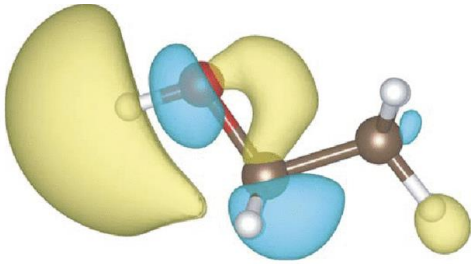
The Magic Methyl Effects

Aaron Roth

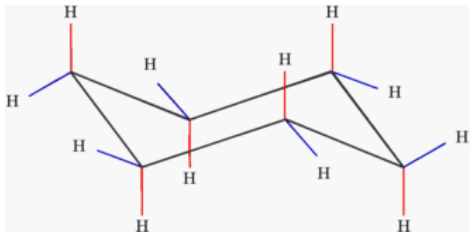
27 Feb 18

The "Magic Methyl Effects"

Stereoelectronics



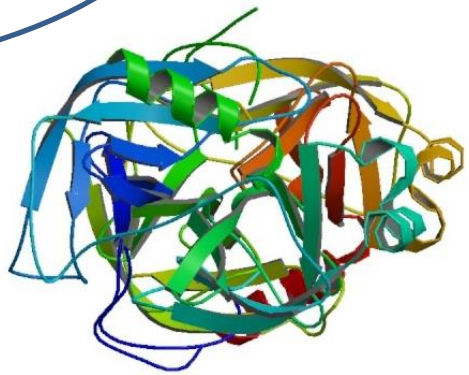
Conformation



"…where the ***seemingly mundane*** change of C-H to C-Me improves the IC_{50} value of a drug candidate more than 100-fold"

Angew. Chem. Int. Ed. ***2013****, 52, 12256*

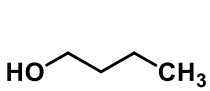
Solubility



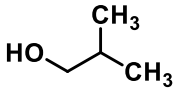
Metabolism

Effects of Methyl Groups on Solvation

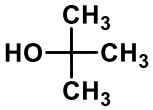
- ***Solubility in water of different alcohols at 20 C***



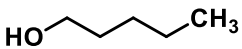
n-butanol
8.2 g/100g



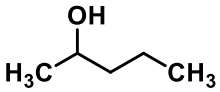
Isobutanol
5g/100g



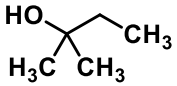
tert-butanol
miscible



n-pentanol
2.4g/100g

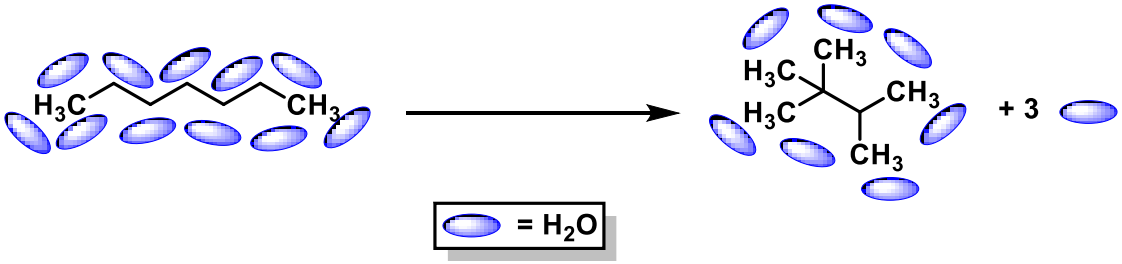


2-pentanol
4.9g/100g



neopentanol
12.2g/100g

- ***Solubilization of Branched Compounds***

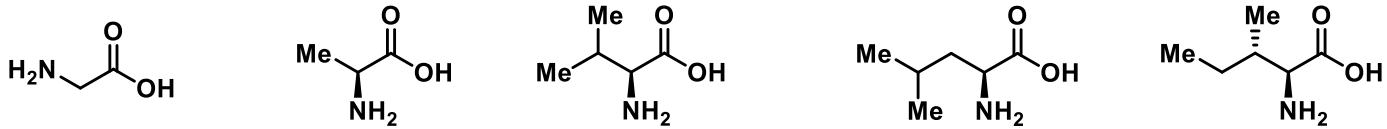


Fewer water molecules required to create an ordered solvent shell results in an entropic gain and increased solubility

Angew. Chem. Int. Ed. **2013**, *52*, 12256; *Chem. Rev.* **2011**, *111*, 5215.

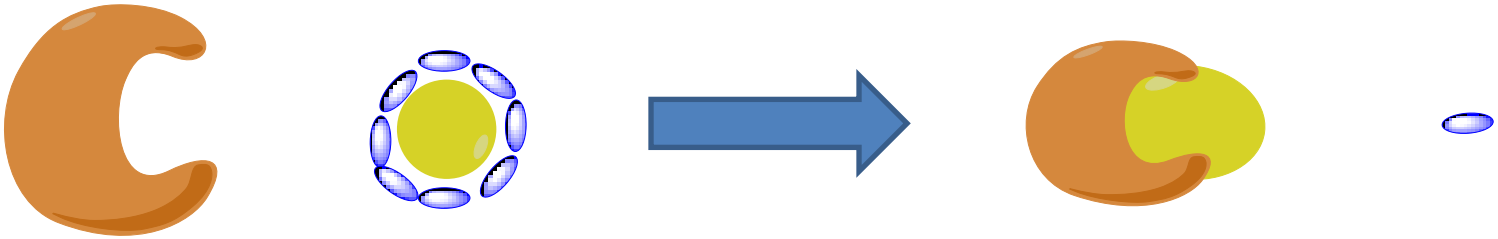
Branching in Amino Acids

Solvation energies of Amino Acid side chains to transfer between Water and Ethanol



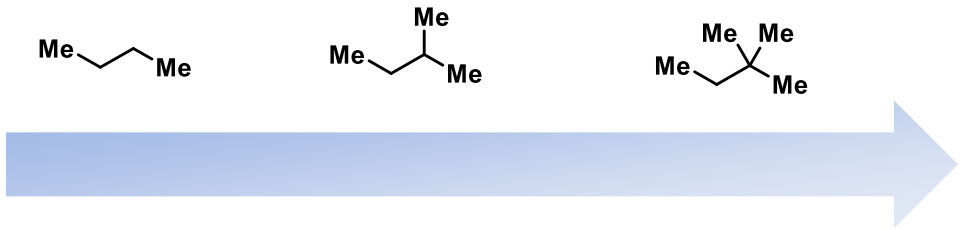
	Glycine	*Alanine*	*Valine*	*Leucine*	*Isoleucine*
$\Delta G_{transfer}$ (kcal/mol)		***-0.73***	***-1.69***	***-2.42***	***-2.79***

Ethanol is selected because it approximately represents the polarity of the inside of a protein



When a ligand binds to a protein it must become desolvated

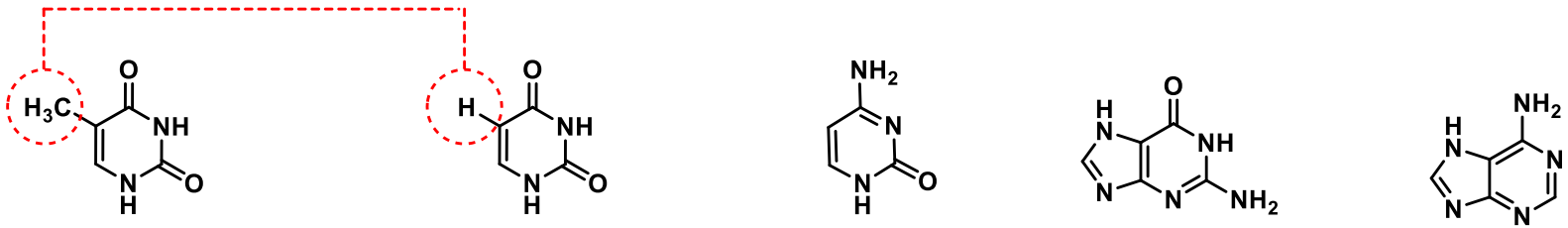
Decreasing $\Delta G_{solvation}$



- **$\Delta\Delta G_{transfer}$ by replacing a C-H with a C-Me is ~0.8 kcal/mol**
- **This translates to a theoretical 3.5-fold increase in potency**

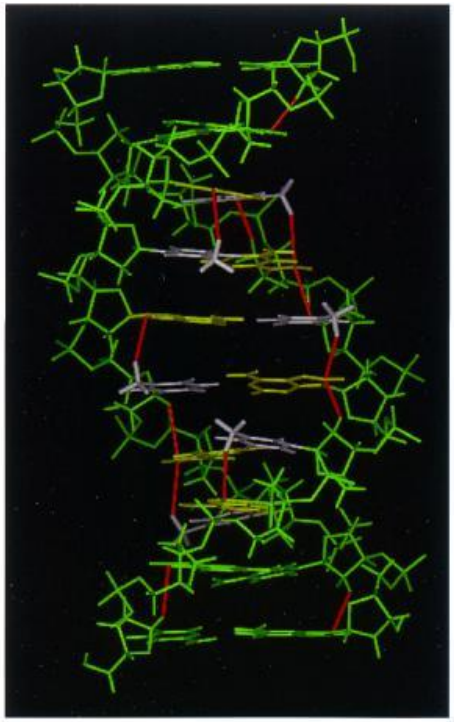
Angew. Chem. Int. Ed. **2013**, 52, 12256.

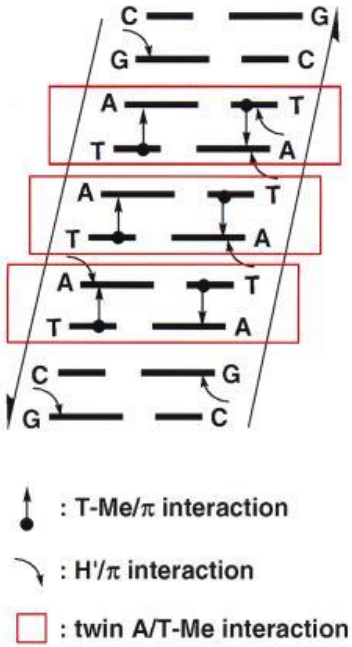
Thymine vs. Uracil



thyamine ***uracil*** ***cytosine*** ***guanine*** ***adenine***

DNA strand ***RNA strand*** ***Conserved between DNA and RNA***



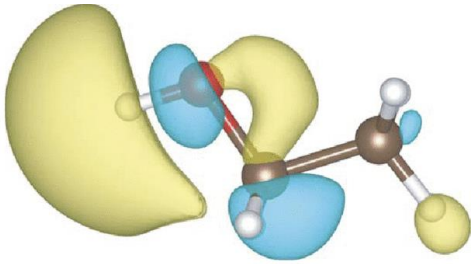


This change in DNA conformation is implicated in site recognition

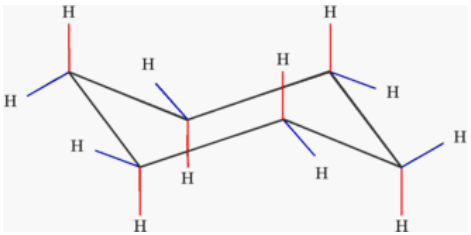
Chem. Rev. **2011**, *111*, 5215.; *Nucleic Acids Research* **2002**, *30*, 2183.

The "Magic Methyl Effects"

Stereoelectronics



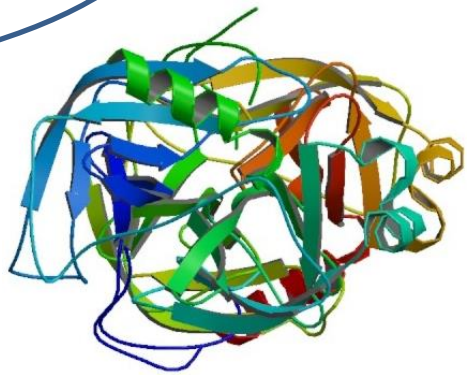
Conformation



"…where the ***seemingly mundane*** change of C-H to C-Me improves the IC_{50} value of a drug candidate more than 100-fold"

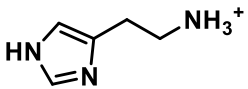
Angew. Chem. Int. Ed. ***2013****, 52, 12256*

Solubility

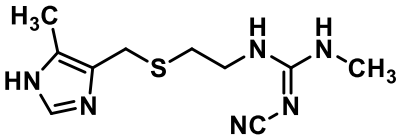


Metabolism

Case 1: Cimetidine



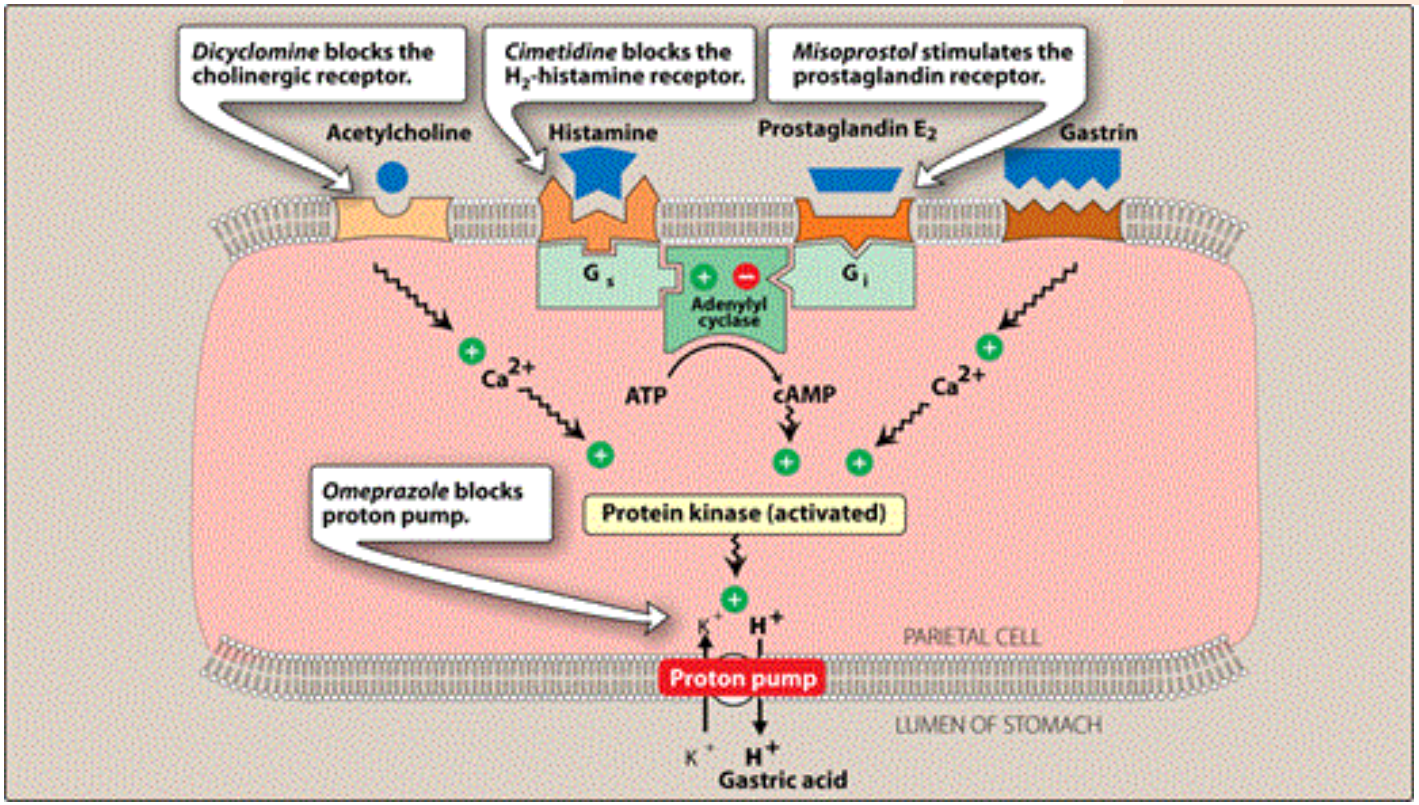
Histamine
H2-receptror Agonist



Cimetidine
H2-receptror Antagonist

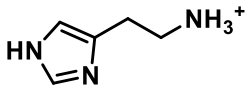
***Receptor Agonist** – A drug (ligand) that binds to a receptor site and induces a biological response.*

***Receptor Antagonist** – A drug (ligand) that binds to a receptor site and stops or reduces a biological response.*

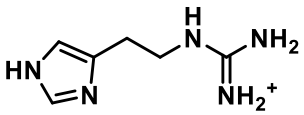


https://healtheappointments.com/chapter-28-gastrointestinal-and-antiemetic-drugs-essays/

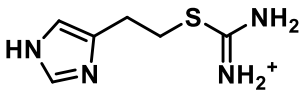
Rational and Early Leads



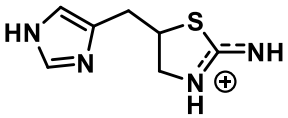
Histamine



Initial Hit

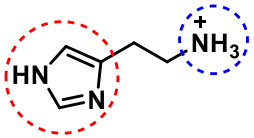


Isosteric modifications showed modest improvement

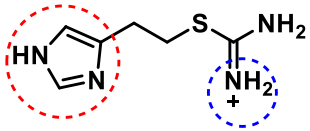


Rigid analog was less potent

- *Necessary to distinguish the receptor agonist activity of histamine from any putative drug candidate*



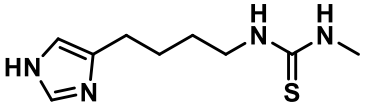
Histamine



New Lead

- *Both the natural ligand and putative inhibitor contain an imidazole core and a positively charged tail*

Necessary for recognition →



← Can be substituted

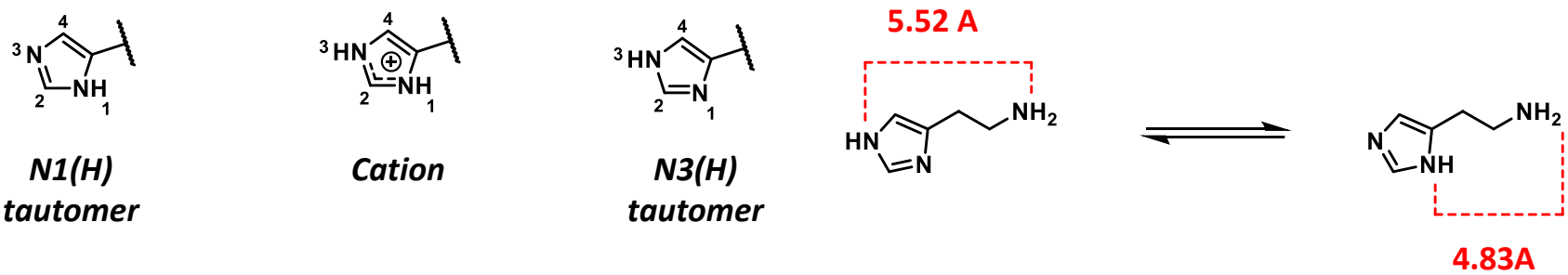
Burimamide

- *An effective antagonist but lacked sufficient oral activity in humans*

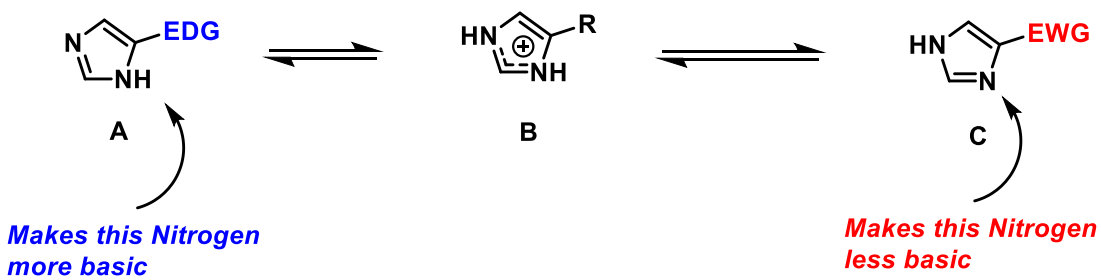
Chem. Rev. **2011**, *111*, 5215; *The Organic Chemistry of Drug Design (3rd Edition)*, R. Silverman, **2014**, 123-163.

Tautomers of Imidazole

- **In an aqueous solution there can be three different forms of imidazole present**



- *What is the predominant tautomer currently and what tautomer is necessary for inhibition?*

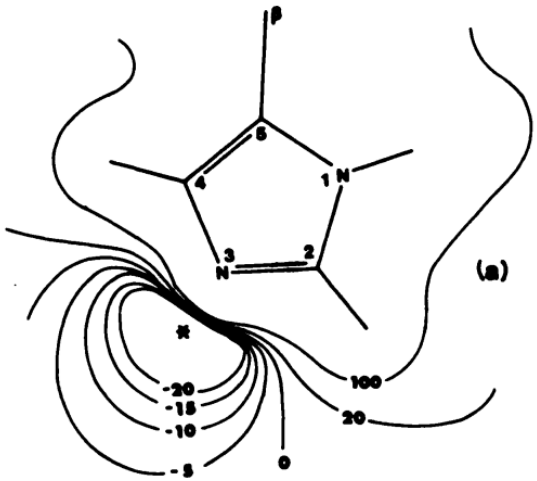


- *The population of these species cannot be measured directly*
- *Estimates can be made from the electronic influence of the side chain where an EDG favors C and an EWG favors A*

Chem. Rev. **2011**, *111*, 5215; *The Organic Chemistry of Drug Design (3rd Edition)*, R. Silverman, **2014**, 123-163.

Electrosatatic Potential of Different Imidazole Tautomers

- **Electrostatic potential minima of the N1(H) and N3(H) tautomers**



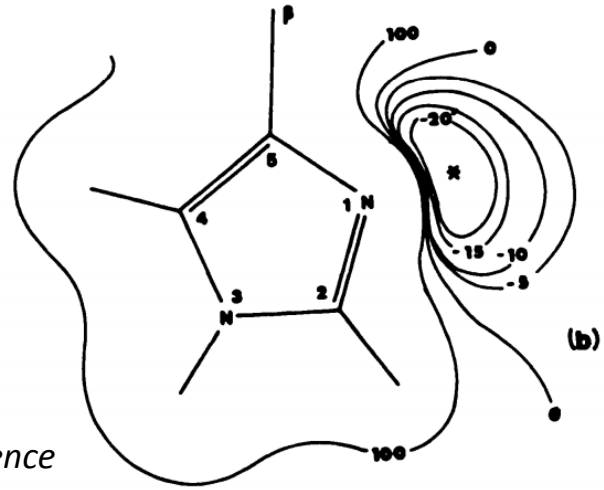
Electrostatic potential map for N(1)H

Electrostatic potential minima

Species	N(1)H tautomer minimum near N(3)	N(3)H tautomer minimum near N(1)
	kcal/mole	*kcal/mole*
Cation	−37.9	−28.2
Neutral (free base)	−96.5	−98.7

High electrostatic potential → relative absence of electrons

Low electrostatic potential → relative abundance of electrons

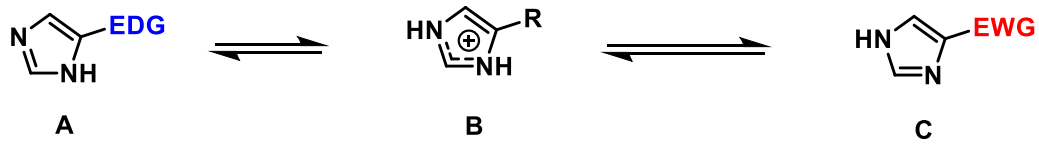


Electrostatic potential map for N(3)H

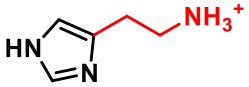
- **The calculated electrostatic potential indicates which site is more likely to be reactive depending on the protonation state of the side chain**

Molecular Pharmacology, **1976**, *12*, 738.

Electronic Effect of the Side Chain

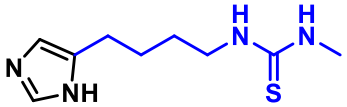


	R_1	R_2	pK_a	*Tautomer*
Histamine	H	$-CH_2CH_2NH_3^+$	5.90	*C*
Imidazole	H	$-H$	6.80	
Burimamide	H	$-(CH_2)_4NHCSNHCH_3$	7.25	*A*
4(5)-Methylimidazole	H	$-CH_3$	7.40	*A*



Histamine
pKa = 5.90
3 mol% cationic
Favor tautomer C

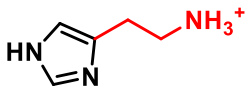
Thus, although both histamine and burimamide are mono-substituted imidazoles, the structural similarity is misleading in that the predominant species of the respective imidazole rings are chemically different. If the active form of the an-



Burimamide
pKa = 7.25
40 mol% cationic
Favor tautomer A

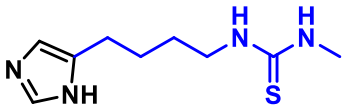
Nature, **1974**, *248*, 65.

Modifying the Electronic Properties



Histamine
pKa = 5.90
3 mol% cationic
Favor tautomer C

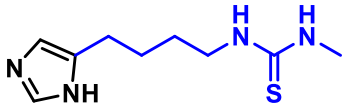
What are the necessary modifications to change the primary form of the imidazole in Burimamide to resemble that of Histamine?



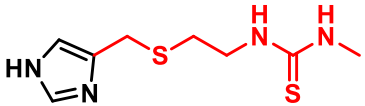
Burimamide
pKa = 7.25
40 mol% cationic
Favor tautomer A

Two strategies were employed -

Strategy 1 – installation of an electron withdrawing side chain



Burimamide



Thiaburmamide

~ 3x as potent

	R_1	R_2	pK_a
Histamine	H	$-CH_2CH_2NH_3^+$	5.90
Thiaburimamide	H	$-CH_2SCH_2CH_2NHCSNHCH_3$	6.25
Imidazole	H	$-H$	6.80
Burimamide	H	$-(CH_2)_4NHCSNHCH_3$	7.25
4(5)-Methylimidazole	H	$-CH_3$	7.40

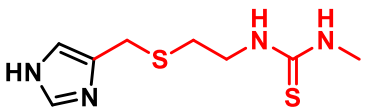
Δ 0.35

Δ 1.0

Nature, **1974**, *248*, 65.

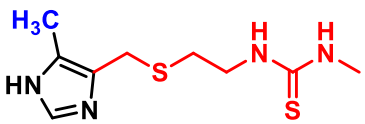
Modifying the Electronic Properties

Strategy 2 – installation of an electron donating substituent



Thiaburmamide

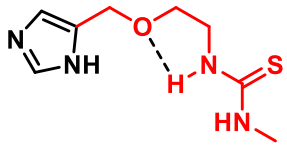
The introduction of an electron donating group should serve to increase the basicity of the adjacent nitrogen



Metiamide

	R_1	R_2	pK_a
Histamine	H	$-CH_2CH_2NH_3^+$	5.90
Thiaburimamide	H	$-CH_2SCH_2CH_2NHCSNHCH_3$	6.25
Metiamide	CH_3	$-CH_2SCH_2CH_2NHCSNHCH_3$	6.80
Imidazole	H	$-H$	6.80
Burimamide	H	$-(CH_2)_4NHCSNHCH_3$	7.25
4(5)-Methylimidazole	H	$-CH_3$	7.40
Methylburimamide	CH_3	$-(CH_2)_4NHCSNHCH_3$	7.80

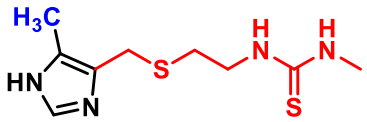
*While adding the methyl substituent resulted in approximately 20% of Metiamide to exist in the cationic form, it was **8-9 times more efficacious than Thiaburmamide**.*



Oxaburimide was less potent than thiaburmamide likely due to an intramolecular hydrogen bond

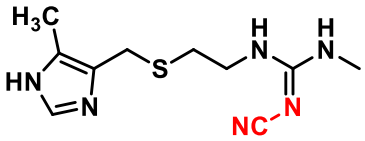
Nature, **1974**, *248*, 65.

Finishing Touches



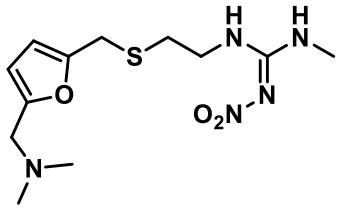
Metiamide

Minimizing toxic Side-effects

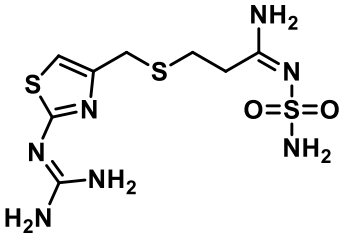


Cimetidine

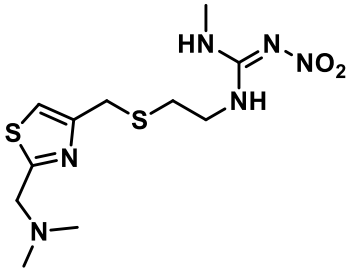
- ***Cimetidine was the first drug to profit more than $1 billion dollars a year making it the first blockbuster drug***



Ranitidine



Famotidine

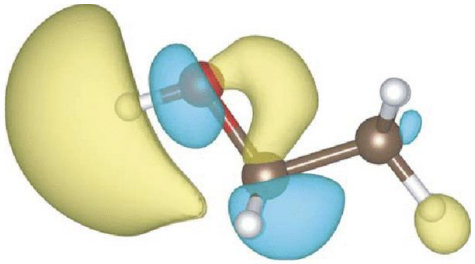


Nizatidine

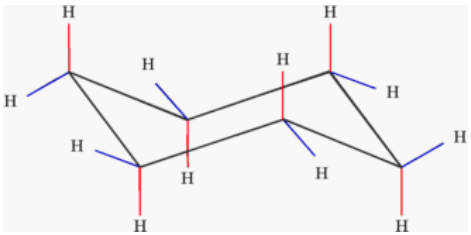
Chem. Rev. **2011**, *111*, 5215; *The Organic Chemistry of Drug Design (3rd Edition)*, R. Silverman, **2014**, 123-163.

The "Magic Methyl Effects"

Stereoelectronics



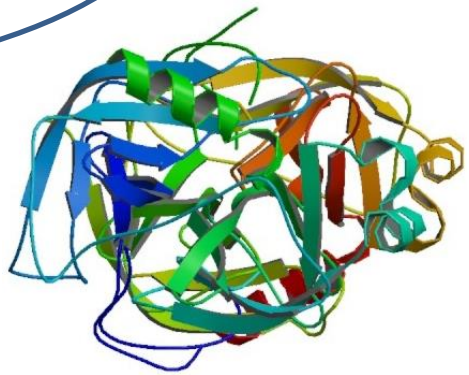
Conformation



"…where the ***seemingly mundane*** change of C-H to C-Me improves the IC_{50} value of a drug candidate more than 100-fold"

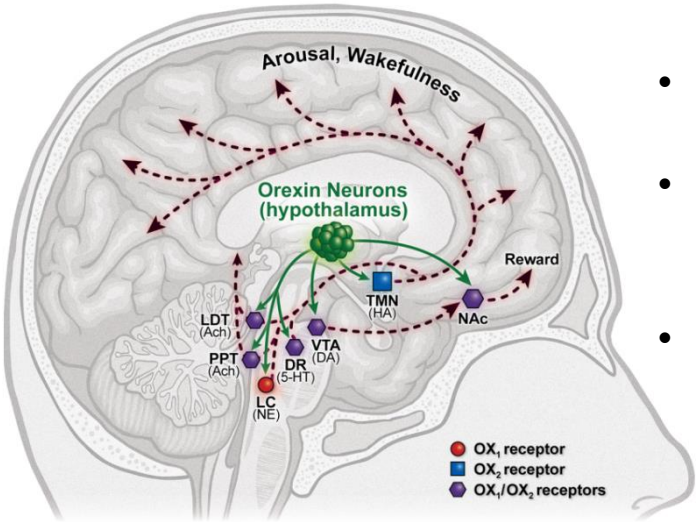
Angew. Chem. Int. Ed. ***2013****, 52, 12256*

Solubility



Metabolism

Case 2: Filorexant (MK-6096)

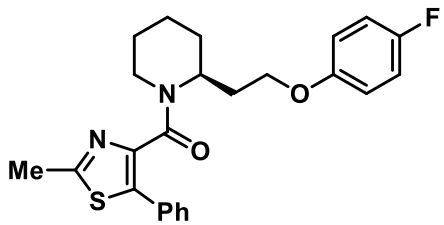


Location of Orexin Receptors

- ~50% of adults do not get a good night sleep during the week
- Absenteeism and lost productivity caused by improper sleep is estimated to cost approximately **$40 billion annually**.
- Indirect costs from accidents caused by lack of rest are estimated to cost **$92 – 107 billion dollars a year**.

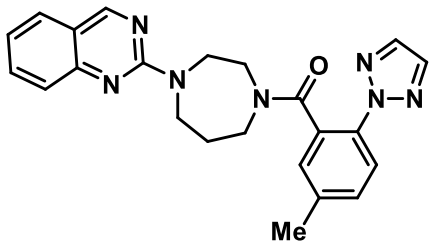
Angew. Chem. Int. Ed. **2013**, 52, 12256.; *J. Med. Chem***, 2016***, 59,* 504.

Conformational Analysis of 1,4-diazepane Orexin Receptor Anatagonist

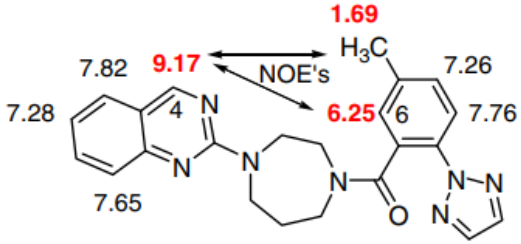


Initial hit from Merck (~2005)

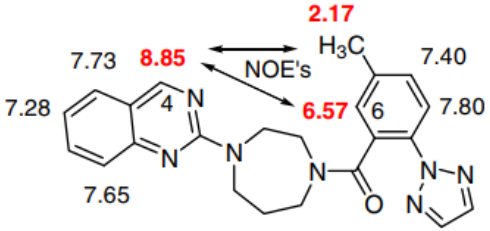
Hit to Lead optimization



During the synthesis of the above Diazepane, 1H-NMR indicated a number of different conformations



Significant upfield shifts from expected positions due to shielding

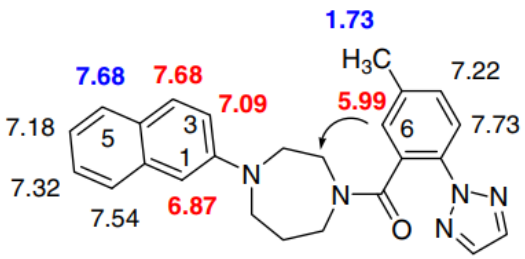


Slightly less dramatic upfield shifts suggest weaker π-stacking interactions

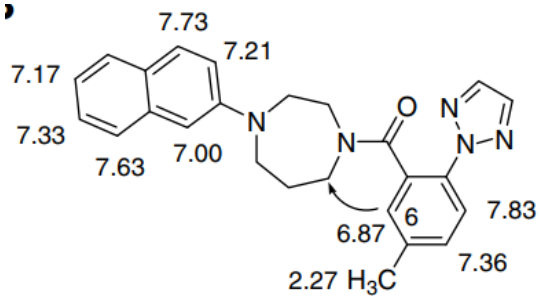
Bioorg. Med. Chem. Lett., **2009**, *19*, 2997.; *J. Med. Chem.* **2016**, *59*, 504.

The Driving Forces Behind This Unusual Conformation

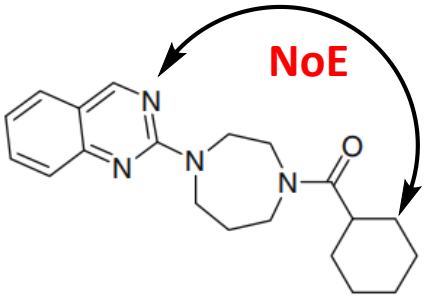
- **Napthyl derivatives were synthesized to simplify the analysis**



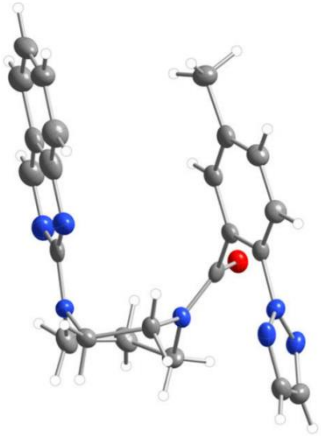
Significant upfield shifts from expected positions due to shielding and strong NoE's



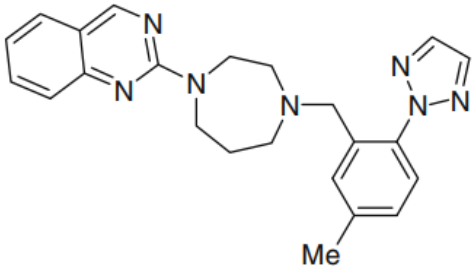
No significant NoE's were observed indicating greater separation between the aromatic moieties



- ***Exists in a 1:1:1:1 ratio of conformers***



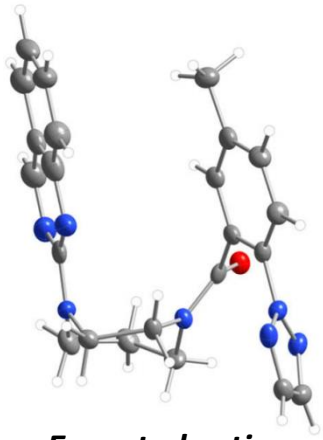
X-ray structure showing the Diazapane in a twist boat conformation



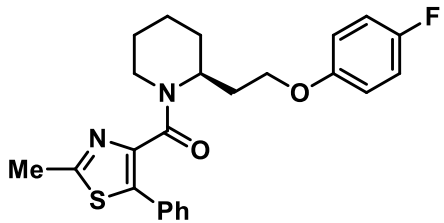
- ***No evidence of π-stacking***

Bioorg. Med. Chem. Lett., **2009**, *19*, 2997.; *J. Med. Chem.* **2016**, *59*, 504.

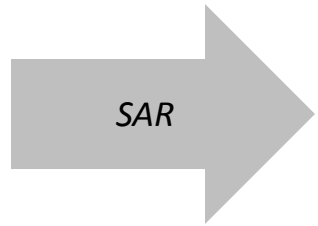
The Beginnings of MK-6096

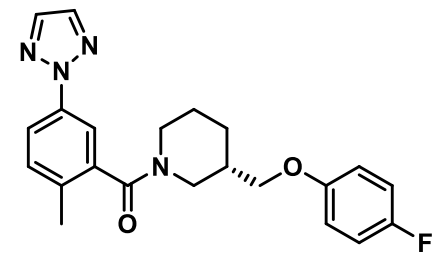


Expected active conformer of 1,4-diazapane

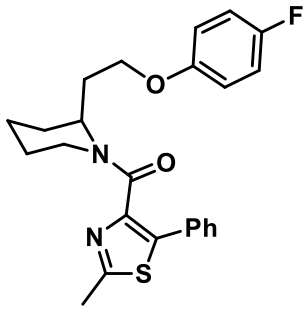


Initial Lead for MK-6096
OX1R Ki = 0.06 nM
OX1R Ki = 1.0 nM

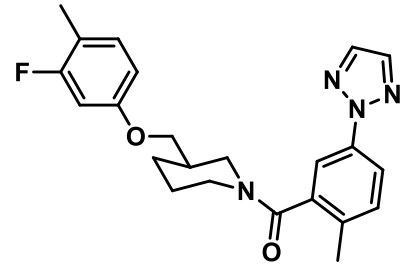




Substituent transposition
OX1R Ki = 23 nM
OX1R Ki = 44 nM



Adopts a pseudo axial orientation to minimize eclipsing interactions

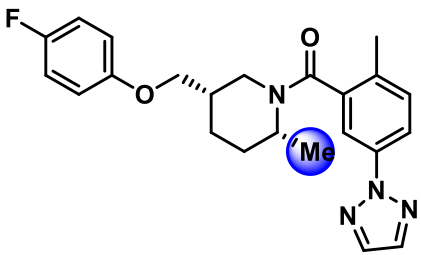


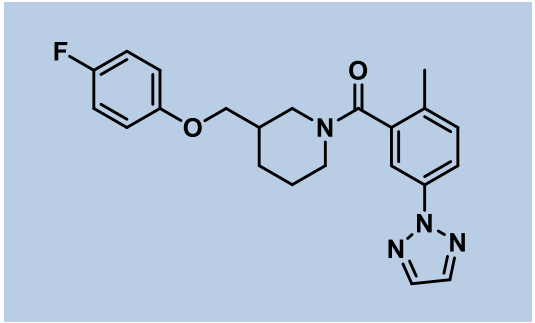
Lowest energy conformation does not adopt the requisite U-shaped conformer

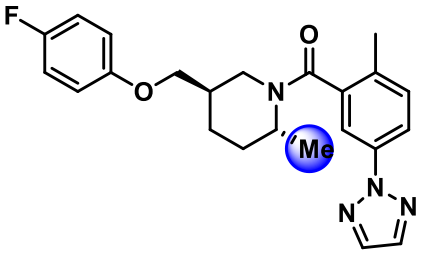
Can a simple modification be made to force the lead compound into the correct conformation?

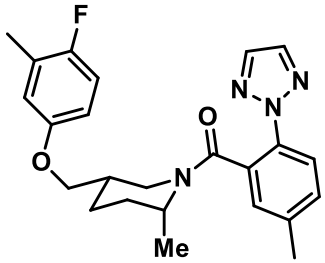
Bioorg. Med. Chem. Lett., **2009**, *19*, 2997.; *J. Med. Chem.* **2016**, *59*, 504.

Employing A Strategic Methyl Group To Induce A Conformational Change

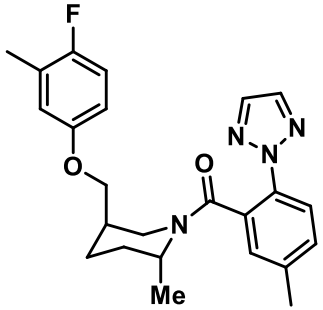








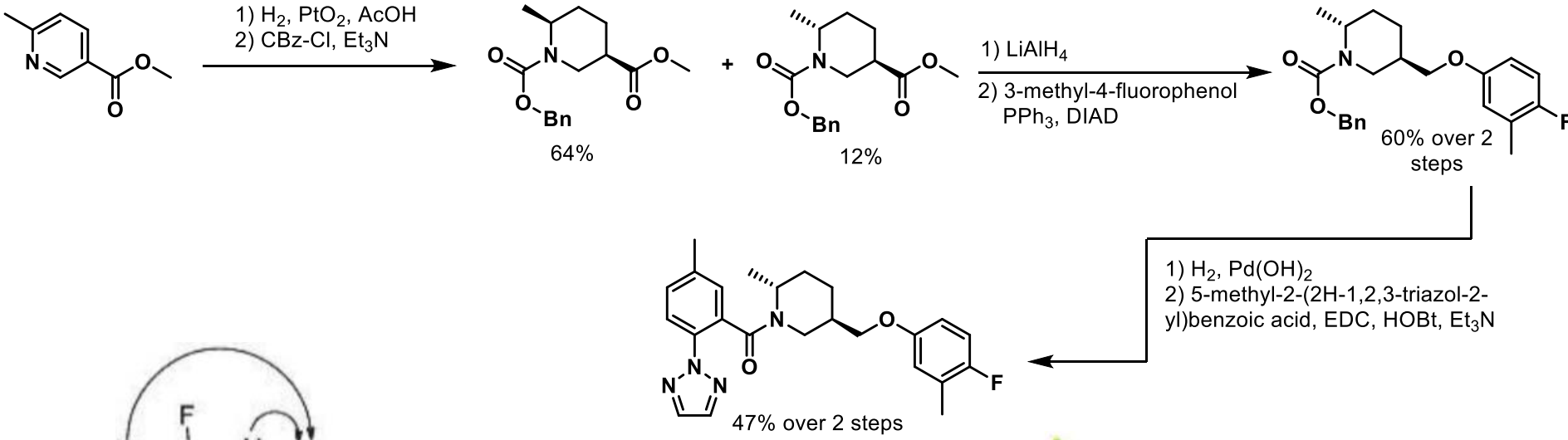
Anticipated low energy conformation of the Cis isomer

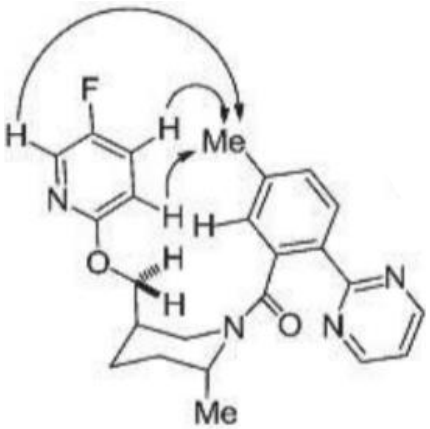


Anticipated low energy conformation of the Trans isomer

Bioorg. Med. Chem. Lett., **2009**, *19*, 2997.; *J. Med. Chem.* **2016**, *59*, 504.

Synthesis of the Cis and Trans Isomers



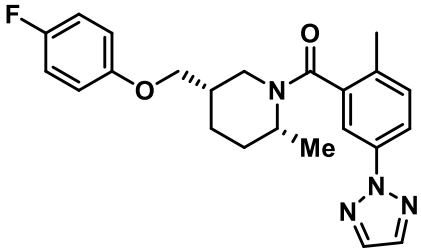


NMR analysis indicated equilibrating isomers in 74:16:7:3 ratio

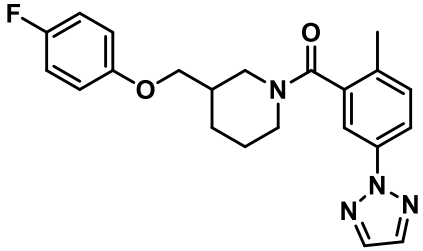
The solid state structure shows the same diaxial orientation but a different orientation of the pyridine ring.

ChemMedChem, **2012**, *7*, 415.; *J. Med. Chem.* **2016**, *59*, 504.

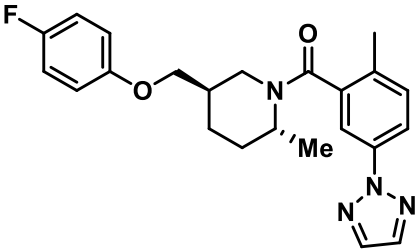
Biological Activities of Both Isomers



Cis, racemic
OX1R = 34 nM
OX2R = 22 nM

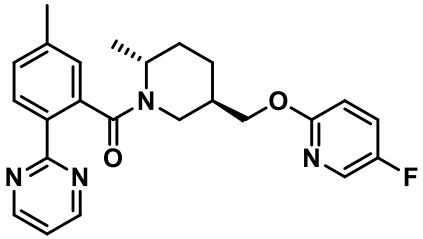


Desmethyl, racemic
OX1R = 6 nM
OX2R = 9 nM

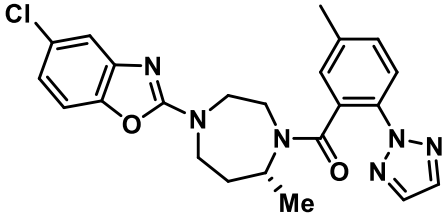


Trans, racemic
OX1R = 0.3 nM
OX2R = 0.1 nM

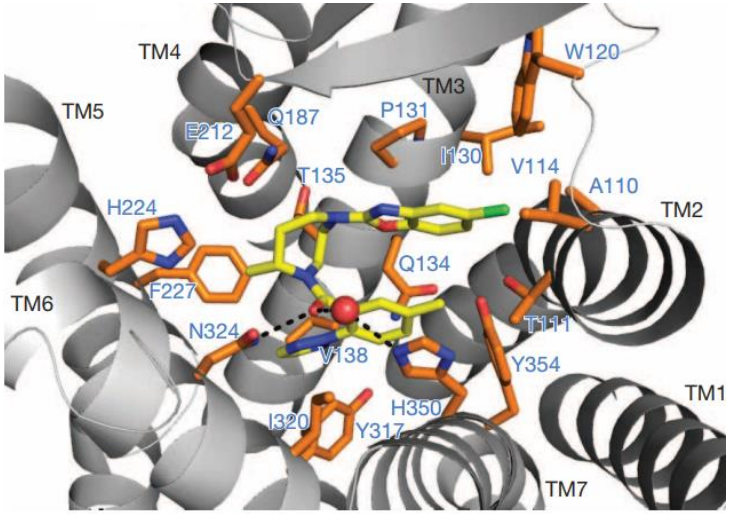
MK-6096 today…



Entered Phase II clinical trials but seems to have been discontinued…



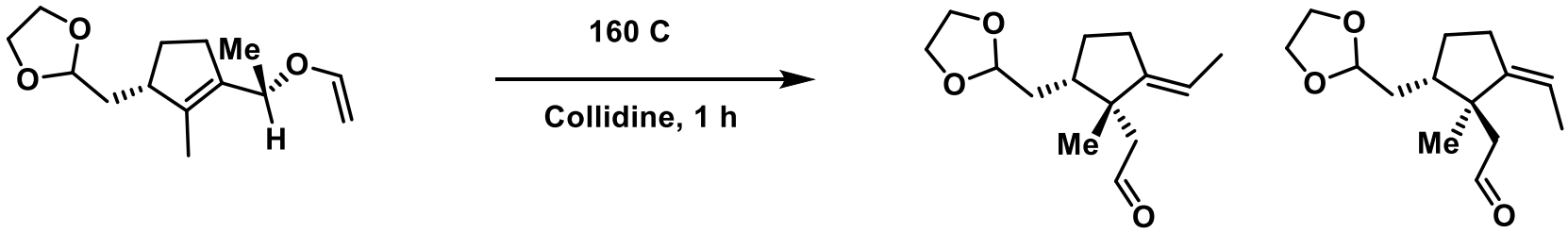
Crystal structure of Suvorexant in OX2R



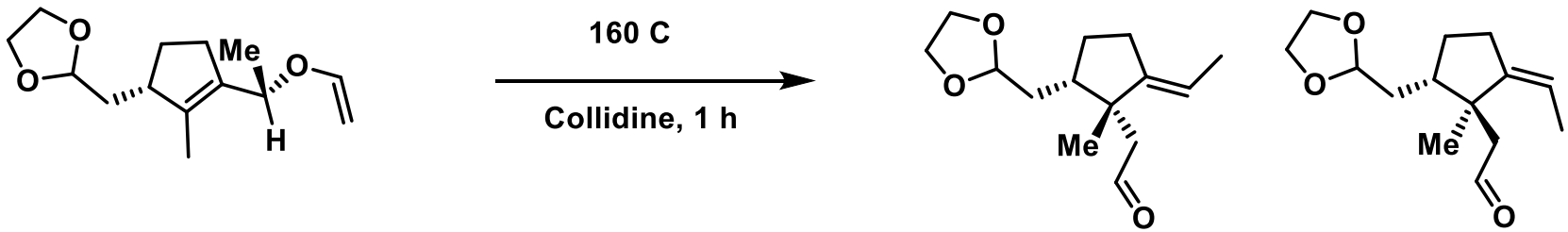
ChemMedChem, **2012***, 7*, 415.; *J. Med. Chem.* **2016***, 59*, 504; *Nature,* **2015**, *519*, 247.

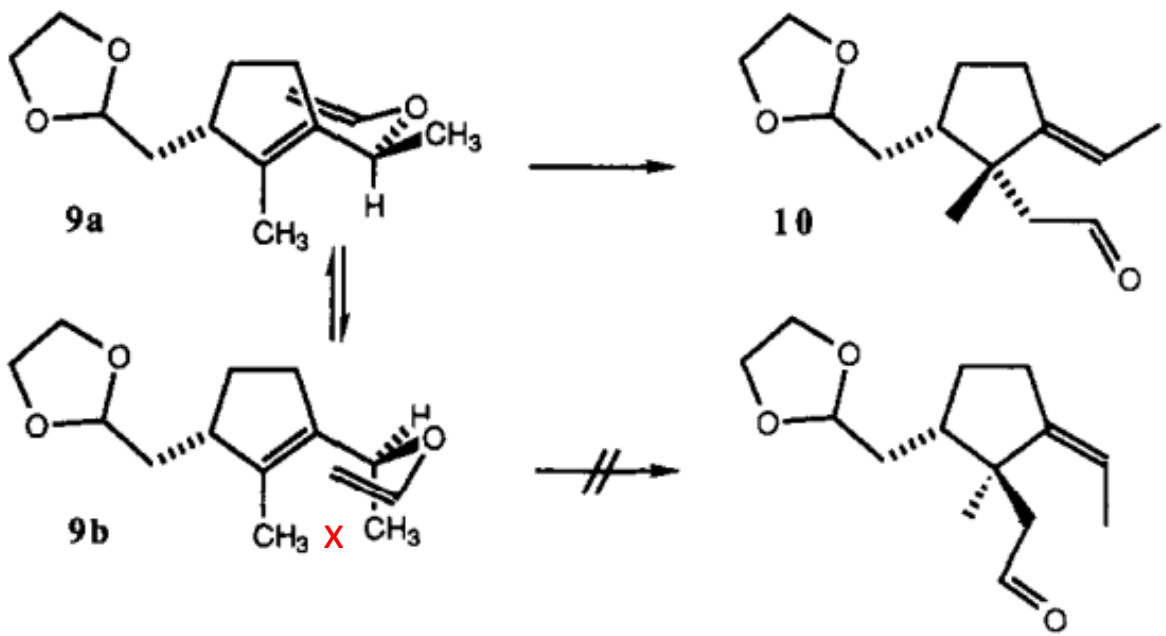
Group Problem

For the reaction below, which is the major product. Why?



Group Problem Solutions

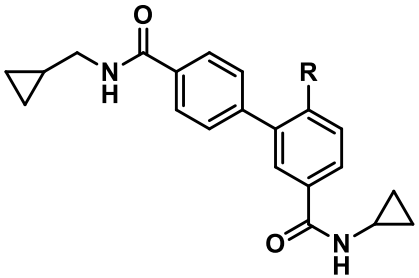




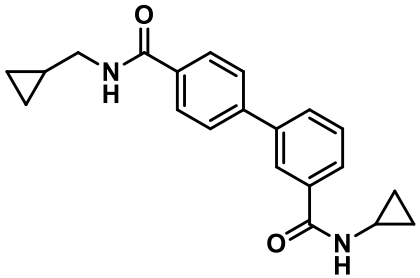
Chem. Rev., **1989**, *89*, 1841

Case 3: Biphenyl amine p38 Kinase Inhibitors

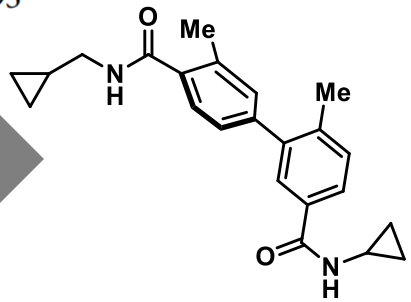
Putative p38 Kinase Inhibitor



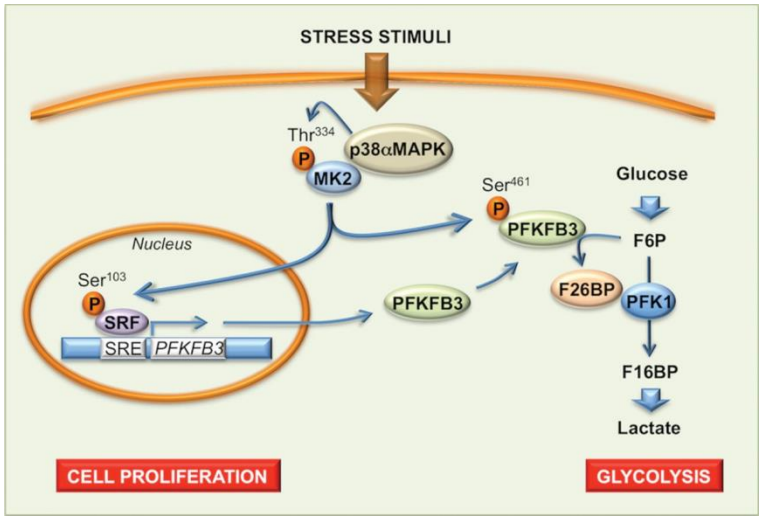
R	K_i^a (nM)	$\Delta\Delta G_{exp}$ (kcal/mol)
H	>2500	0.0
Me	12	<−3.16
F	460	<−1.00
Cl	25	<−2.77
OMe	520	<−0.93

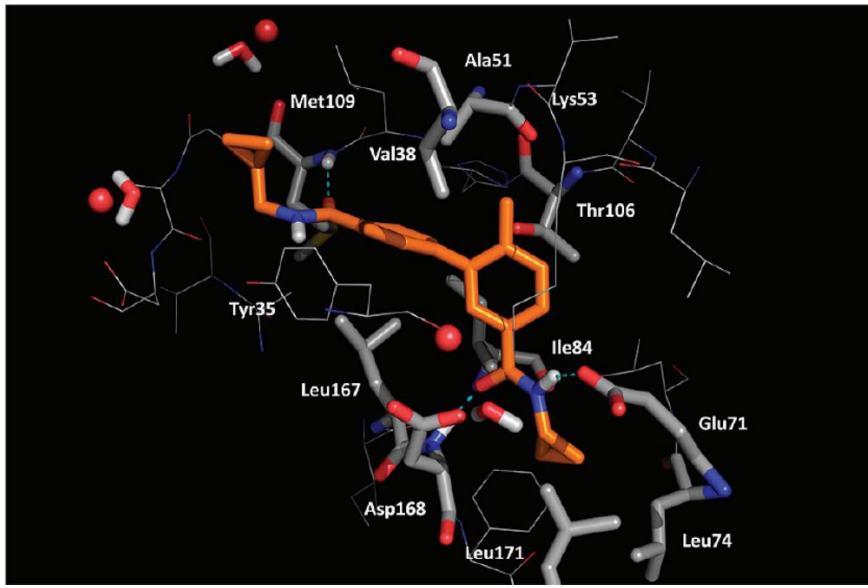


Low rotational barrier



High rotational barrier

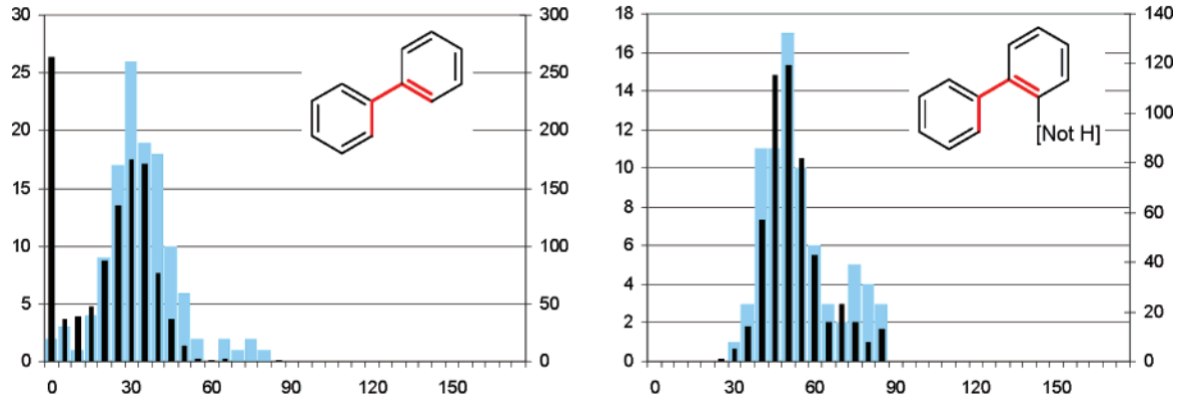




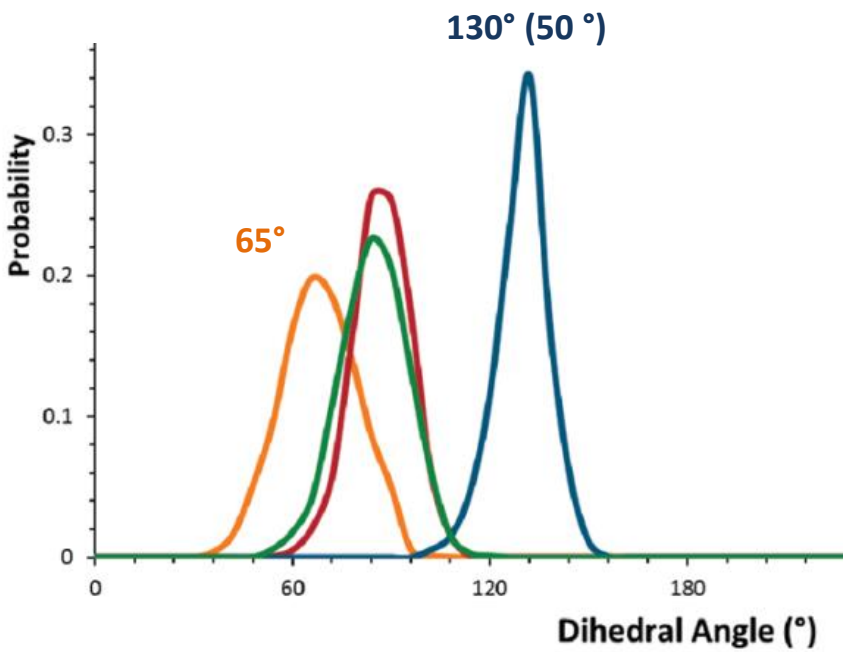
J. Med. Chem. **2012**, *55*, 4489; *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4428.

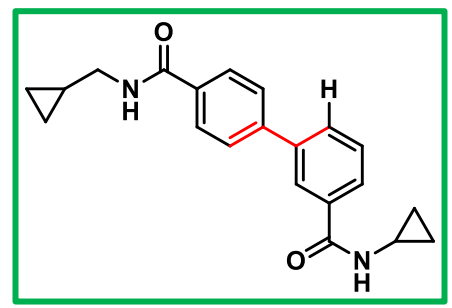
Effect of the Methyl Group on the Dihedral Angle

Dihedral angles from crystal structures in *Protein Database* and *Cambridge Structural Database*

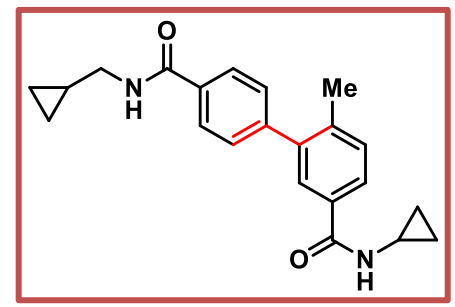


- *Introduction of an ortho substituent generally increases the dihedral angle ~ 15 degrees*

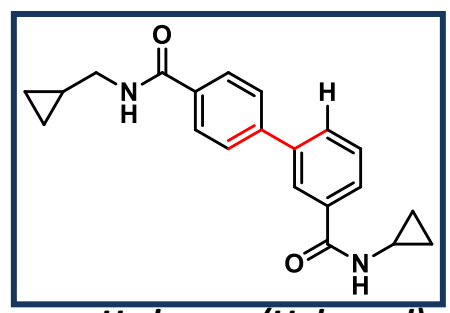




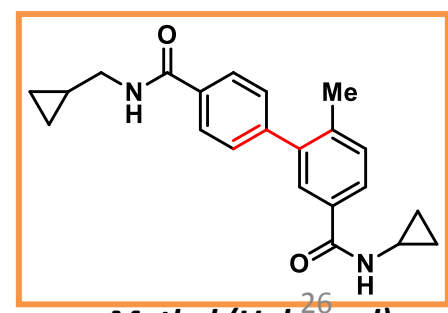
Hydrogen (Bound)



Methyl (Bound)



Hydrogen (Unbound)

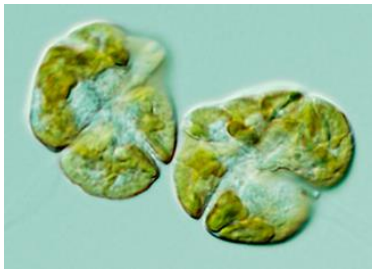


Methyl (Unbound)

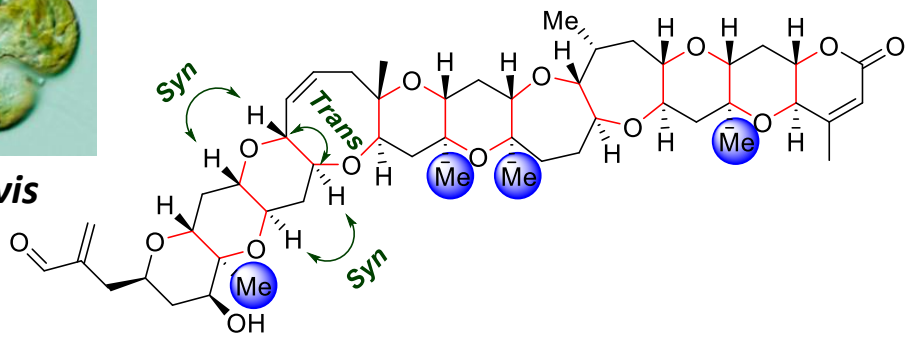
J. Chem. Inf. Model., **2008**, *48*, 1.; *J. Med. Chem.* **2012**, *55*, 4489

Case 4: Synthesis of Brevetoxin A – A Not So Magic Methyl

Red Tide Event



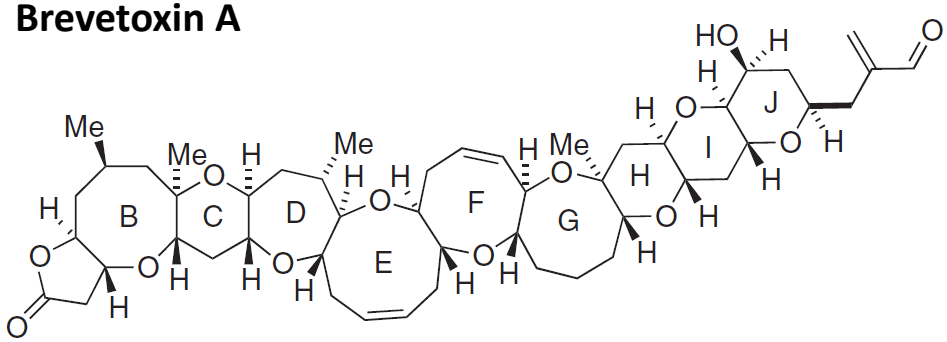
Karenia brevis

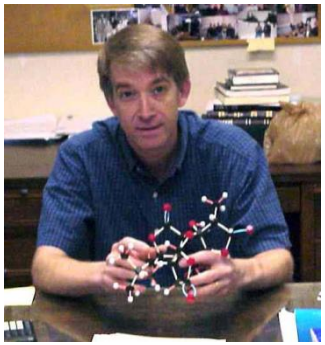


Brevetoxin B

Causative agent in Neurotoxic Shellfish Poisoning

Brevetoxin A





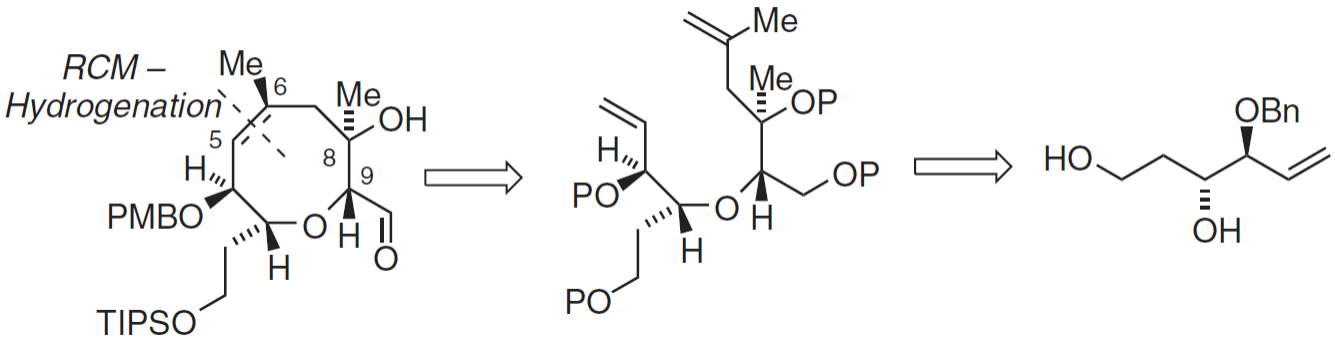
Michael Crimmins
UNC Chapel Hill

KC Nicolaou
Rice University

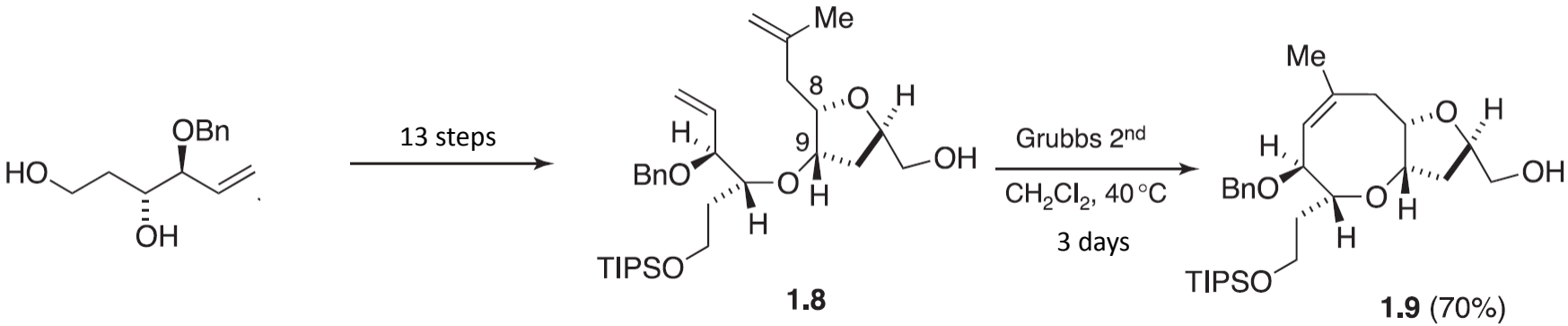
- *Total synthesis of Brevetoxin A have been completed by Nicolaou (1998) and Crimmins (2009)*

Strategy to Access the E-Ring of Brevetoxin A

Crimmins E-Ring Strategy



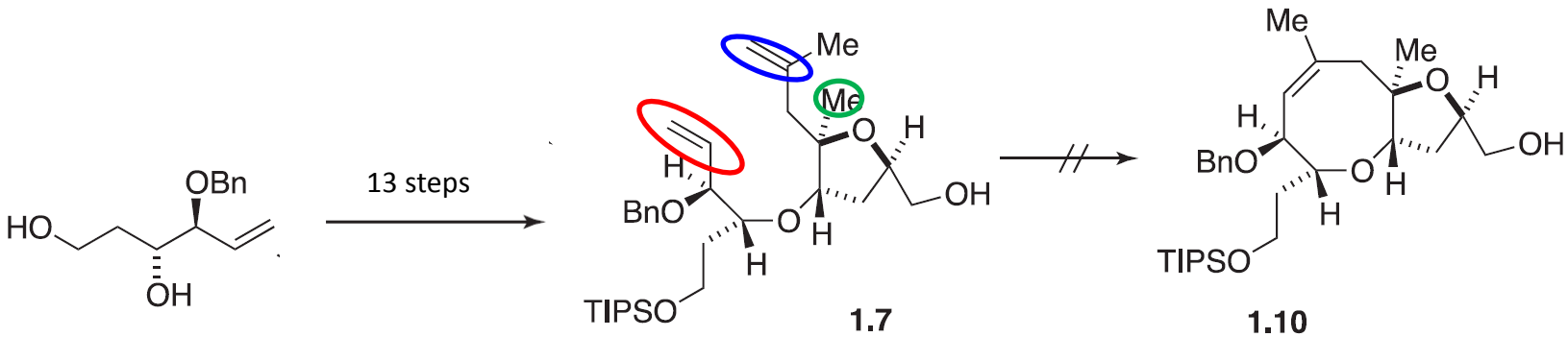
- *Both hydrogenation and RCM are robust reactions and no problems should be anticipated*



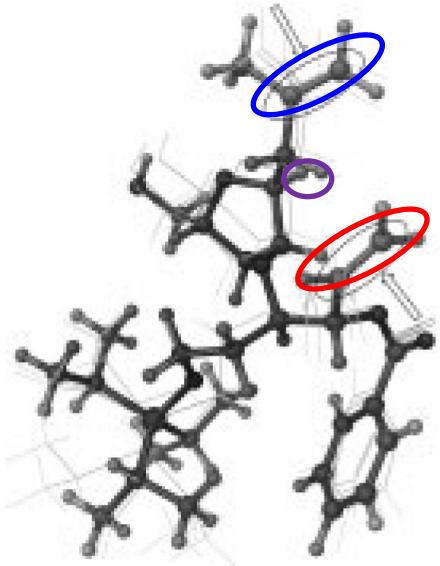
- *In the Desmethyl (C-8) system, the ring closing metathesis proceeded in good yield.*

Chem. Eur. J. **2009**, *15*, 9223; More Dead Ends and Detours: En Route to Successful Total Synthesis, *Wiley*, **2013,** 1-13.

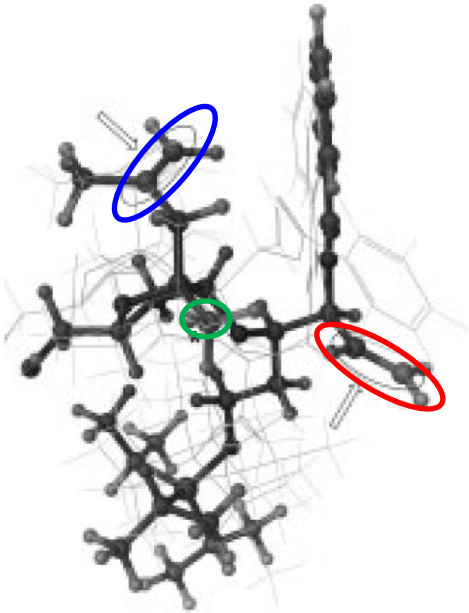
Computational Investigation into the failure of the RCM



- *When the C-8 methyl group was introduced the ring closing metathesis failed completely*

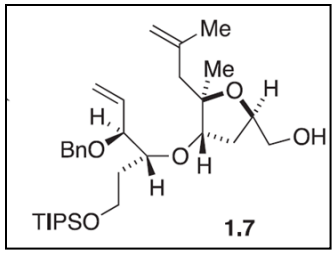


Flexible structure with methyl groups in close proximity



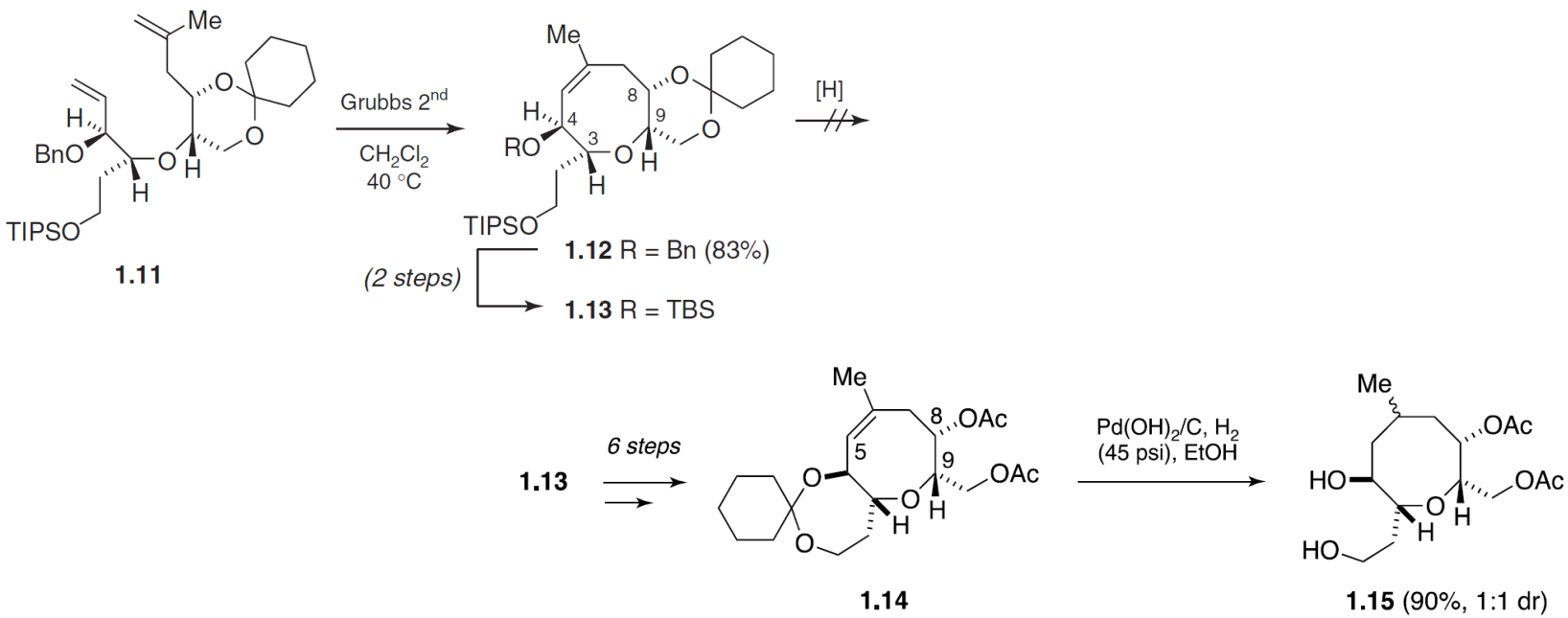
Rigid structure with methyl groups further apart

Chem. Eur. J. **2009**, *15*, 9223; More Dead Ends and Detours: En Route to Successful Total Synthesis, *Wiley*, **2013,** 1-13.



Alternative Solutions after RCM Failure

Alternative Strategy – Late Stage Methylation

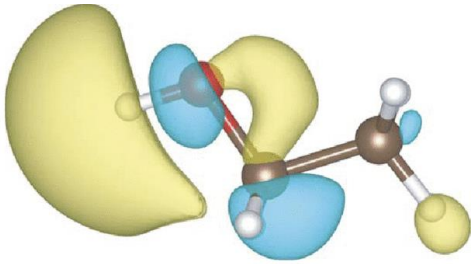


- *As a result of the unselective hydrogenation this route was ultimately abandoned*

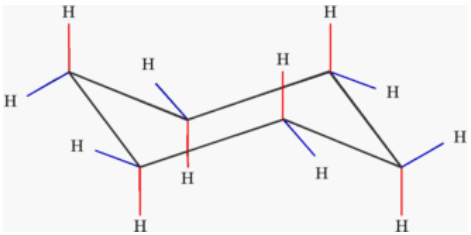
Chem. Eur. J. **2009***, 15*, 9223; More Dead Ends and Detours: En Route to Successful Total Synthesis, *Wiley*, **2013,** 1-13.

The "Magic Methyl Effects"

Stereoelectronics



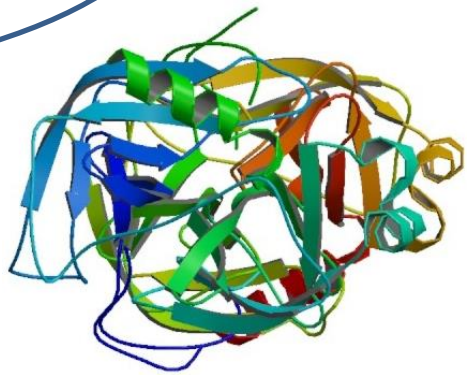
Conformation



"…where the ***seemingly mundane*** change of C-H to C-Me improves the IC_{50} value of a drug candidate more than 100-fold"

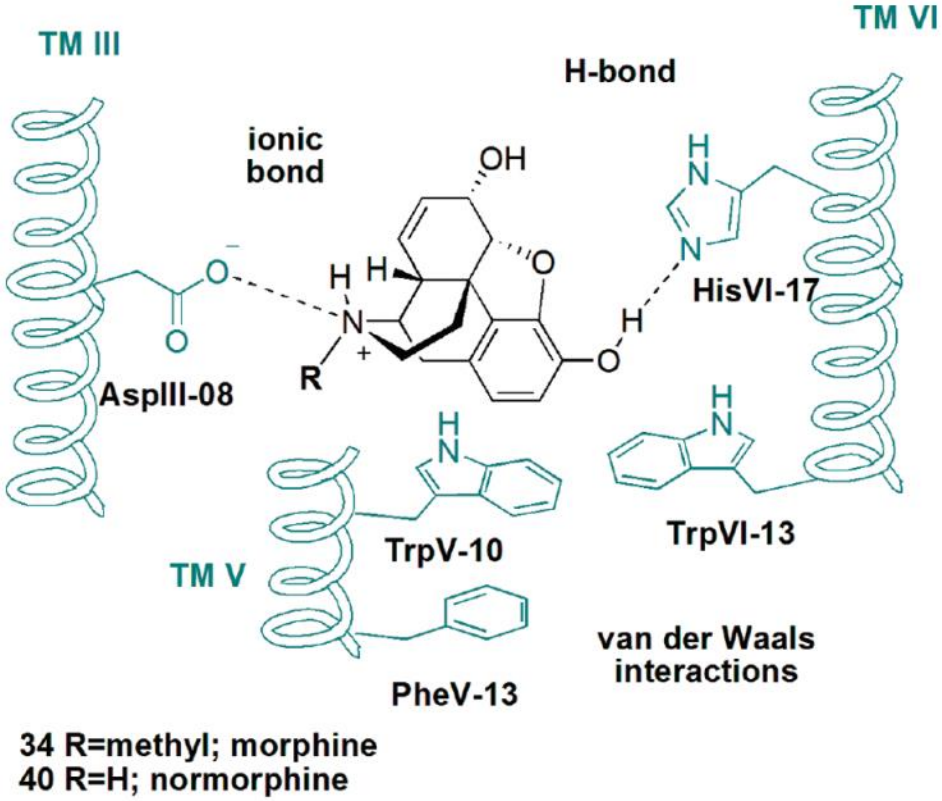
Angew. Chem. Int. Ed. ***2013****, 52, 12256*

Solubility



Metabolism

Lipophilicity and the Methyl Group

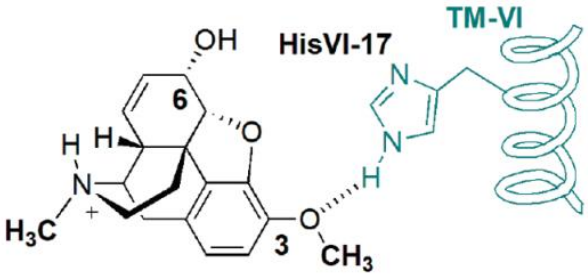


- *Normorphine is 6-fold less potent in vivo than Morphine despite preserving the necessary binding contacts in the opioid receptor active site*

- *Because the secondary nitrogen is more polar, Normorphine is less able to pass through the BBB*

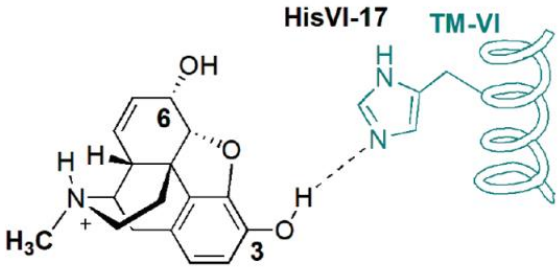
Chem. Rev. **2011**, *111*, 5215.

Methylated Morphine Analogs – Effect on Efficacy

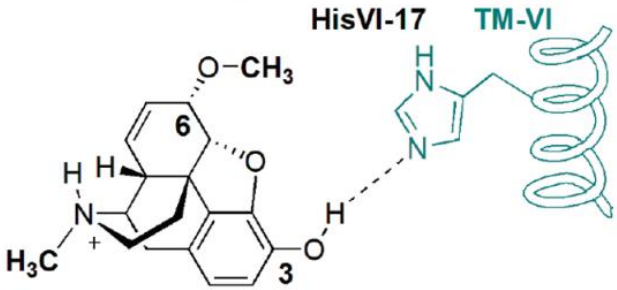


41
codeine

200 fold less potent than morphine in vitro



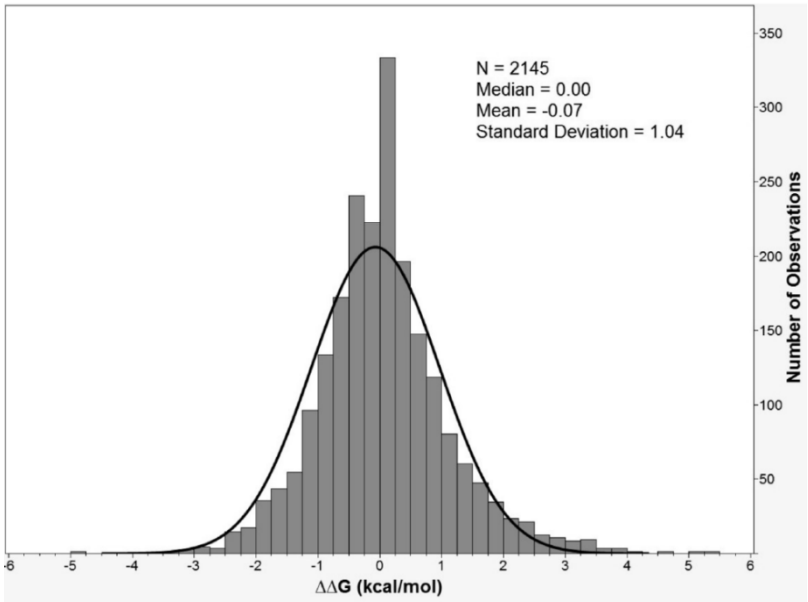
34
morphine



42
heterocodeine

Twice as potent as morphine in vitro

- *Strategic placement of a methyl group is crucial for increases in activity*

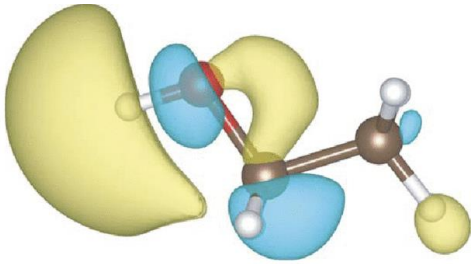


A methyl group installed randomly is just as likely to increase binding affinity as it is to decrease it

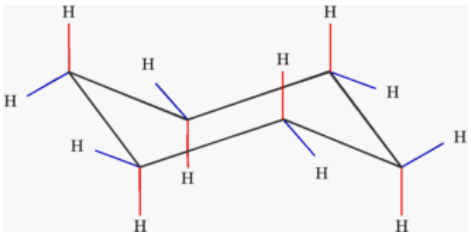
Chem. Rev. **2011**, *111*, 5215.; *J. Med. Chem.* **2012**, *55*, 4489

The "Magic Methyl Effects"

Stereoelectronics



Conformation



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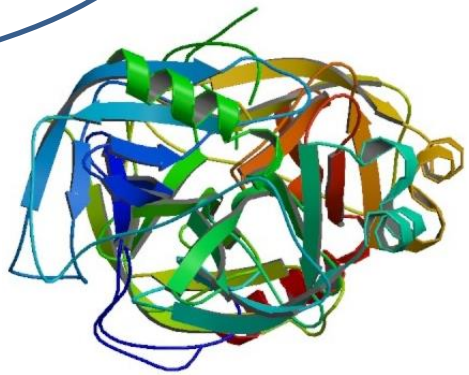
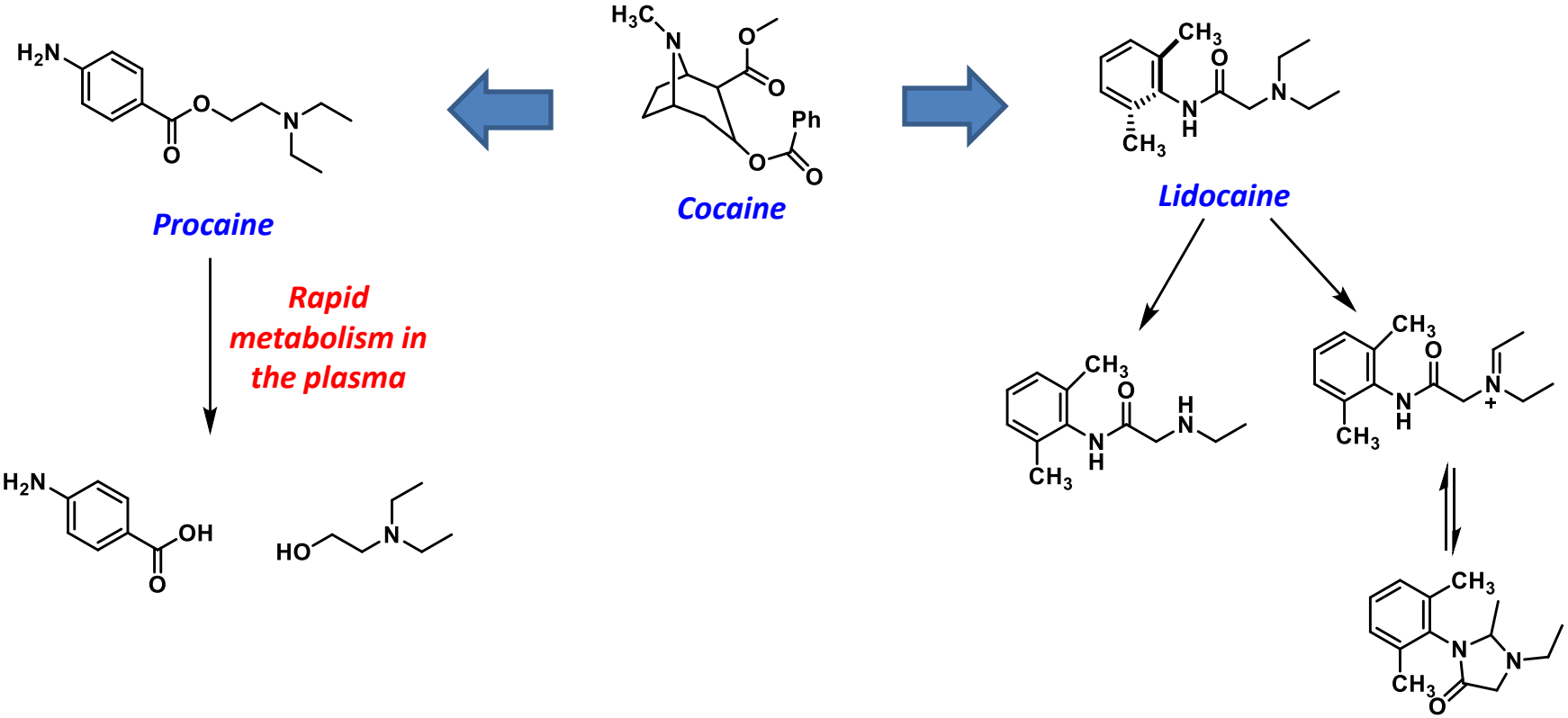
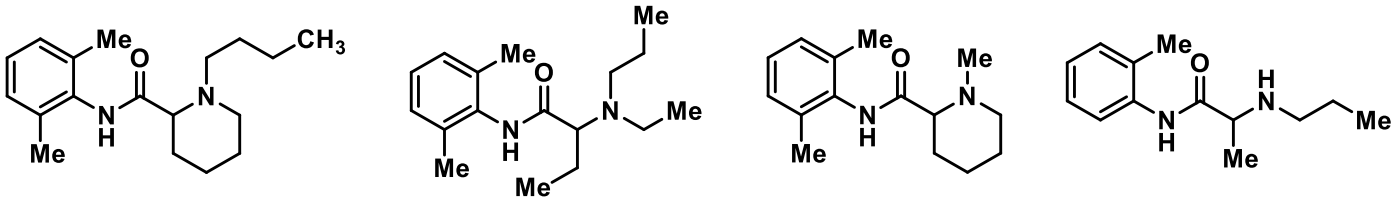


Metabolism

Using the Methyl Group to Control Metabolism - Lidocaine



Conformational twist induced by the ortho methyl groups create steric protection against metabolism

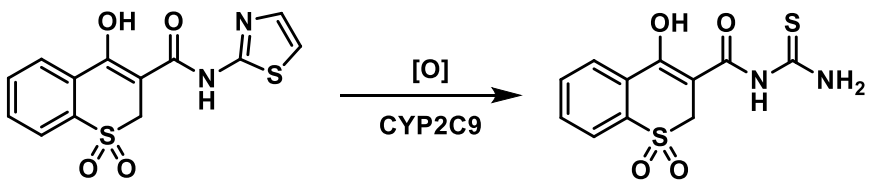


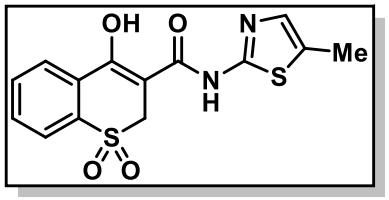
Bupivacaine ***Etidocaine*** ***Mepivacaine*** ***Prilocaine***

Chem. Rev. **2011**, *111*, 5215

Oxidation Sites on Drug Molecules

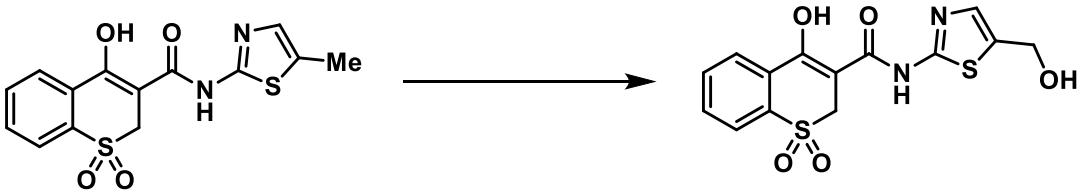
- **Heterocycle preservation**





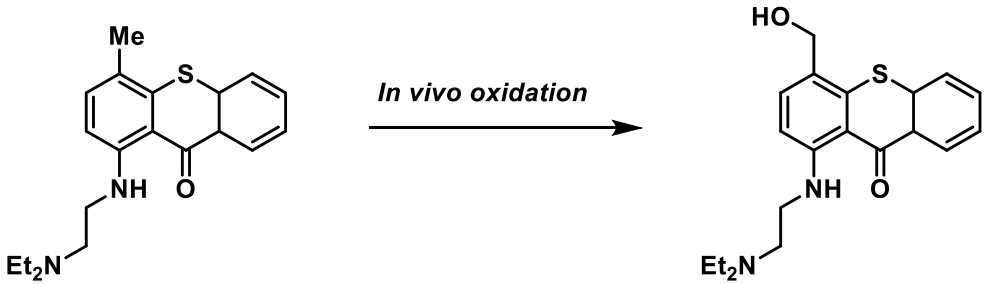
Sudoxicam ***Meloxicam***

New Oxidation Pathway



Increase potency through increased half life

- **Methyl groups can function as prodrugs**



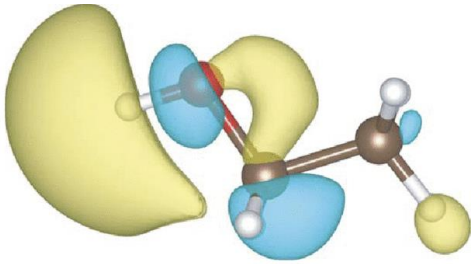
Hycanthone

Increase potency through increased bioavailability

Chem. Rev. **2011**, *111*, 5215

The "Magic Methyl Effects"

Stereoelectronics



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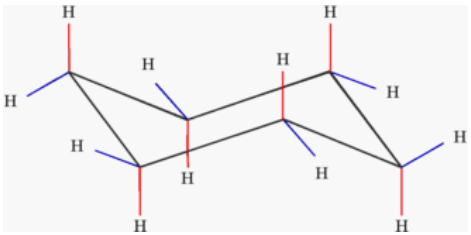

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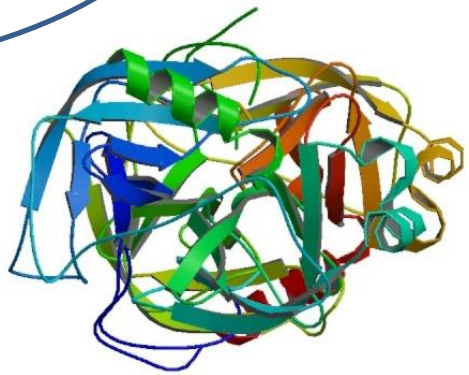
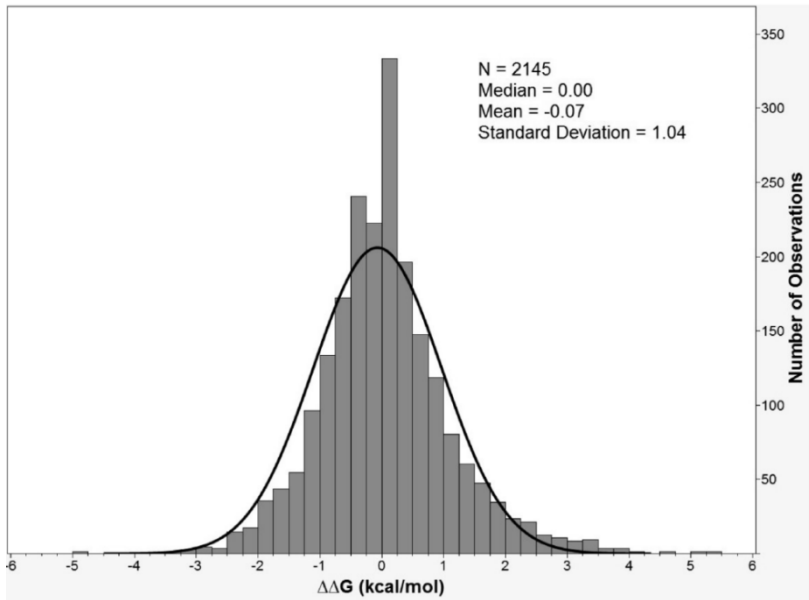


Metabolism

Concluding Thoughts…



Statistically, adding a methyl is just as likely to increase activity as it is to decrease it

Main Resources

CHEMICAL REVIEWS

REVIEW

pubs.acs.org/CR

The Methylation Effect in Medicinal Chemistry

Eliezer J. Barreiro,[*,†,‡,§] Arthur E. Kümmerle,[∥,†,§] and Carlos A. M. Fraga[†,‡,§]

Profound Methyl Effects in Drug Discovery and a Call for New C–H Methylation Reactions

*Heike Schönherr and Tim Cernak**