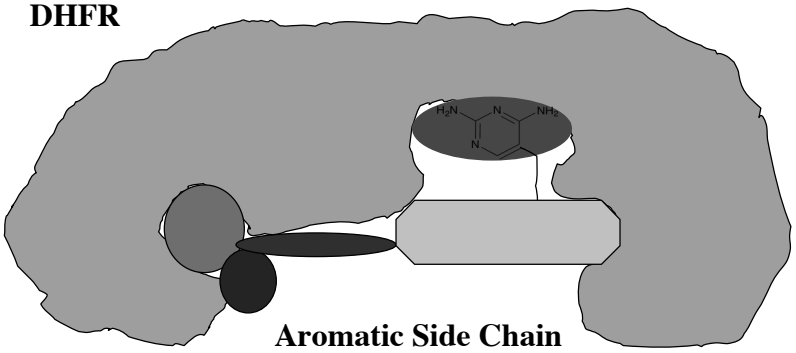




No Reaction due to lack of Hydrogen on $N^{10}$

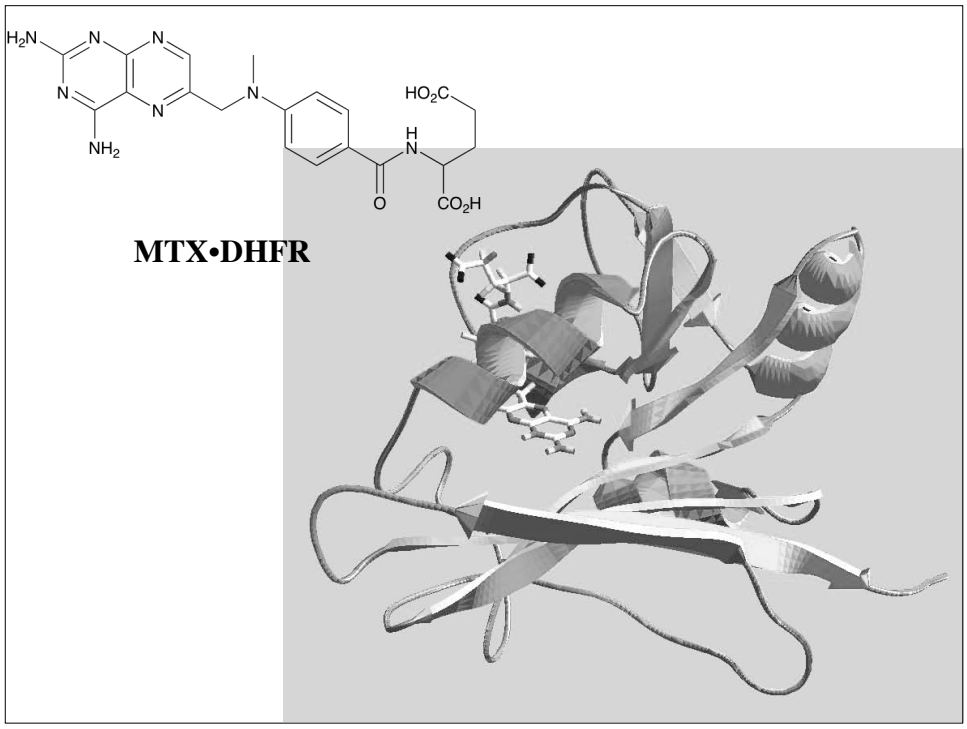
# DHFR Inhibitors - Schematic Design

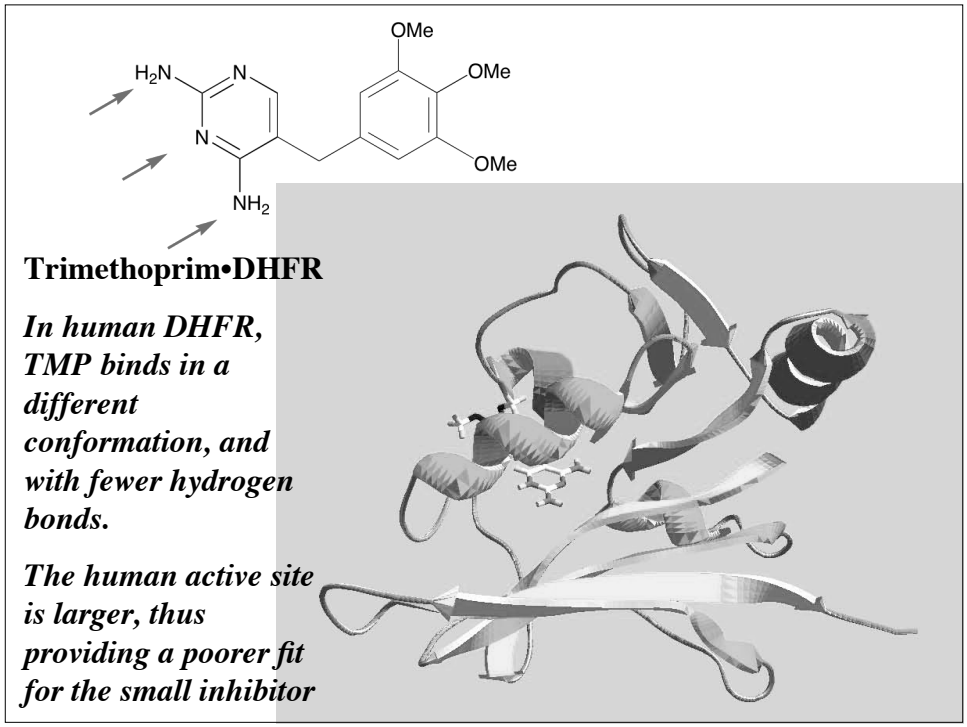
***The mammalian DHFR has a larger active site than bacterial DHFR, thus allowing specific interactions.***

**DHFR**



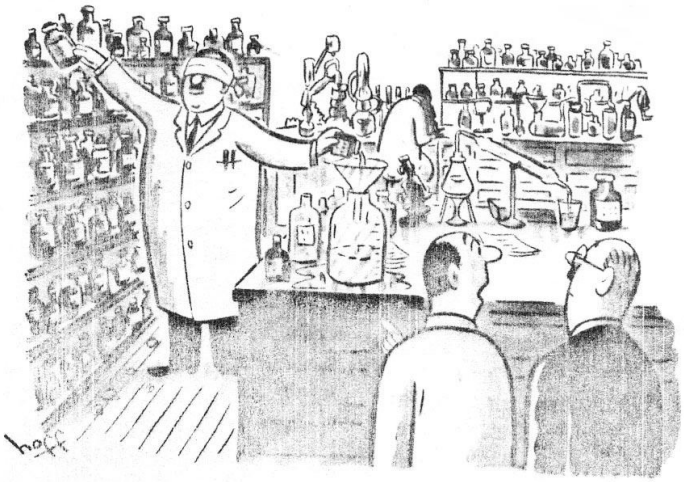
**Aromatic Side Chain**
**Solvent Exposed Groups**
**Spacer**
**Central Core**
**Head**


MTX•DHFR


Trimethoprim•DHFR
In human DHFR, TMP binds in a different conformation, and with fewer hydrogen bonds.
The human active site is larger, thus providing a poorer fit for the small inhibitor

# Serendipitous 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, 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 dye for cheap wines; after a heroic self-experiment, a pharmacologist experienced its drastic diarrheic activity.

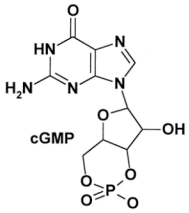
• Warfarin was used a rat poison.

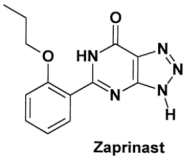
• Viagra……

## "Me Too" Compounds

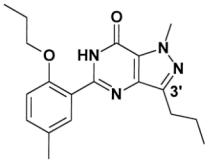
Copying existing drugs with only minor chemical variations is usually referred to as "me too" research. Interestingly, sometimes these close analogs demonstrate major (usually unexpected) advantages, like the bioavailable, broad-spectrum lactamase-resistant penicillins, polar H1 antihistamines without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

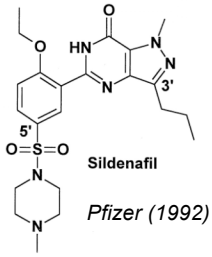



*May & Baker Ltd. (1972)*




*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)

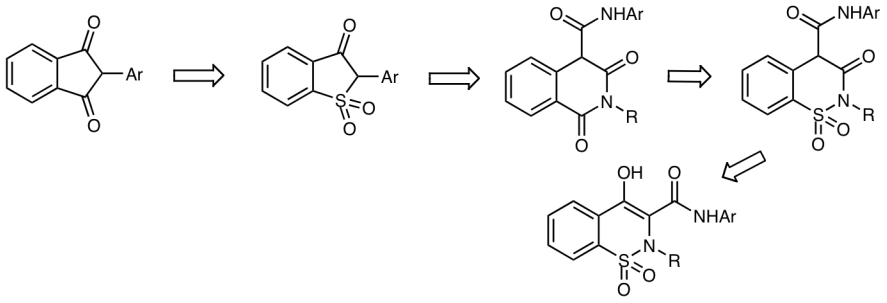
## Rational Drug Discovery - Piroxicam

• 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)

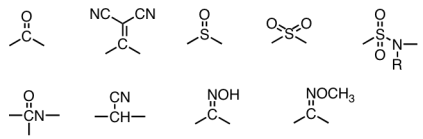
b. $-CH=$ $-N=$ (e.g., benzene, pyridine)

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

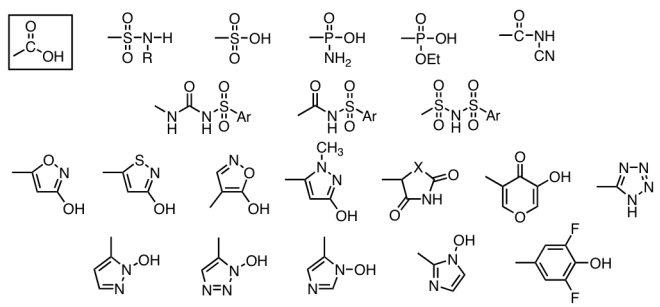
# Non-Classical Isosteres

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

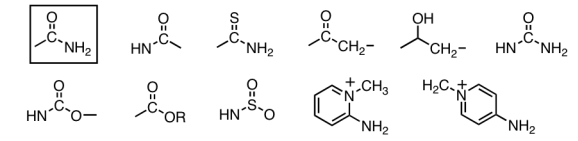
1. Carbonyl group



2. Carboxylic acid group

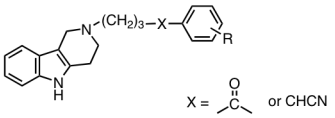


3. Amide group

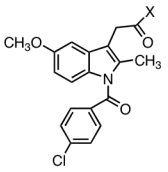


## Examples of Bioisosteric Analogues

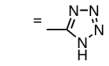
**Neuroleptics (antipsychotics)**

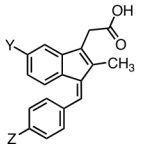



**Anti-inflammatory agents**




X = OH (indomethacin)
= NHOH






Y = $CH_3O$ Z = Cl

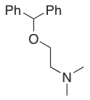
Y = F Z = $SCH_3$ (sulindac)

**Antihistamines**

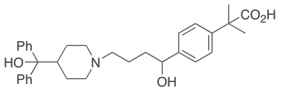
$R–X–(CH_2)_n–Y$ X = NH, O, $CH_2$

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





Diphenhydramine
(Benadryl)



Fexofenadine
(Allegra)

## Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

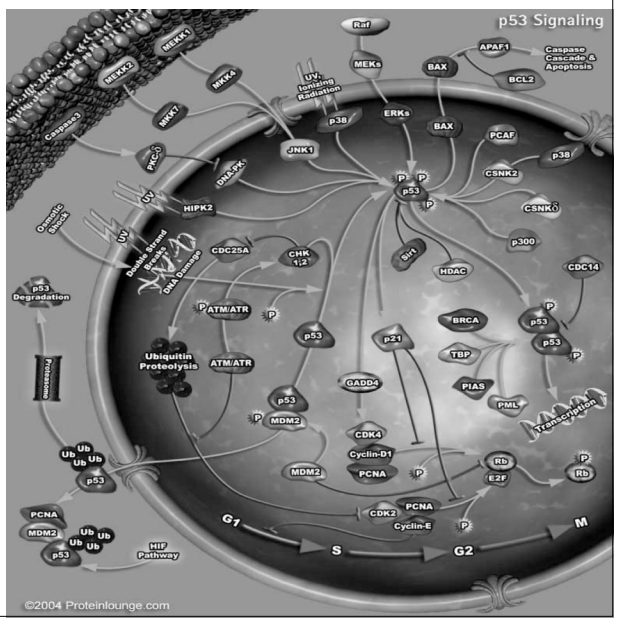
### *The pharmacological hypothesis:*

**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.**



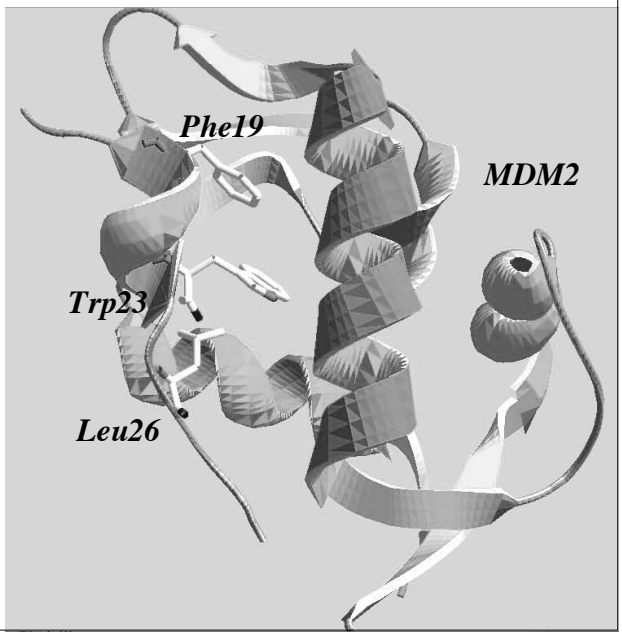
## Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

***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").**

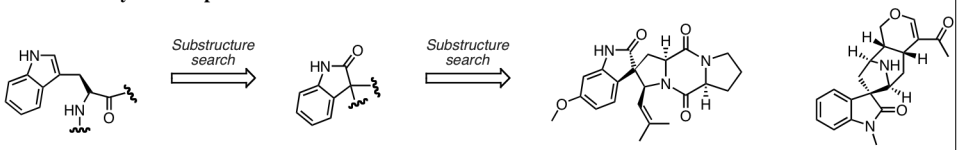
***What other chemical moieties can mimic the indole ring?***




## Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

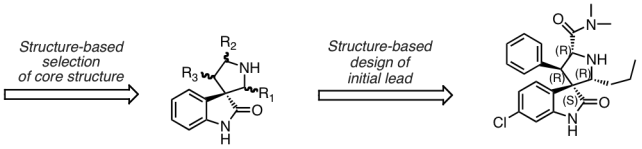
***Structure-Based Strategy:***

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.**

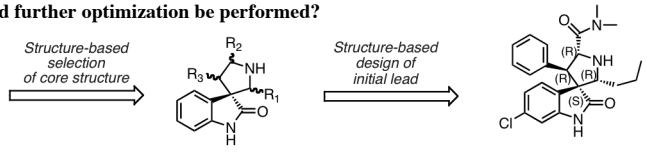



Spiro-oxindole core structure | Initial lead compound

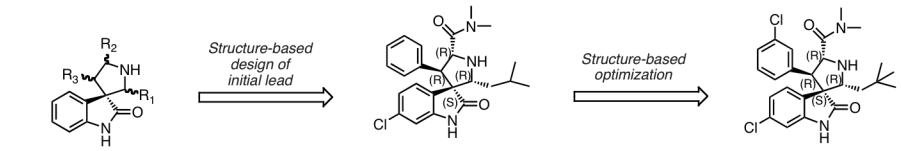
# Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

***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?**

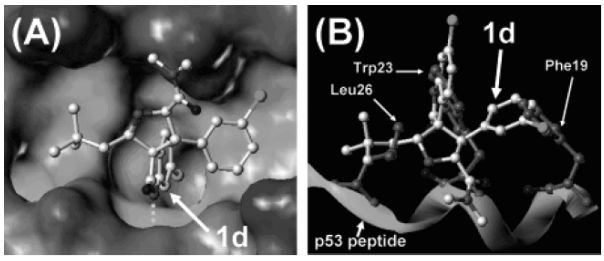



4. **Additional room in the MDM2 cavity could be exploited by larger hydrophobic groups (supported by modeling studies).**
5. **After several rounds of SAR, where the modeling was tested both by the synthesis of supposedly improved as well as inferior molecules, a new compound with $K_d$ 86 nM was identified.**

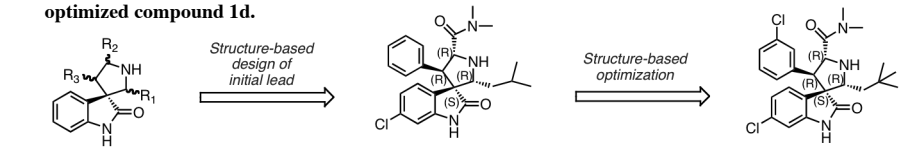



# Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

***Structure-Based Strategy:***




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

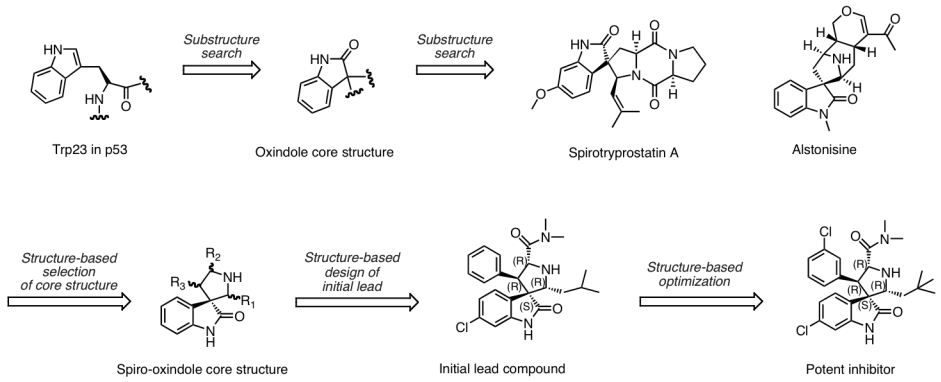



2. **What are the potential issues with MDM2 inhibitors?**

## Structure-Based Design of Potent Non-Peptide MDM2 inhibitors

***(Limited) Validation of Pharmacological Hypothesis:***

1. **MDM2 inhibitors may be equally toxic to normal cells as to cancer cells.**
2. **Evaluation of the potent inhibitor in normal human prostate epithelial cells with wild-type p53 demonstrated an IC50 value of 11 uM in growth inhibition, 13 times higher than LNCaP cancer cells.**
3. **Is this selectivity sufficient?**
4. **What comes next?**




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

Course Links

*Undergraduate Courses*

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

*Graduate Courses*

Advanced Organic Chemistry 2320; Spring 2007
Molecular Pharmacology 3360; October 2006
Pharm 5119 Drug Development 1; Spring 2006
Advanced Organic Chemistry 2320; Spring 2006
Molecular Pharmacology 3360; October 2005
Pharm 5119 Drug Development 1; Spring 2005
Molecular Pharmacology 3360; November 2004
ESPCI Paris; Heterocyclic Chemistry; September 2004
Pharm 5119 Drug Development 1; Spring 2004