Natural Products-Inspired Drug Discovery

Three Short Stories

Youssef Bennani, PhD

Vice-President Research & Development
Vertex Pharmaceuticals-Canada

The State of Affairs in Drug Discovery

Drug Discovery Today • Volume 175 • February 2012 REVIEWS

ELSEVIER

Culture of innovation-ASAP (iASAP): Ask powerful questions; Seek the outliers; Accept defeat; Populate astutely.

Reviews • KEYNOTE REVIEW

Drug discovery in the next decade: innovation needed ASAP☆

Youssef L. Bennani

Vertex Pharmaceuticals, 130 Waverly St., Cambridge, MA 02139, USA

Pharmaceutical companies must find a better way to increase their output of truly new drugs for the benefit of patients and for their business survival. Here, I highlight a general perspective from within pharmaceutical research as it pertains to research advances in chemistry, biology, pharmacology, pharmacokinetics and toxicology that, if well integrated, stands to put the industry on a productive path. In addition, I provide a complementary perspective on the corporate culture aspect of innovation. I also introduce a new concept, termed 'innovation ASAP' (iASAP; asking powerful questions, seeking the outliers, accepting defeat and populating astutely) and provide support for it using examples of several successful drugs.

Youssef L. Bennani obtained his undergraduate (BSc) and graduate degrees (MSc and PhD) in chemistry from the Université de Montréal, under the guidance of Professor Stephen Hanessian, and conducted post-doctoral studies at The Scripps Research Institute with Professor K. Barry Sharpless. He also holds an MBA from Lake Forest Graduate School of Management. In 1993, he joined the pharmaceutical industry, as a researcher, working for Ligand Pharmaceuticals, Abbott Laboratories and Athersys Inc. He currently serves as Vice President of Drug Innovation at Vertex Pharmaceuticals, heading both the discovery chemistry and pharmacokinetics departments. He has led programs in immunology, oncology, neurology, metabolic and infectious diseases, resulting in numerous preclinical and clinical molecular entities; and has published over 170 papers and patents in the field of drug discovery.

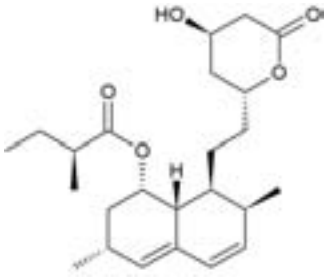
NPV: Natural Products Value

56% of Rx-drugs in US are NPs or NP-Inspired drugs

NPs or NP-Inspired drugs: 27% of revenue from Top 50 drugs

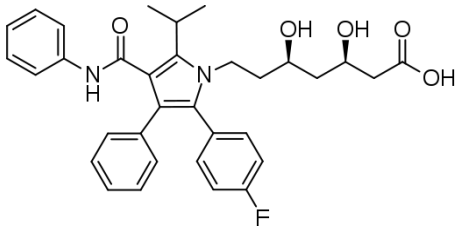
>80% of all categorized human diseases treated by NPs or NP-Inspired drugs
Antibiotics, Antifungals, Anticancer, Antiparasitics, Anticoagulants, Immunosuppressants ....

Natural products as drugs

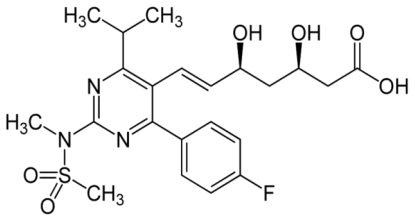


Lovastatin
Mevacor

Natural products-inspired drugs



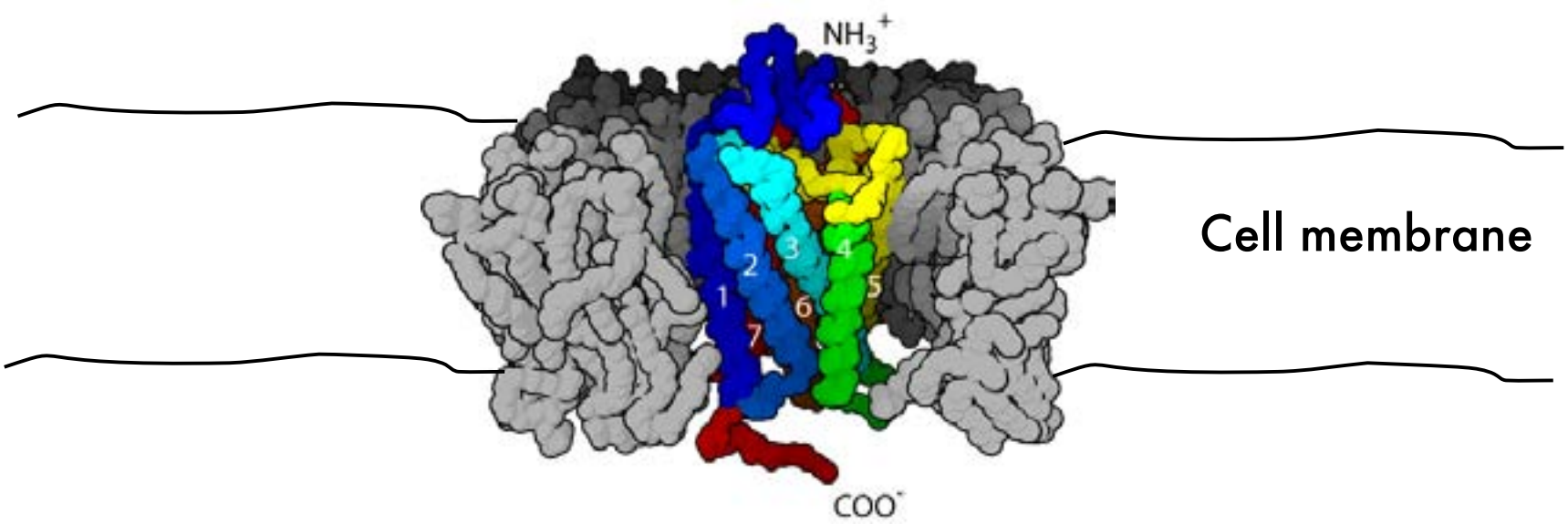
Lipitor®
$ 13.7 Bn/2008



Crestor®
$ 3.9 Bn/2008

Koehn, F. and Carter G. T. Nature Drug Discovery, 2005, 4, 206, Chin Y-W et al., AAPS Journal, 2006, 8, E239

The Histamine Receptor Family of GPCRs



Four GPCRs evolved from different ancestral genes to common end point of high histamine affinities:

- H_1 : CNS and periphery for allergy (Claritin, Zyrtec, Allegra etc.)
- H_2 : GI tract for ulcer (Tagamet, Zantac etc.)
- H_4 : Potential link to inflammatory/pain processes

- H_3- Antagonists for neurological diseases and obesity

H3-Mediated Neurotransmitter Release

H_3 Autoreceptors modulate release of Histamine:

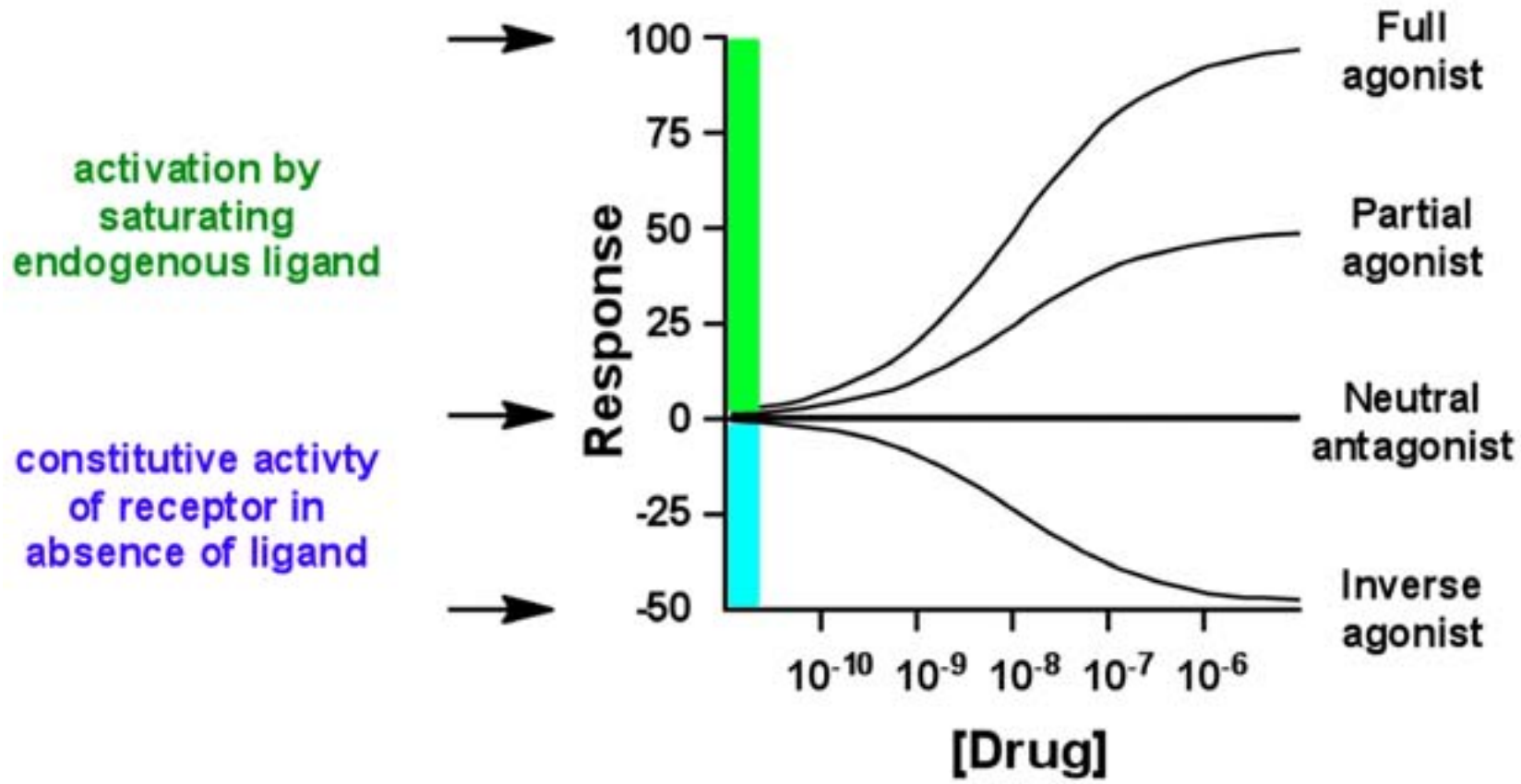
- Attention
- Vigilance-wakefulness
- Impulsivity control
- Pain

H_3 Heterereceptors modulate release of Ach, NE, DA, 5-HT:

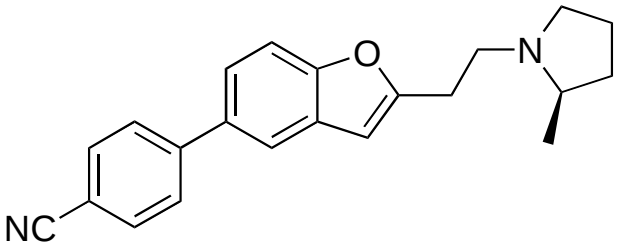
- *Cognition enhancement*
- *Mood regulation*
- *Schizophrenia*
- *Obesity*

Leurs R. *et al.*, *Nature Drug Discovery* 2005; 4:107

Histamine-Mediated Signaling, Constitutive Activity



Natural Products-based H3R-Antagonists

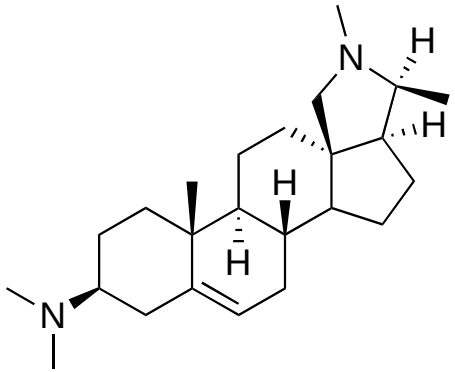


ABT-239

Human H3R:	*Ki = 0.45 nM*
Rat H3R:	*Ki = 1.35 nM*

- Potent and highly selective H3R Inverse Agonist
- Orally active in several animal models as cognition enhancer
- Limited by hERG affinity

J. Medicinal Chemistry, 2005, 48, 38-45

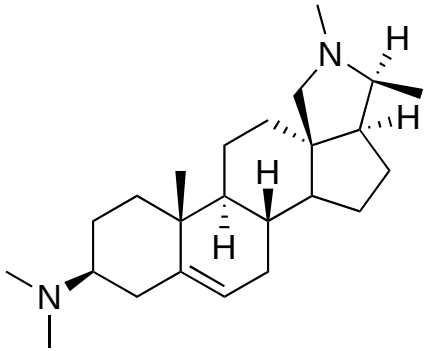


Conessine

Human H3R:	*Ki = 5.3 nM*
Rat H3R:	*Ki = 24.5 nM*

- Aza-steroid from the plant species Apocynaceae family
- Used in herbal traditional medicine for Amoebic Dysentery
- Synthetic challenge!
- Limited commercial supply

Profiling Conessine



H3R-Target Affinity

Human H3R: *Ki = 5.3 nM*

Rat H3R: *Ki = 24.5 nM*

Functional Activity

- hH3 FLIPR IC50= 10 nM
- hH3- GTPγS EC50 = 25 nM (Inverse Agonist)
- *hH3- Adenylate cyclase* IC50 = 8 nM

Selectivity for H3R

- Histamine: H1, H2, and H4 Ki >10 μM
- *h*α2C4, Ki = 10 nM

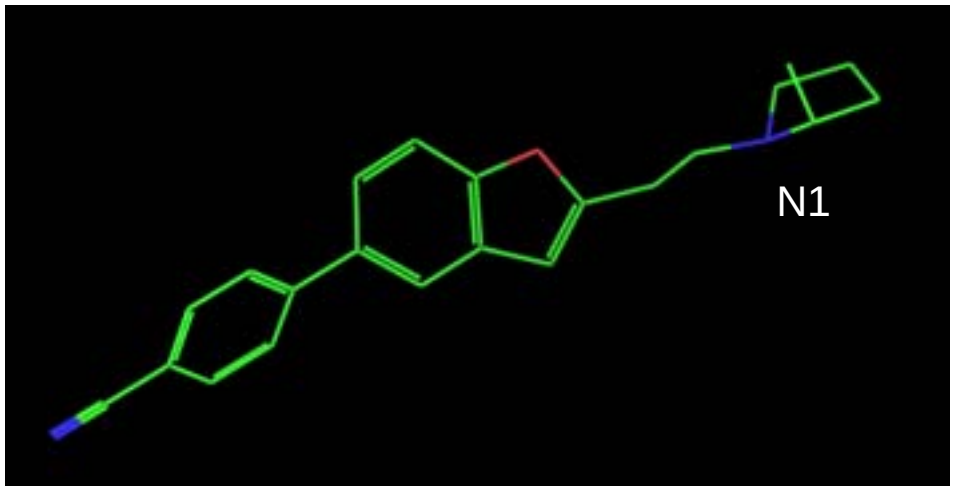
(α2C4/hH3 = 2)

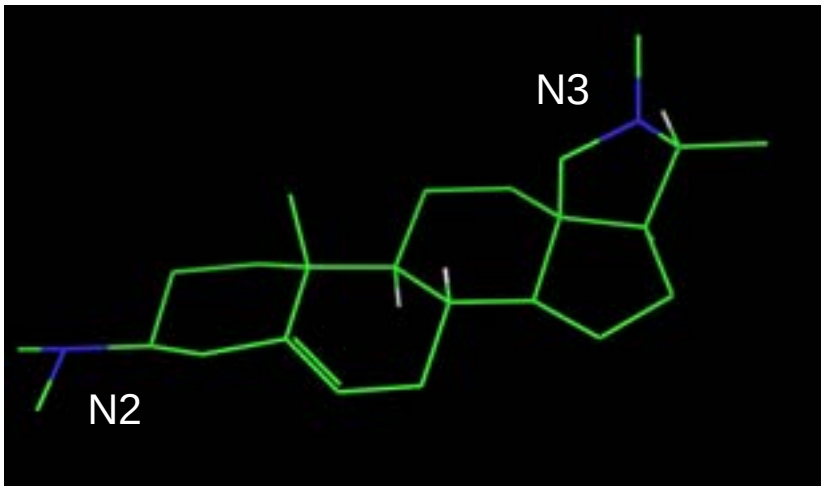
Plasma-Brain Distribution

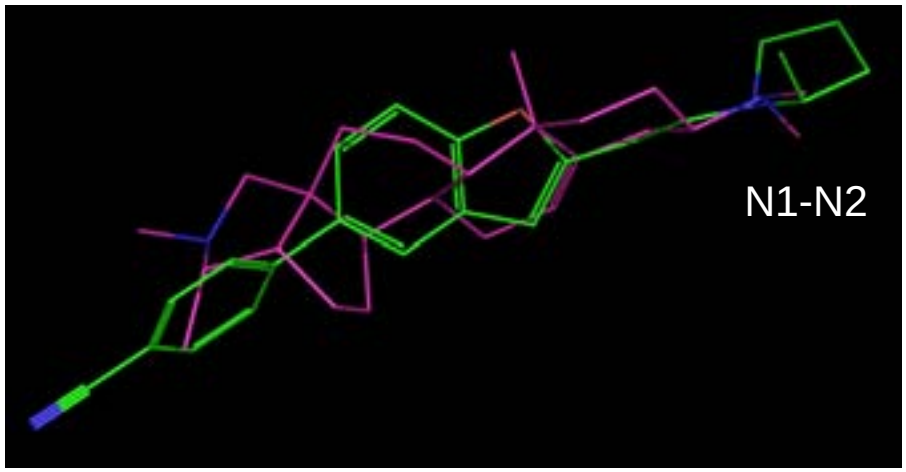
	1 h	5 h	24 h
plasma (μg/mL)	0.148	0.089	0.037
blood (μg/mL)	0.444	0.306	0.102
brain (μg/g)	6.57	6.80	4.14
C_b/C_p (ratio)	46.0	95.4	114.4
FRBC	0.819	0.849	0.805

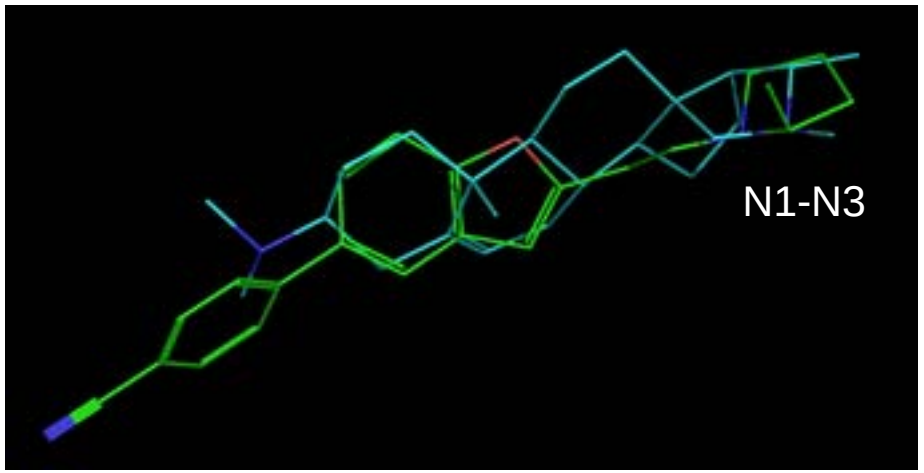
Issues: Selectivity and CNS residence/accumulation

Which basic site is most critical?





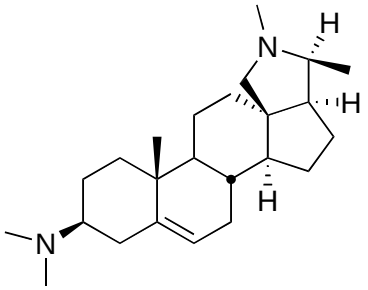




Higher Strain Overlay

Lower Strain Overlay

Conessine: which amine is necessary for affinity?

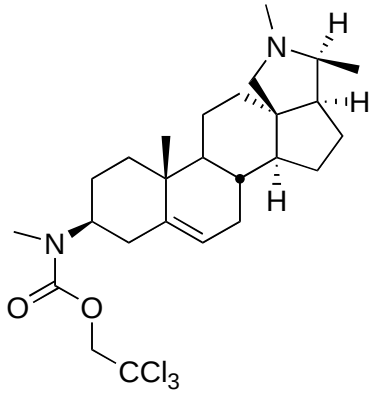


Human H3R: *Ki = 5.3 nM*

Rat H3R: *Ki = 24.5 nM*

Troc-Cl, benzene,

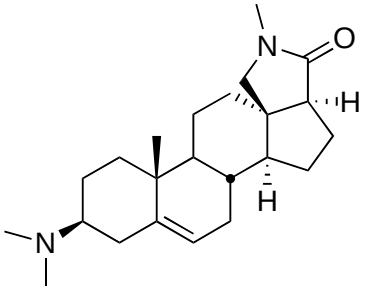
reflux, 4h, 48%



Human H3R: *Ki = 20 nM*

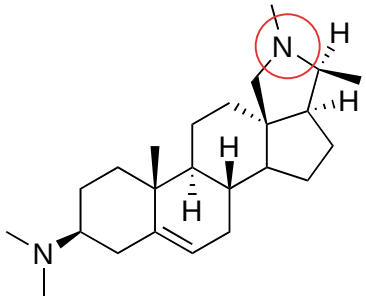
Rat H3R: *Ki = 8 nM*

NBS, acetone/H2O
48h, rt, 85%



Human H3R: *Ki = 543 nM*

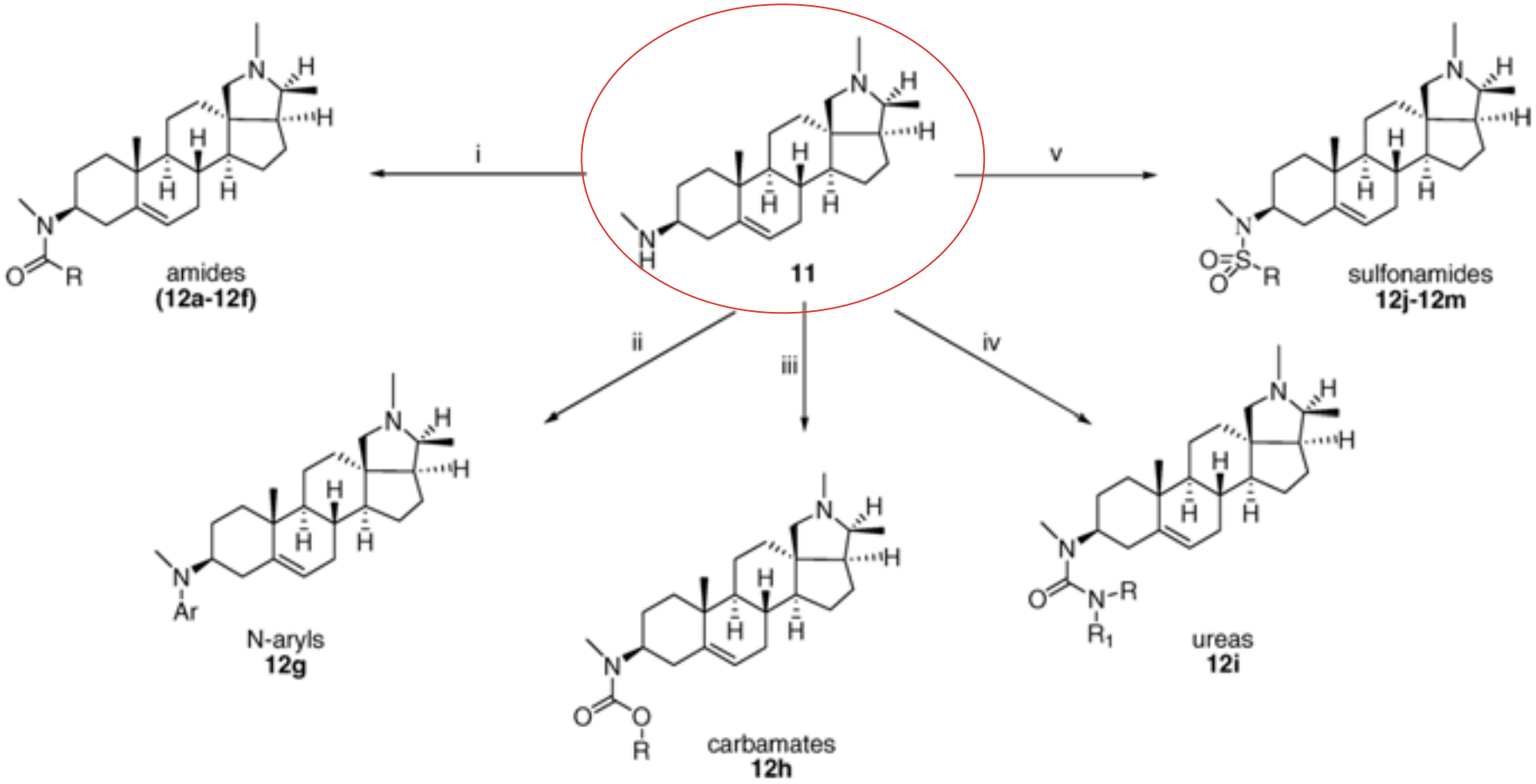
Rat H3R: *Ki = >1000 nM*



Pyrrolidine nitrogen is critical for binding

Kapnang, H.; Chaeles, G. Tetrahedron Lett. 1980, 21, 2951; Bhutani, K. K.; Vaid, R. M. Tetrahedron Lett. 1988, 29, 359

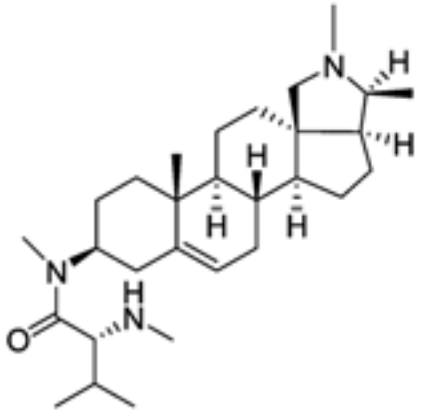
Conessine Derivatives



(i) Acid chloride, Et_3N, CH_2Cl_2; (ii) Aryl bromide, $Pd_2(dba)_3$, BINAP, NaOtBu; (iii) chloroformate, Et_3N, CH_2Cl_2, or 4-nitrophenyl-ester, Et_3N, THF; (iv) carbamoyl chloride, Et_3N, CH_2Cl_2; (v) sulfonyl chloride, Et_3N, CH_2Cl_2.

Zhao, C. et al. J. Med. Chem., 2008, 51 (17), pp 5423–5430

N-Me-(D)-Valine-Conessine



Activity at H3R

hH3R, Ki = 0.21 nM
rH3R, Ki = 2.57 nM

Activity at H3R

Kb = 4 nM; H3 antagonism in adenylate cyclase
Inverse agonism (EC_{50} = 3.3 nM) in a GTPγS assay

Selectivity for H3R

- Histamine: H1, H2, and H4 Ki >10 µM
- Muscarinic-M1, Ki = 11 nM
- *h*α2C4 Ki = 77.6 nM, hH3/α2C4 = 370*
- GR, AR, ER, PR, Ki > 30 µM

Properties

- Orally bioavailable in rats (F = 65%)
- [brain]/[blood] ranges 12–49, with a $T_{1/2}$ (77 h)
- No notable CNS side effects at up to13 mg/kg (ip)
- No inhibition of CYP enzymes (IC_{50} > 10 µM).
- Weakly induced phospholipidosis *at 10 µM*
- High in-vivo potency : PAR-mouse at *0.01 mg/kg (ip)*

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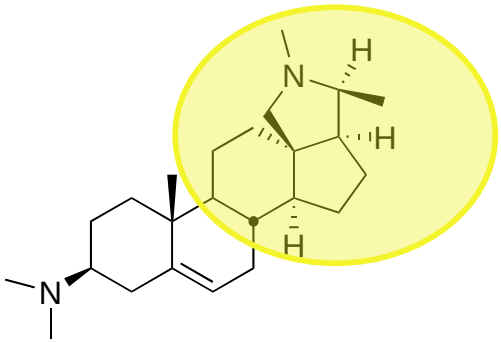
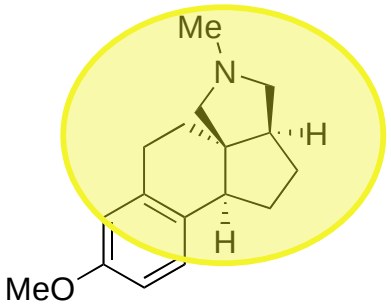
Design of NP-Inspired H3R(s) Antagonists





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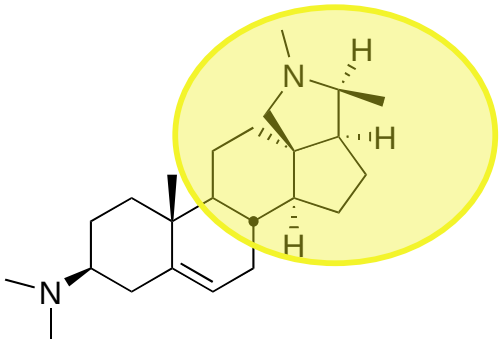
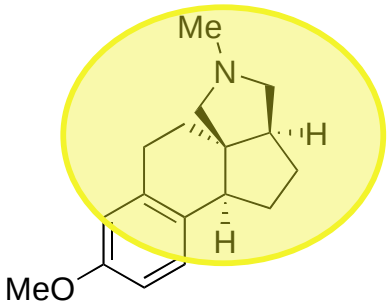
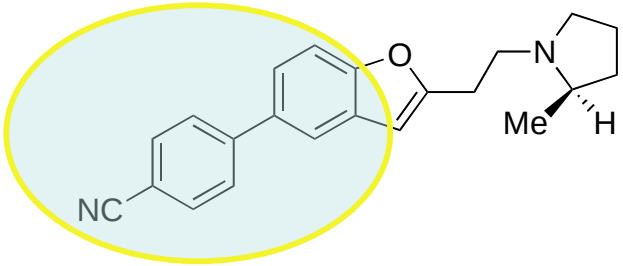
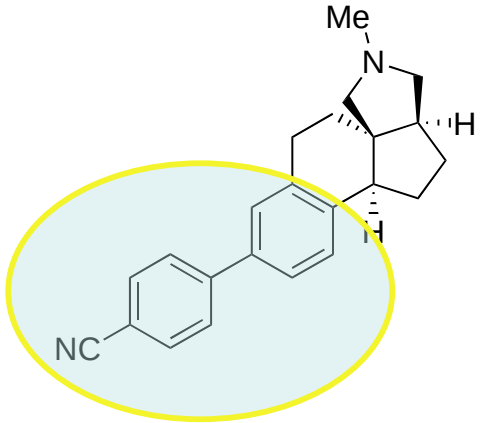


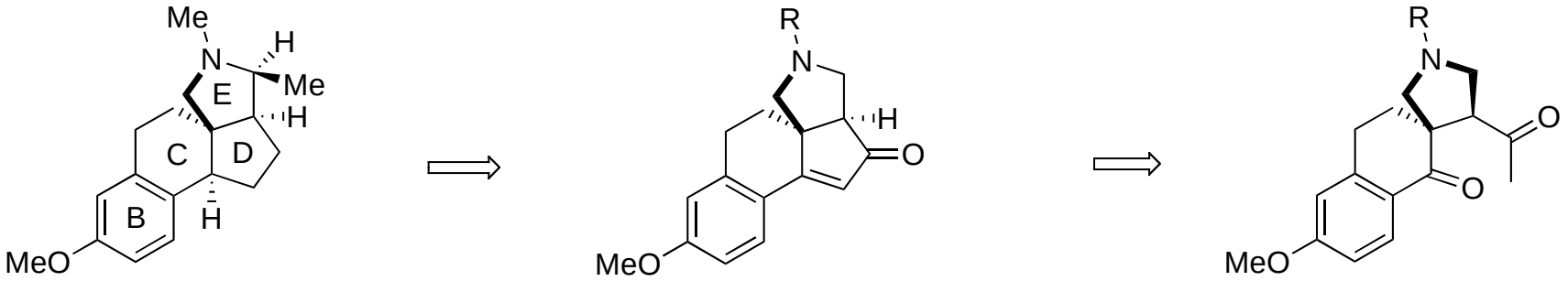






Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

Meyers' Formal Synthesis of (+)-Conessine



Kopach, M. E. et al., J. Am. Chem. Soc., 1996,118, pp 9876

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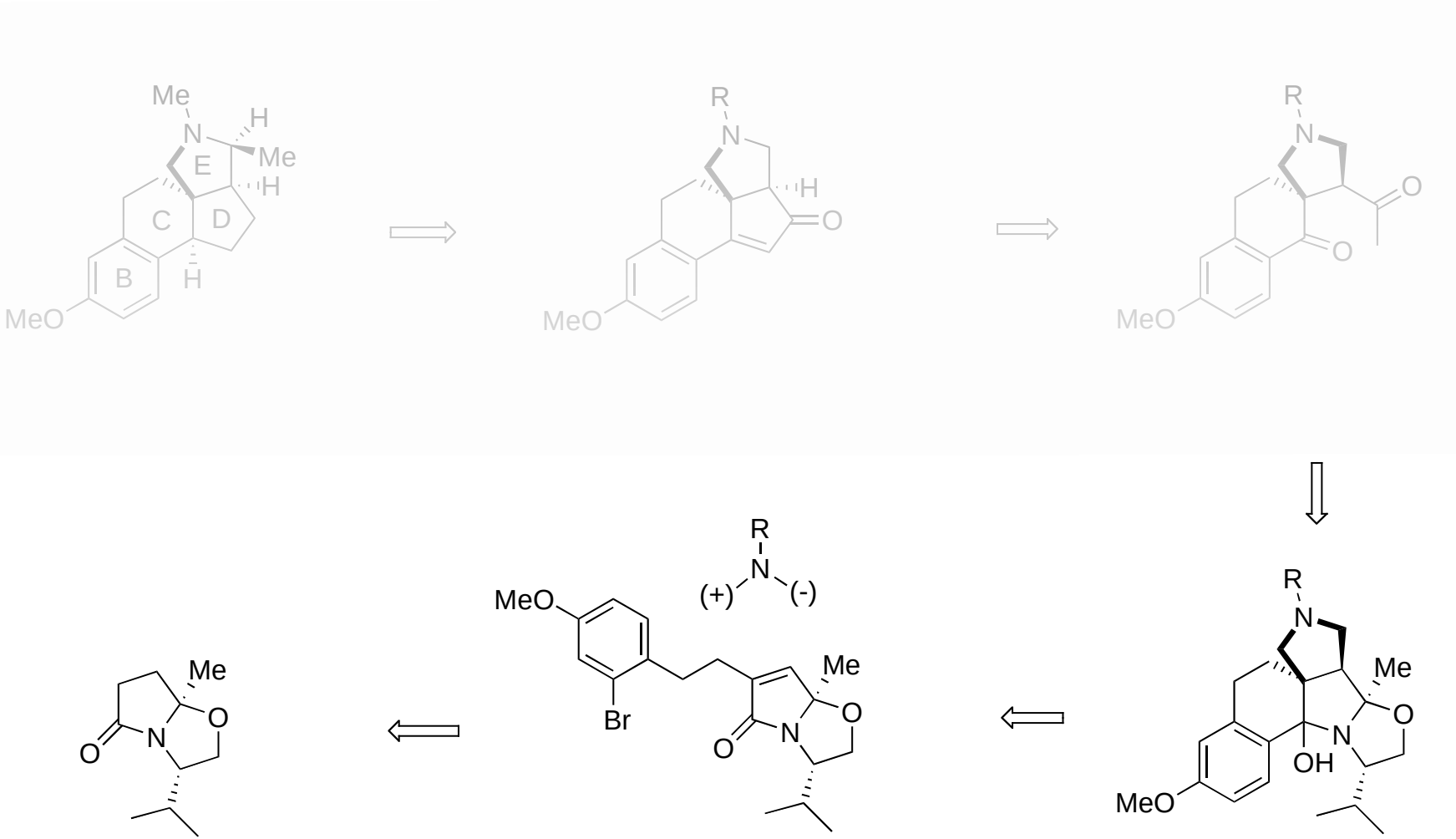
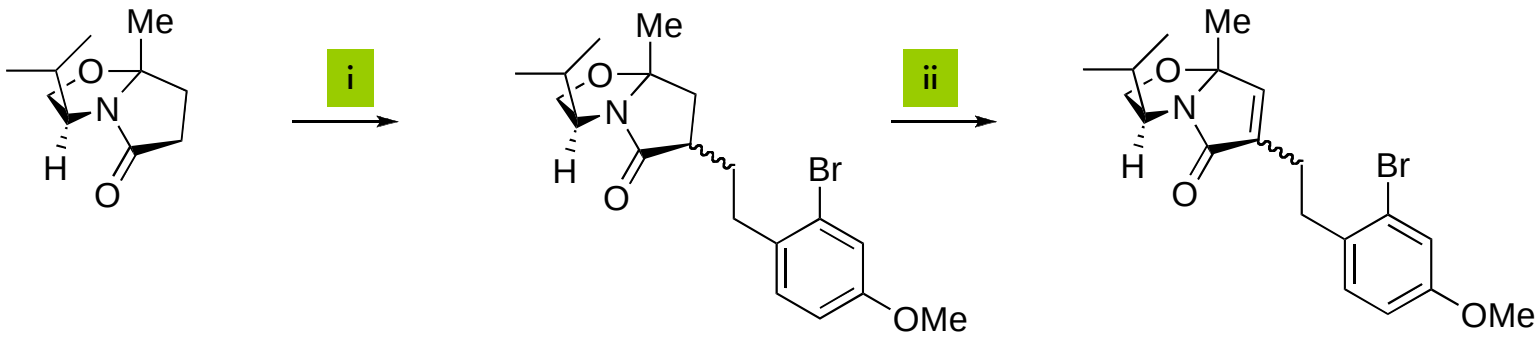


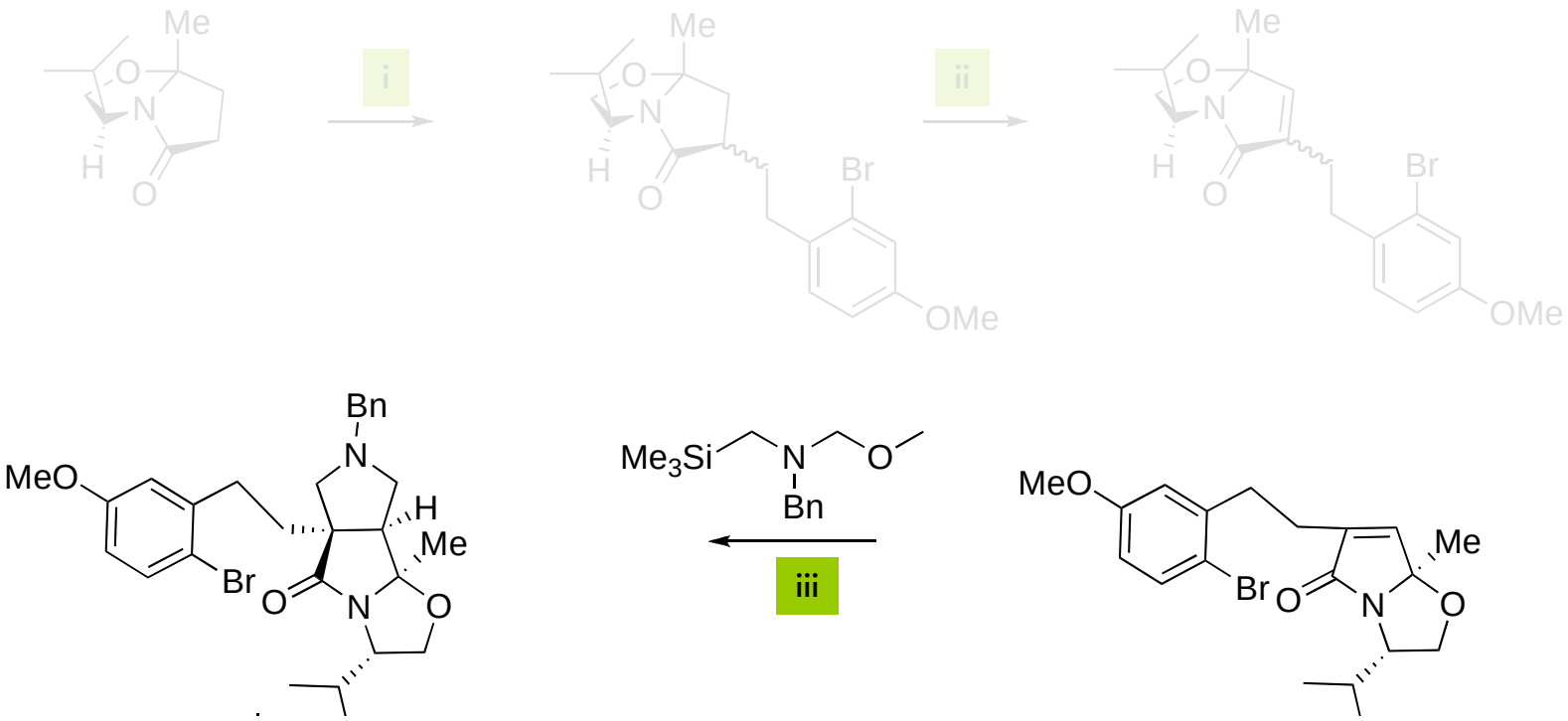
Meyers' Bicyclic lactam

Kopach, M. E. et al., J. Am. Chem. Soc., 1996,118, 9876



i) LDA/THF, -78°C, (2-Br-, 4-MeO-Ph)-CH_2CH_2I; ii) 1)$(PhSe)_2$, KH, THF, 25°C; 2) H_2O_2; iii) 0.01% TFA, 180°C, C_6H_6; iv) t-BuLi, THF, -78°C; v) Acid; vi) NaOEt

Kopach, M. E.; Fray, A. H.; Meyers, A. I., J. Am. Chem. Soc., 1996, 118, pp 9876-9883



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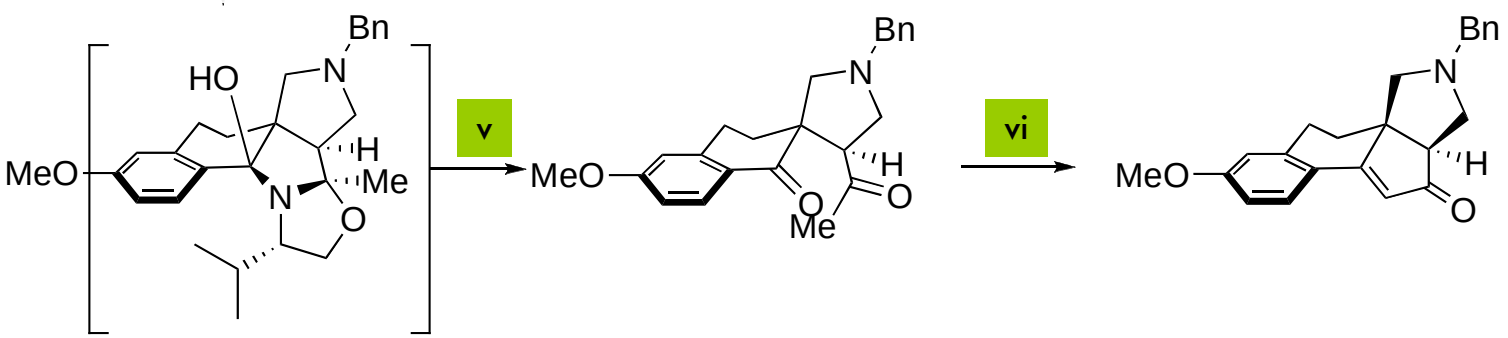

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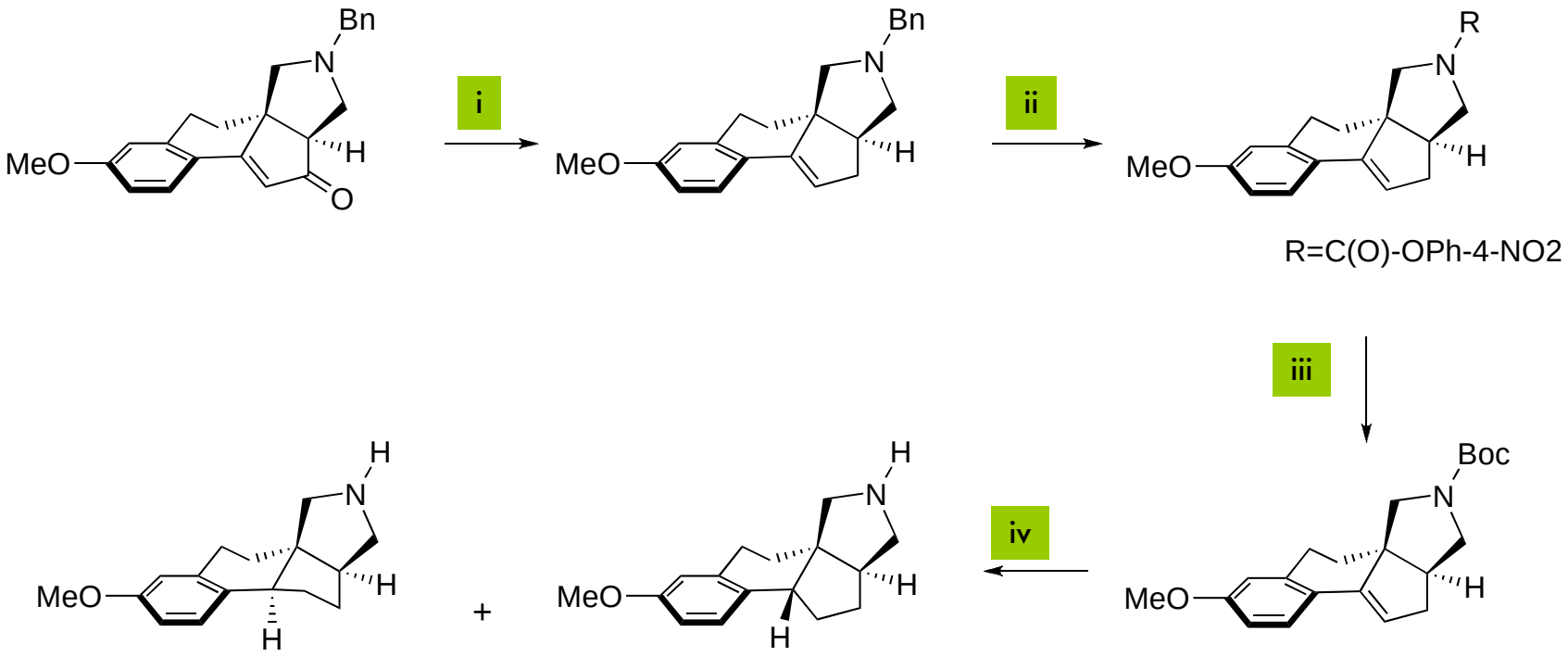
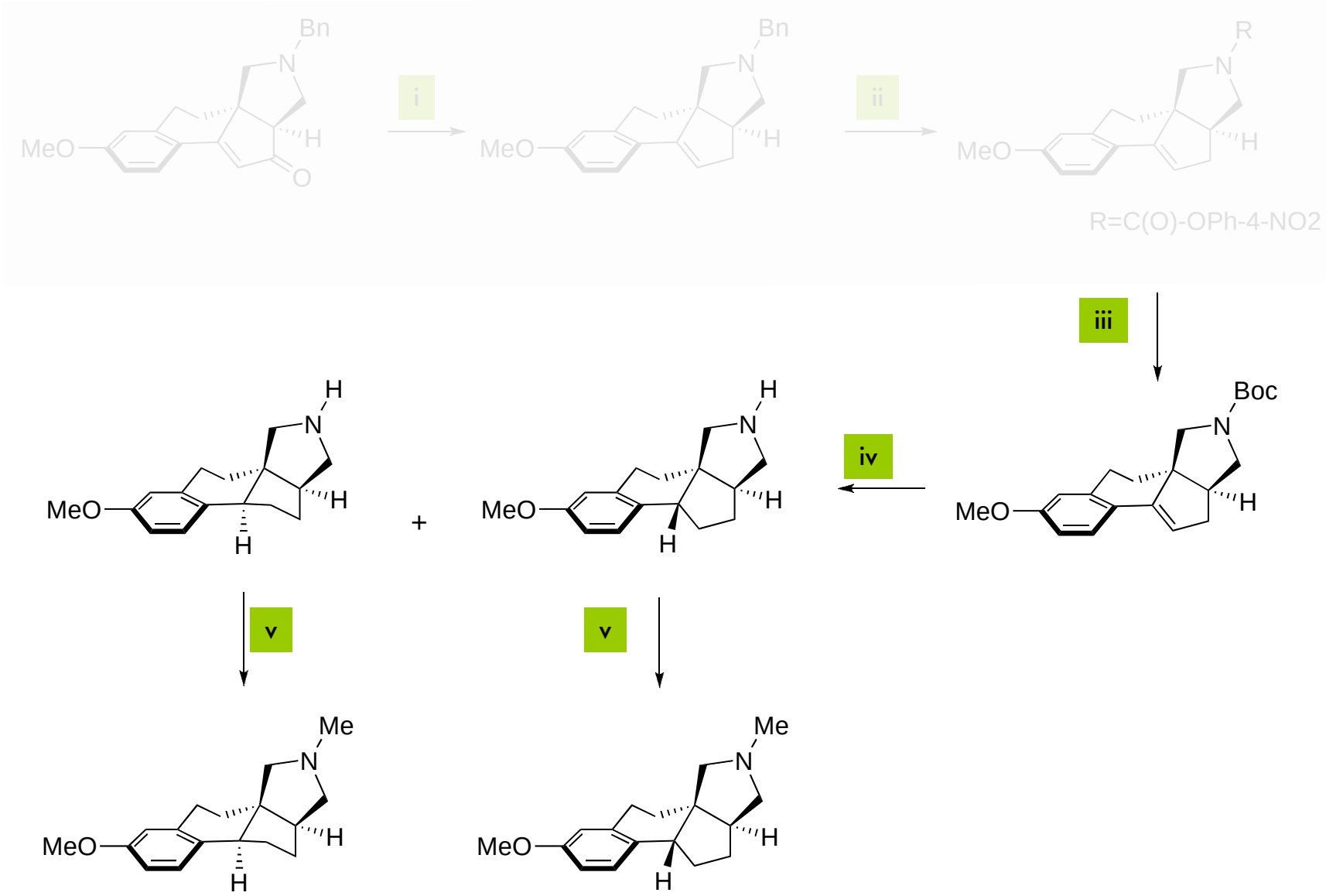


i) LiAlH4,THF; ii) 4-NO2-PhOCOCl; iii) 1) t-BuOK/THF, 2) H2O; iv) TFA; H2/Pd/C

Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649



iv) TFA; H_2/Pd/C ; v) HCO_2H, $NaCNBH_3$

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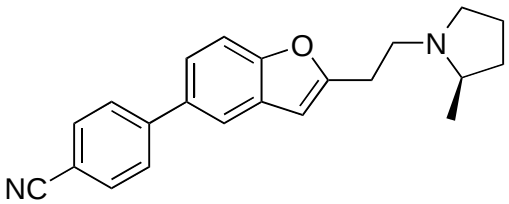
Design of NP-Inspired H3R(s) Antagonists



ABT-239

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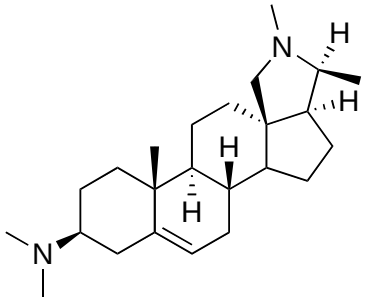
Rat H3R: *Ki = 1.35 nM*



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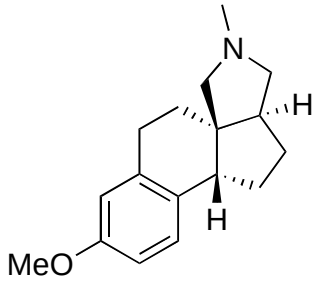
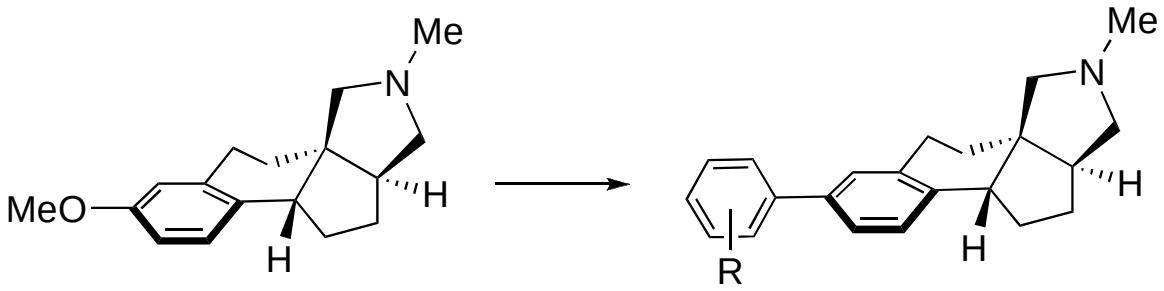


Analogue

Human H3R: *Ki = >1000 nM*
Rat H3R: *Ki = >1000 nM*

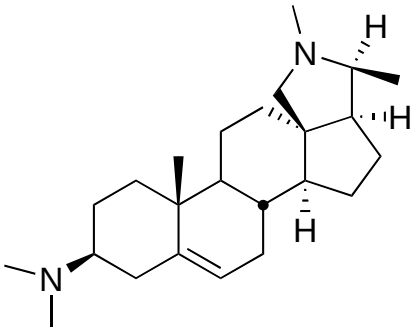
Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649



1) BBr_3, CH_2Cl_2; 2) Tf_2O, Et_3N, CH_2Cl_2; 3) R-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O.

Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

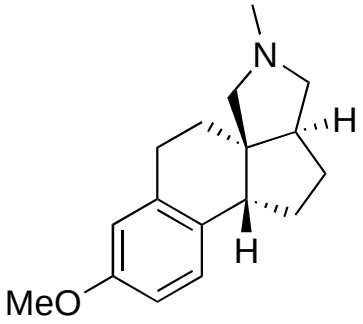
Design of NP-Inspired H3R(s) Antagonists



Conessine

Human H3R: *Ki = 5.3 nM*

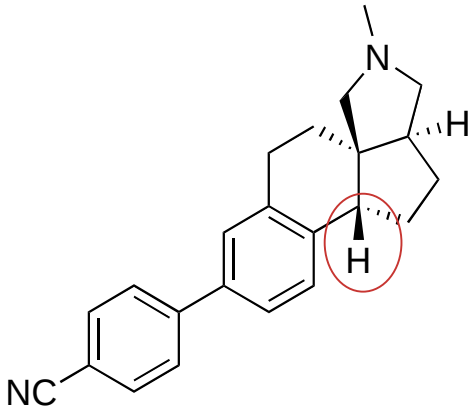
Rat H3R: *Ki = 24.5 nM*



Analogue

Human H3R: *Ki = >1000 nM*

Rat H3R: *Ki = >1000 nM*



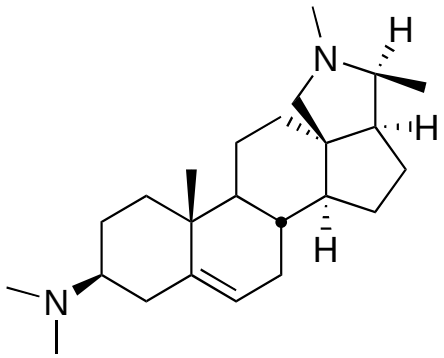
Analogue

Human H3R: *Ki = 14.5 nM*

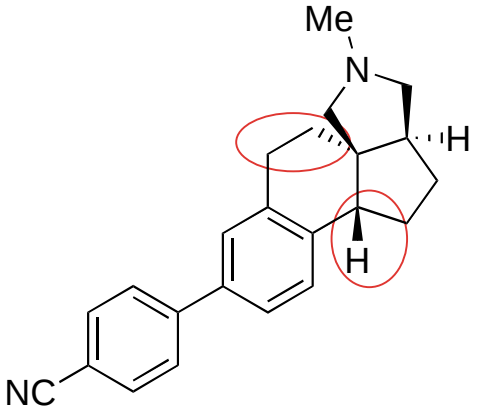
Rat H3R: *Ki = 45 nM*

Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

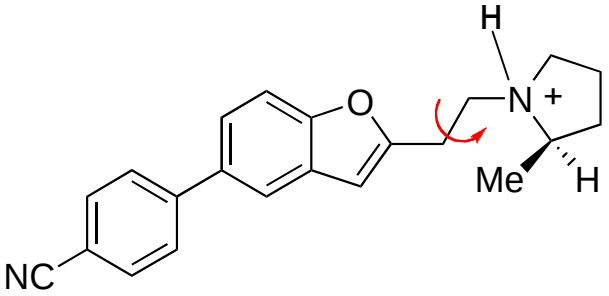
Design of NP-Inspired H3R(s) Antagonists

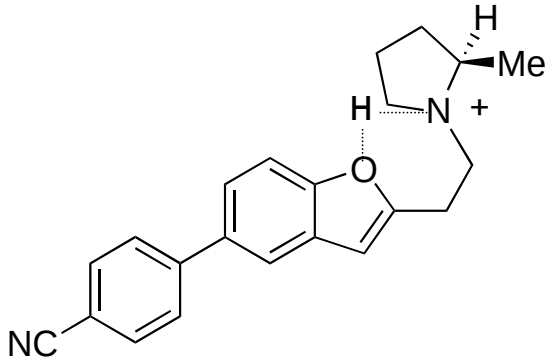


Conessine



Hybrid





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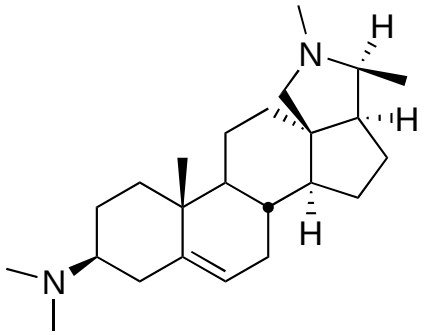
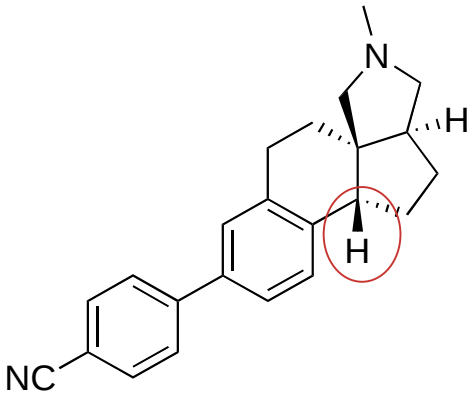
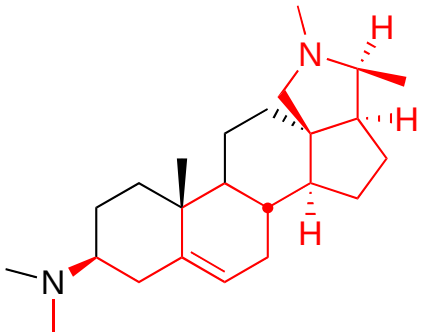
Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

Design of NP-Inspired H3R(s) Antagonists

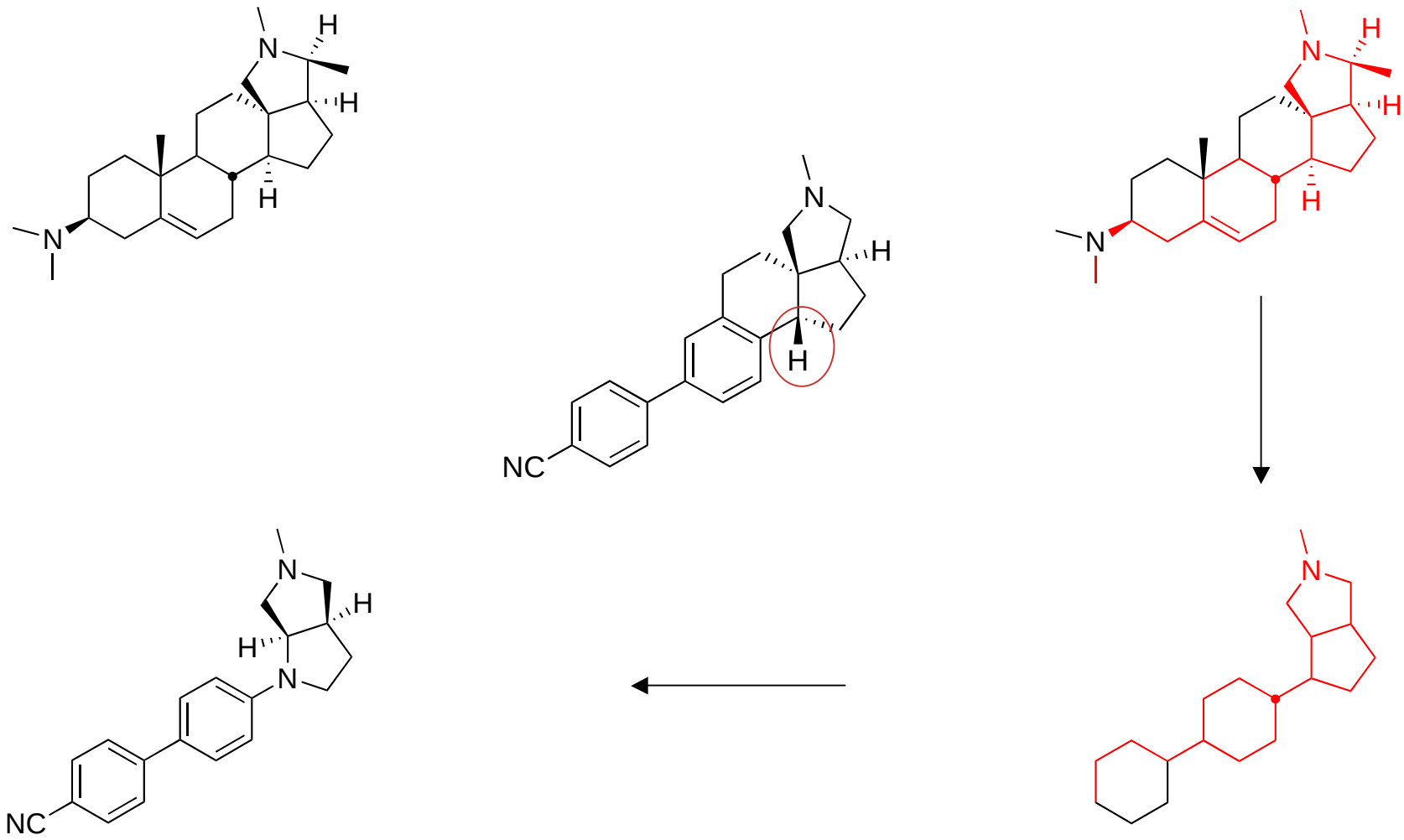






Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

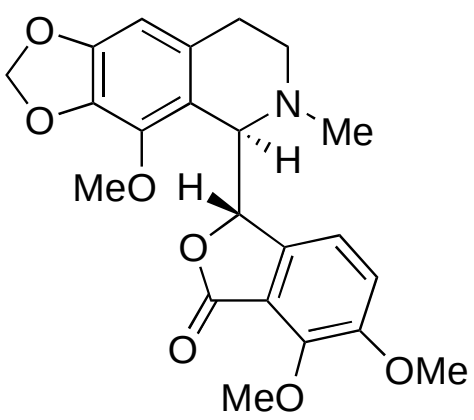
Design of NP-Inspired H3R(s) Antagonists



Human H3R: *Ki = 0.5 nM*
Rat H3R: *Ki = 4.6 nM*
Orally Efficacious H3-Blocker

Zhao, C. et al. J. Med. Chem., 2009, 52 (15), pp 4640–4649

"2nd Short Story"



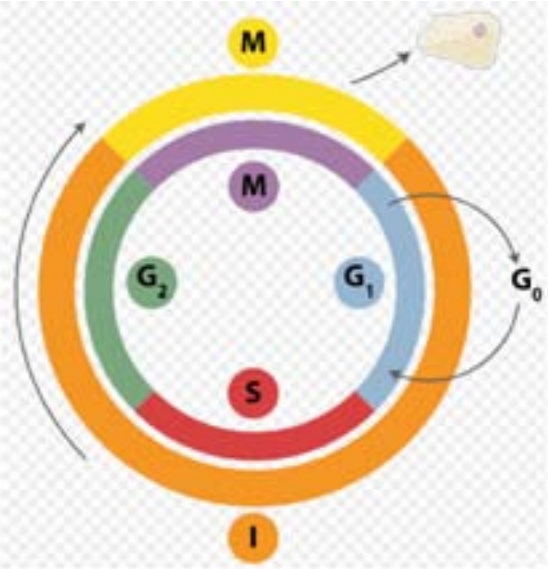
Noscapine

- *Phthalideisoquinoline alkaloid natural product (Opium contains ~7% noscapine- $ 100/25 g)*
- *Approved drug, limited use as an oral antitussive drug (Nitepax)*
- *Possesses non-toxic and non-narcotic properties*
- *First reported by Lettre et al. as Antimitotic agent in 1942 (IC_{50} ~50 μM)*

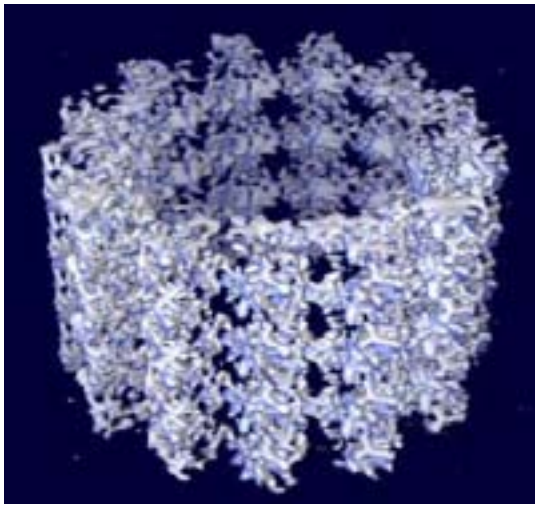
- *Recently profiled by Joshi et al., (1998) as anti-tubulin agent (IC_{50} ~50 μM)*
- *Cougar Biotechnolgy in-licensed asset from Emory University; (2004)*
- *Started human clinical trials for cancer treatment in 2009; currently in Phase 2*

Lettre, H. et al. Naturwissenschaften, 1942, 30, 184; Ann. N. Y. Acad. Sci., 1954, 58, 1264
Joshi, H. C. et al. P.N.A.S., 1998, 95, 1601

Cell Cycle and Microtubules



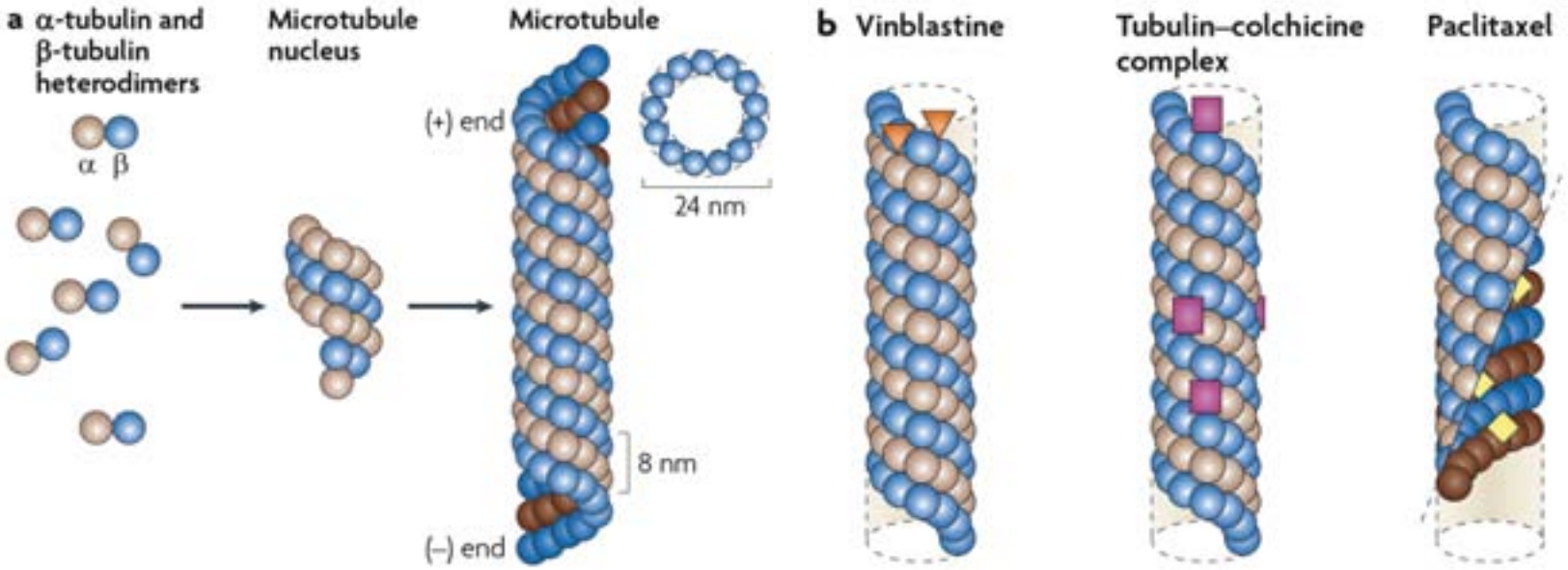
State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	G_0	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	G_1	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication occurs during this phase.
	Gap 2	G_2	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	M	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

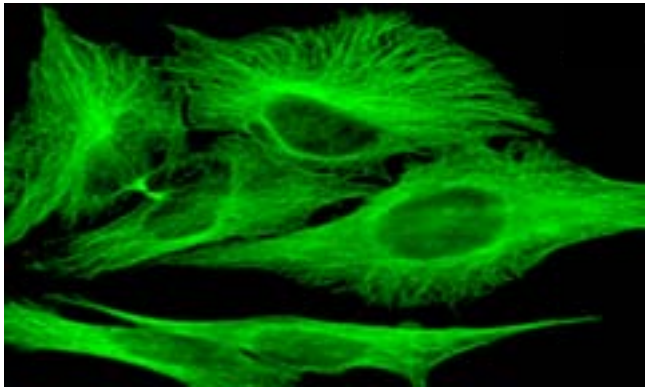


Microtubules

- Provide cell structure maintenance,
- Serve as platforms for intracellular transport
- Help form the spindle during mitosis

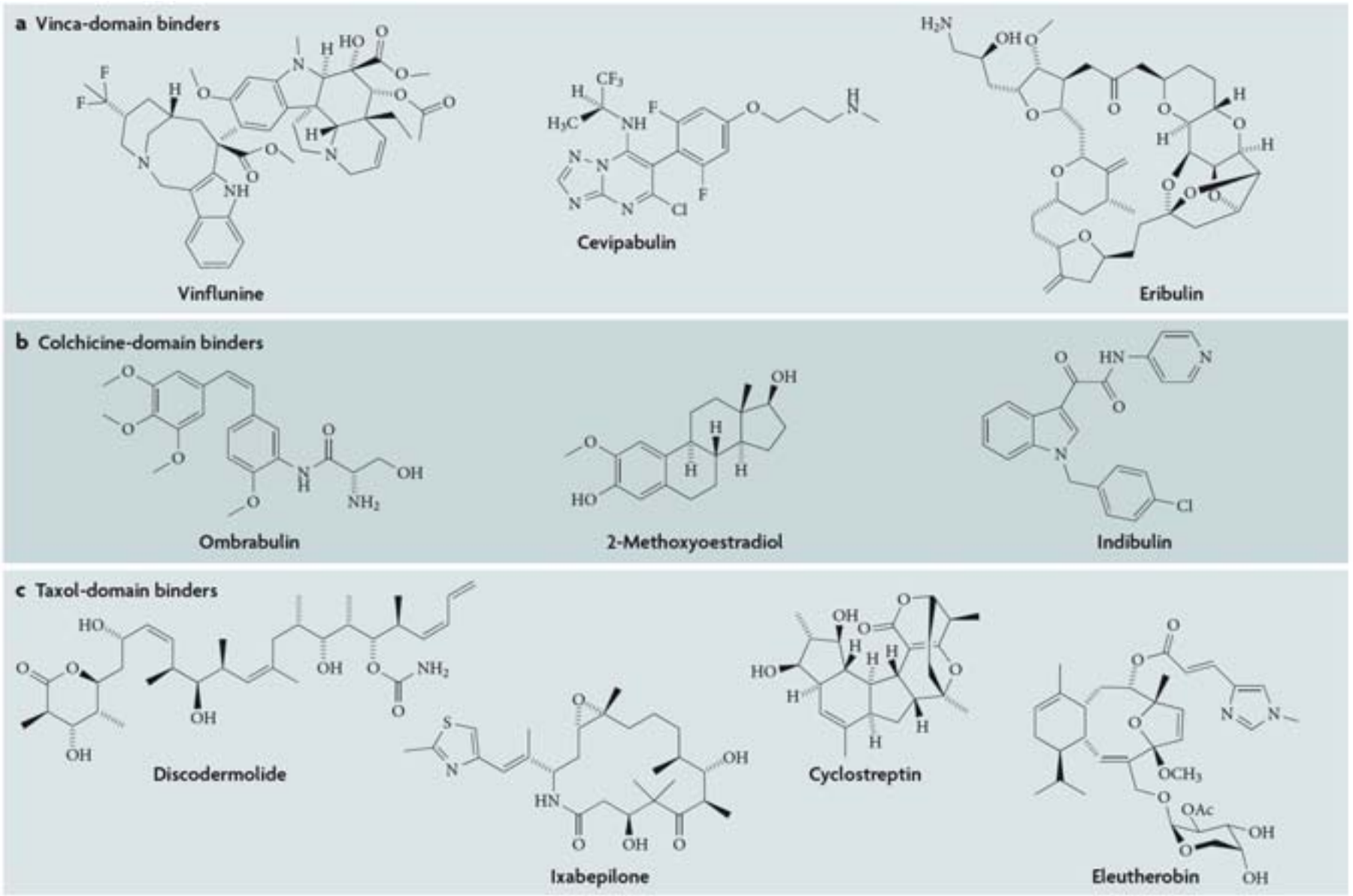
Chemotherapeutic Microtubules binding sites





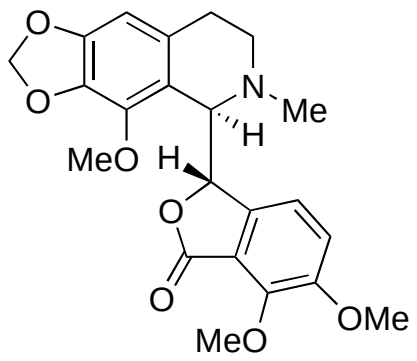
HeLa cells with microtubules Antibody stain,
0.5 % DMSO

Chemical structures of microtubule-binding agents



Dumontet, C. & Jordan M. A.; Nature Drug Discovery; 9, 790, 2010

Need "Entry" into the chemistry



Noscapine

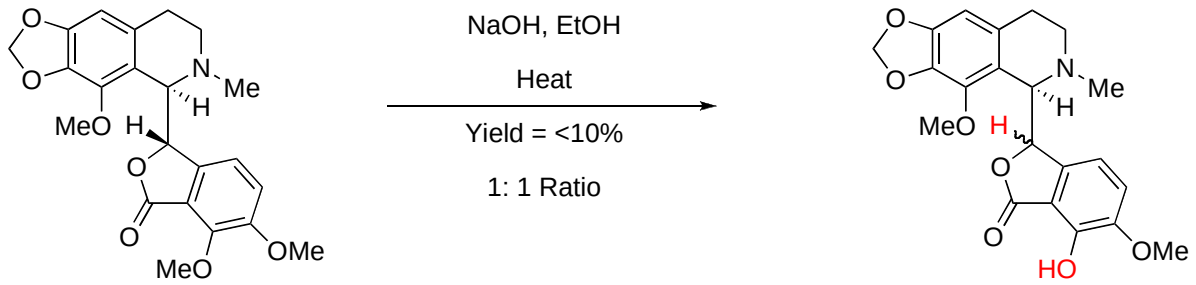
Attractions

- *New Antitubulin-antimitotic agent, opportunity for fixing resistance*
- *Oral bioavailability!*
- *Potential novel binding site*

Issues

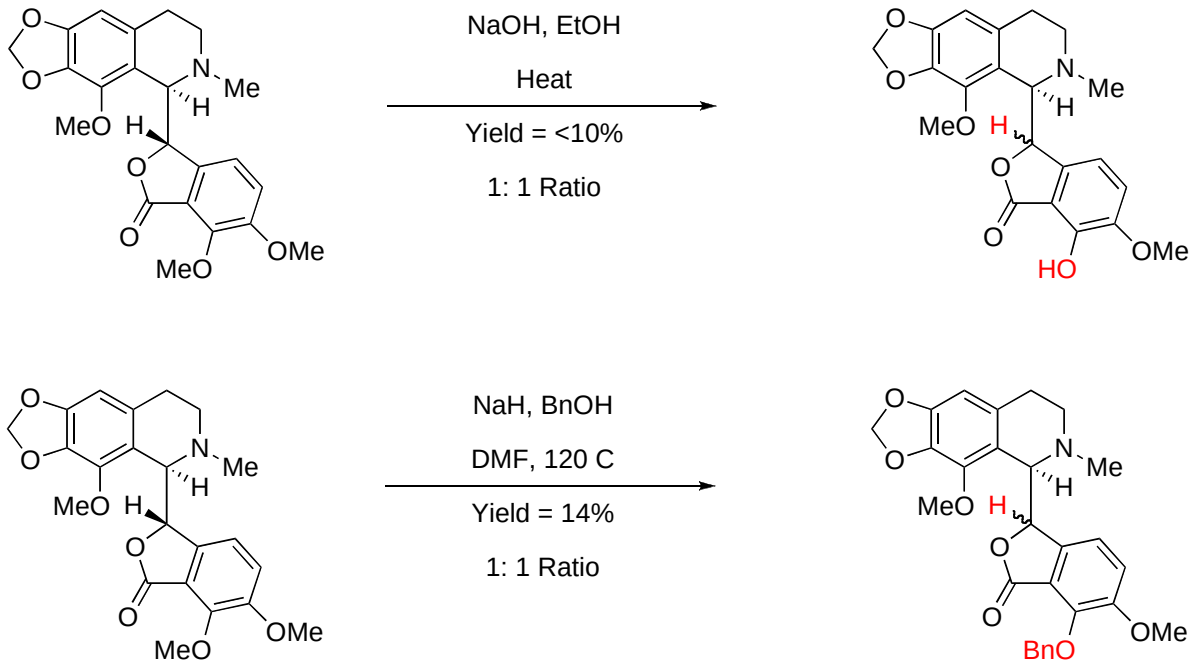
- *IC_{50} ~50 μM*
- *2 benzylic chiral centers; 5 methoxy groups*
- *Lactone-ring opening in acid (Gut)*
- *Need chemical entry*

Regio- and stereoselective O-demethylation



Anderson J. et al. J. Med. Chem. 2005, 48, 2756

Regio- and "stereoselective" O-demethylation



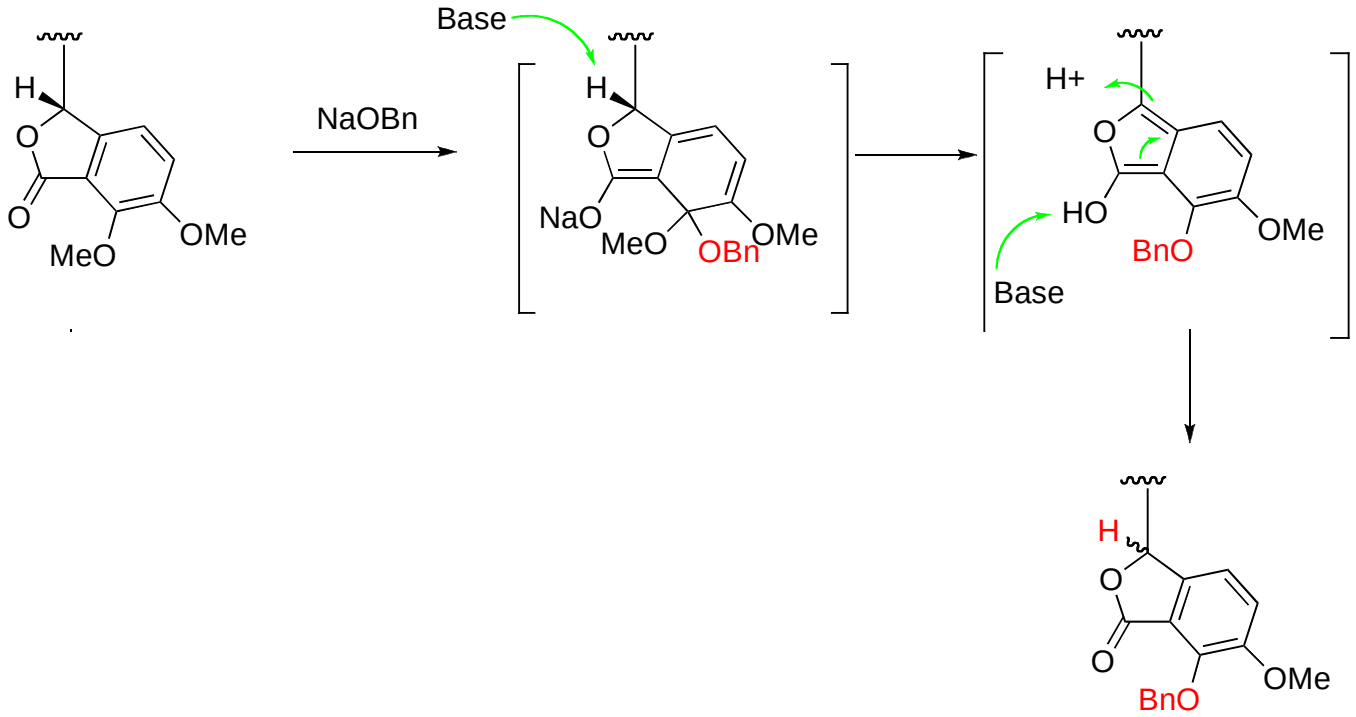
Anderson J. et al. J. Med. Chem. 2005, 48, 2756

Regio- and stereoselective O-demethylation

Anderson J. et al. J. Med. Chem. 2005, 48, 2756

Regio- and stereoselective O-demethylation

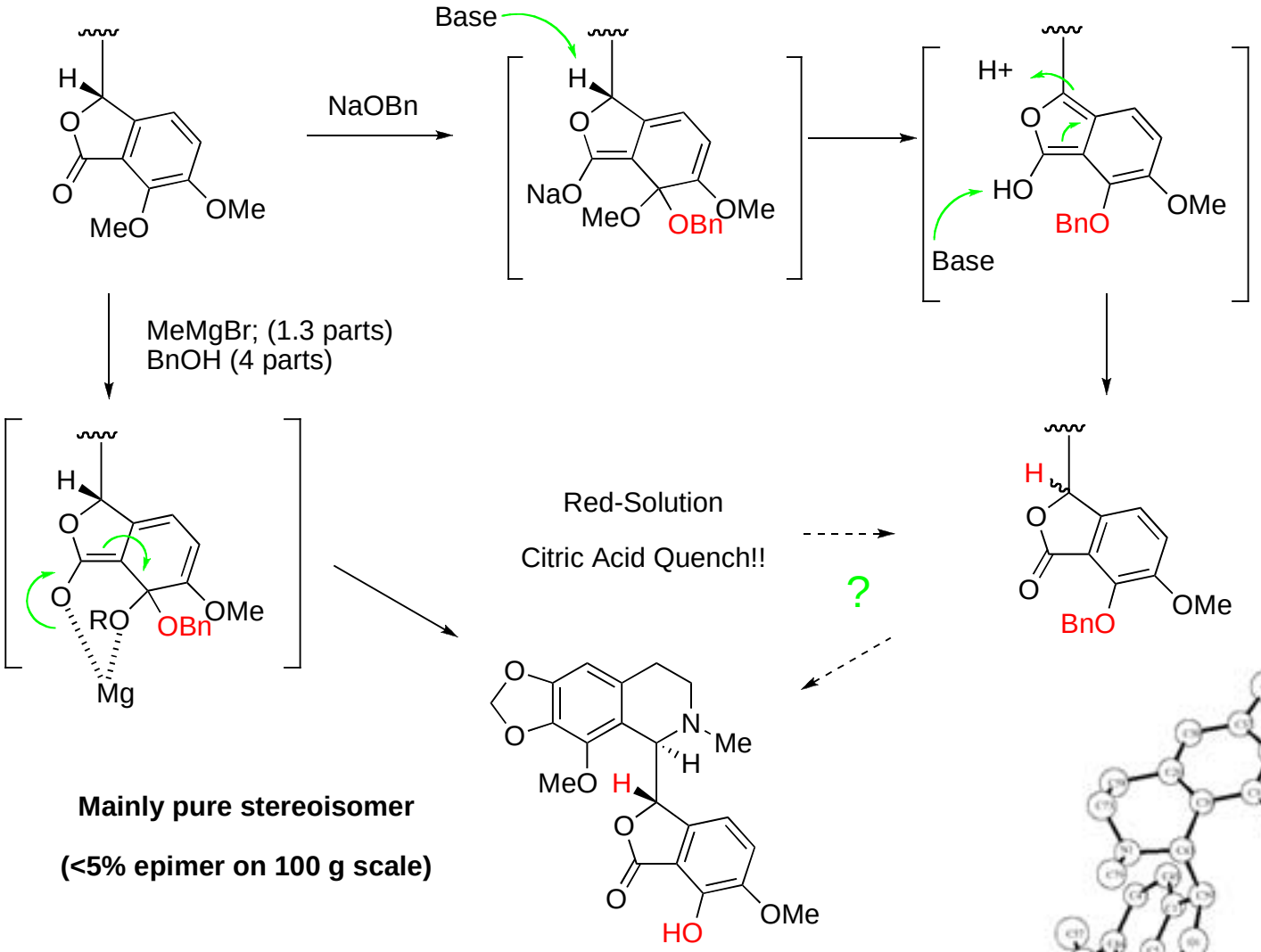
S_NAromatic Reaction



Anderson J. et al. J. Med. Chem. 2005, 48, 2756

Regio- and Stereoselective O-demethylation

$S_NAromatic$ Reaction



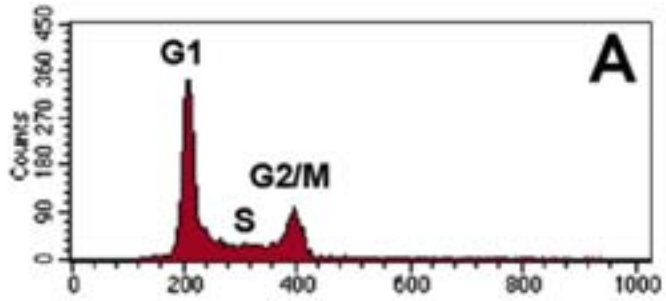
Mainly pure stereoisomer

(<5% epimer on 100 g scale)

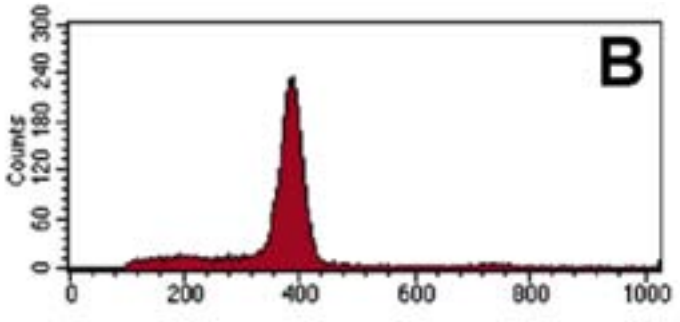
Anderson J. et al. J. Med. Chem. 2005, 48, 2756

Fluorescence-Activated Cell Sorting (FACS) data

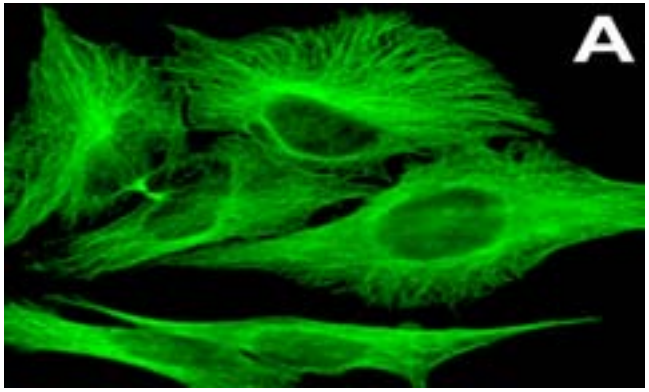
DNA-intercation w/ Propidium Iodide



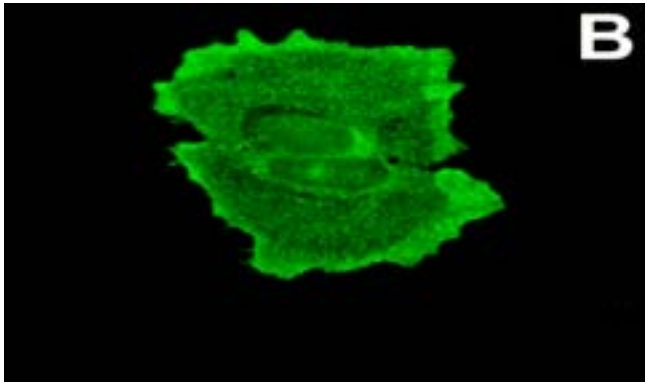
0.5%-DMSO-Control



Noscapine @ 50 µM

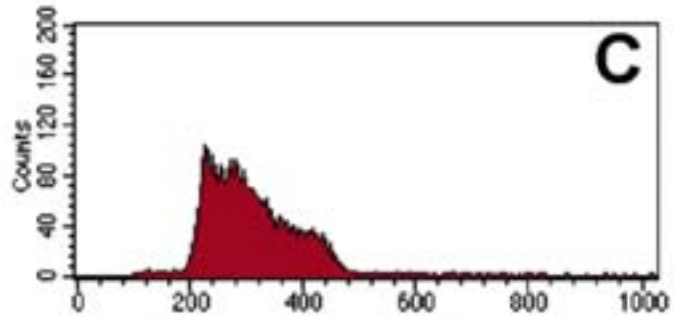


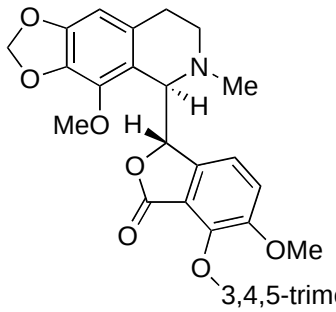
HeLa cells with microtubules antibody stain, 0.5 % DMSO



Disrupted microtubule cytoskeleton

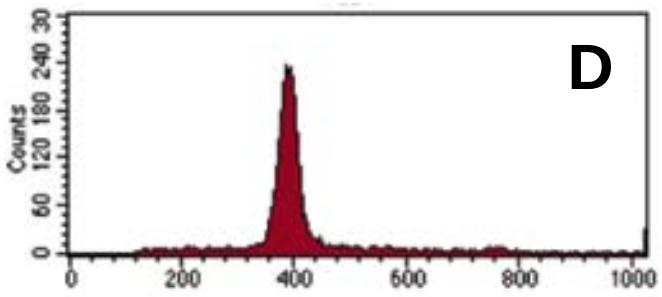
Cell cycle FACS data: "New Biology"

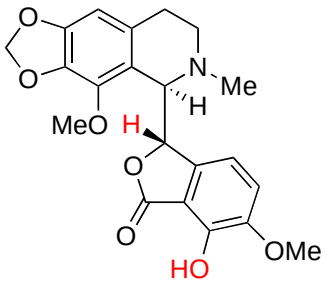




Derivative-1 @ 1 µM

65% S-phase Arrest

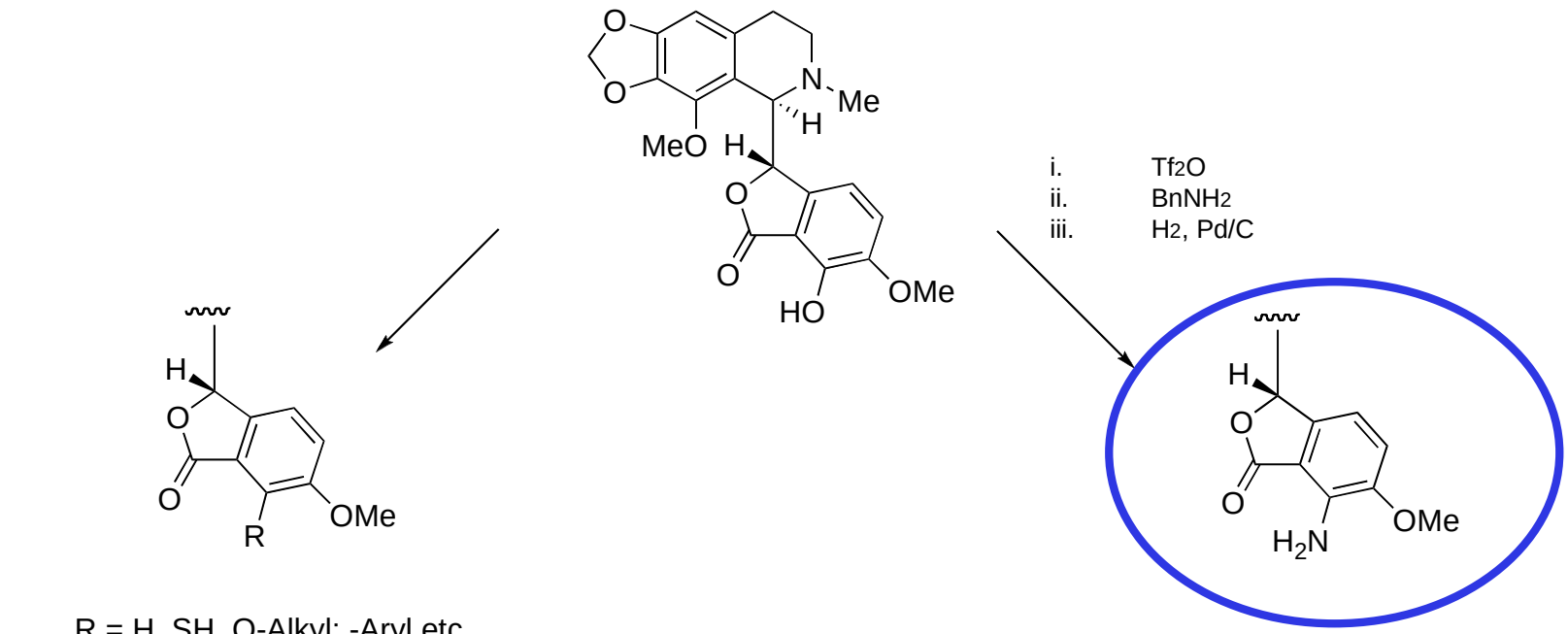




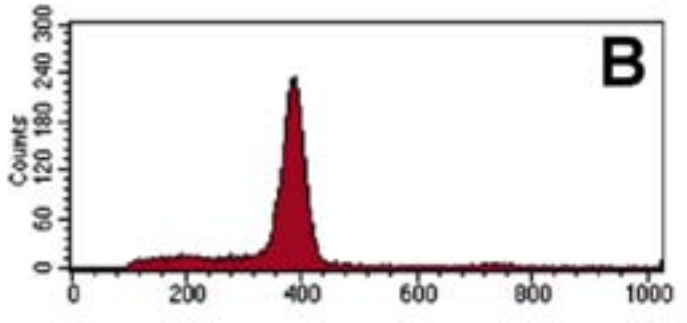
Nosc-Phenol @ 1 µM

88% G2/M-phase Arrest

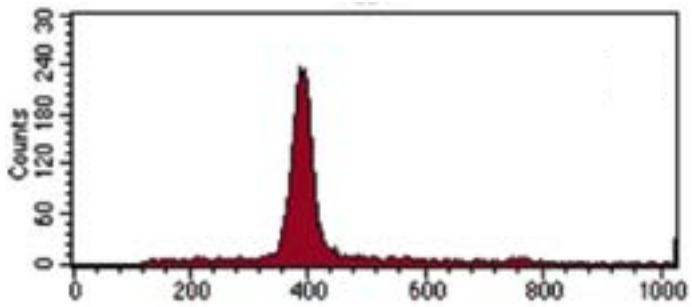
Noscapine derivatives: Increased Potency



R = H, SH, O-Alkyl; -Aryl etc.



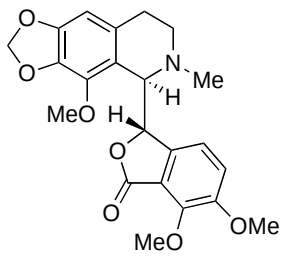
Noscapine @ 50 µM

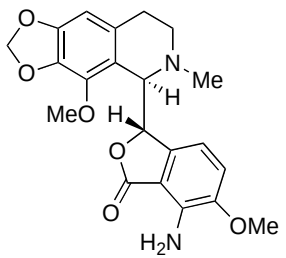


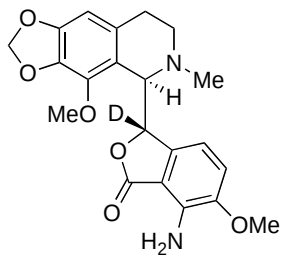
Noscapine-7-Aniline @ 0.1 µM

92% G2/M-Phase Arrest

IC_{50} (nM) on Hepatocellular Carcinoma cell lines



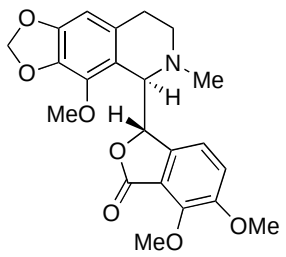


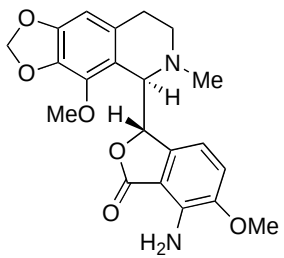


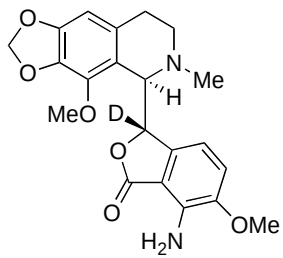
	Noscapine	Nosc.-7-Aniline	
SMMC-7721	> 1000	109.3	93.5
MHCC97H	> 1000	351	347.8
Bel-7402	> 1000	78.4	91.7
Hep3B	> 1000	> 1000	> 1000
HepG2	> 1000	> 1000	> 1000
Huh7	> 1000	218.4	295.7
JHH2	> 1000	> 1000	> 1000
JHH4	> 1000	106.9	104.2
JHH7	> 1000	318	340.4
PLC/PRF/5	> 1000	277.8	366.9
SNU182	> 1000	508.1	617.3
SNU387	> 1000	59.5	69.4
SNU398	> 1000	323.5	382
SNU423	> 1000	226.1	265.7
SNU449	> 1000	283.4	358.2
SNU475	> 1000	584.5	622

HCC cell lines exposed to various agents for 72 hrs, viability assessed by Cell Titer Glo (N=3). IC_{50} values determined using Prism4 and expressed in nM.

IC_{50} (nM) on Hepatocellular Carcinoma cell lines





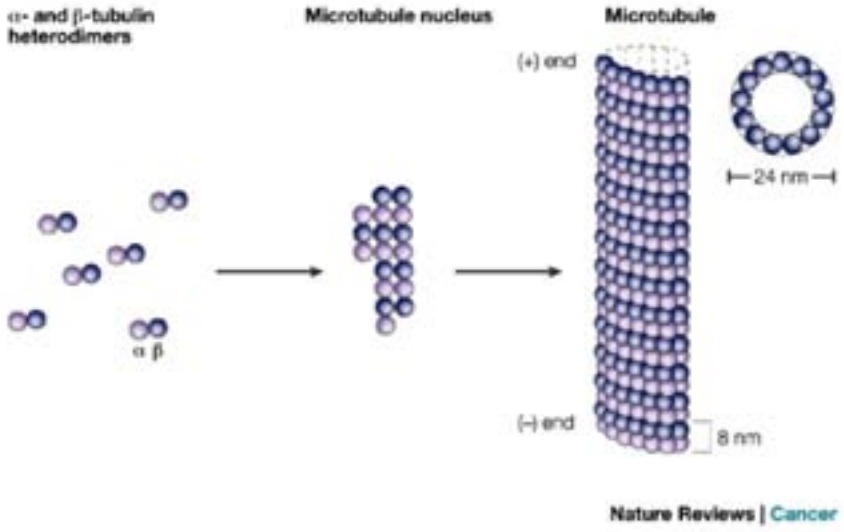


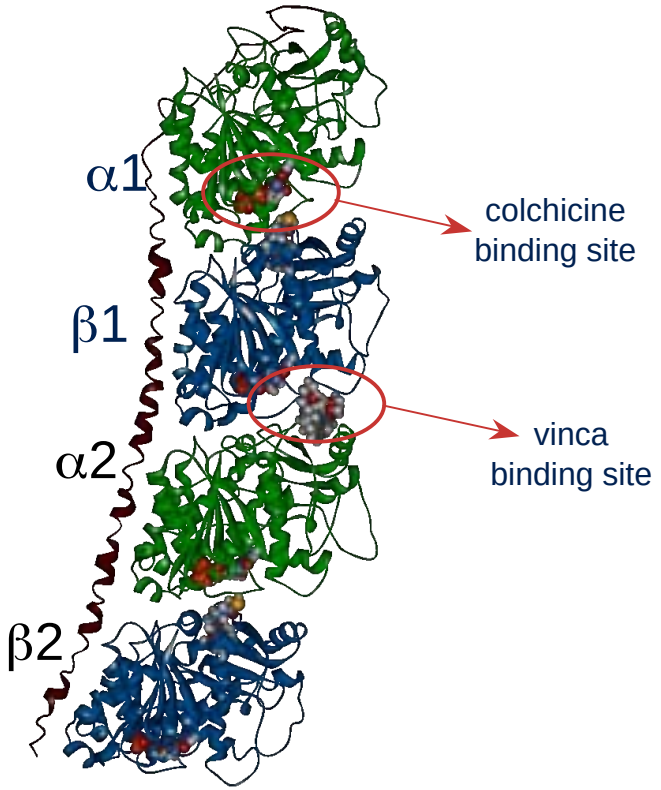
10 - 50 x less potent than clinically relevant chemotherapeutics

	Noscapine	Nosc.-7-Aniline		Docetaxel	Epothilone B	Vincristine
SMMC-7721	> 1000	109.3	93.5	2	2.3	6.5
MHCC97H	> 1000	351	347.8	22.4	6.7	21.2
Bel-7402	> 1000	78.4	91.7	1.9	0.9	4.2
Hep3B	> 1000	> 1000	> 1000	38.2	> 100	> 100
HepG2	> 1000	> 1000	> 1000	> 100	44.7	> 100
Huh7	> 1000	218.4	295.7	12.1	4.5	20
JHH2	> 1000	> 1000	> 1000	> 100	61.3	> 100
JHH4	> 1000	106.9	104.2	14.1	1.8	7.2
JHH7	> 1000	318	340.4	24.7	14.8	28.2
PLC/PRF/5	> 1000	277.8	366.9	16.7	12.7	22.1
SNU182	> 1000	508.1	617.3	39.2	22.3	35.9
SNU387	> 1000	59.5	69.4	4.3	20	40.9
SNU398	> 1000	323.5	382	17.6	2.9	< 0.13
SNU423	> 1000	226.1	265.7	10.8	10.3	18.4
SNU449	> 1000	283.4	358.2	> 100	19.9	> 100
SNU475	> 1000	584.5	622	20.5	11.6	29.3

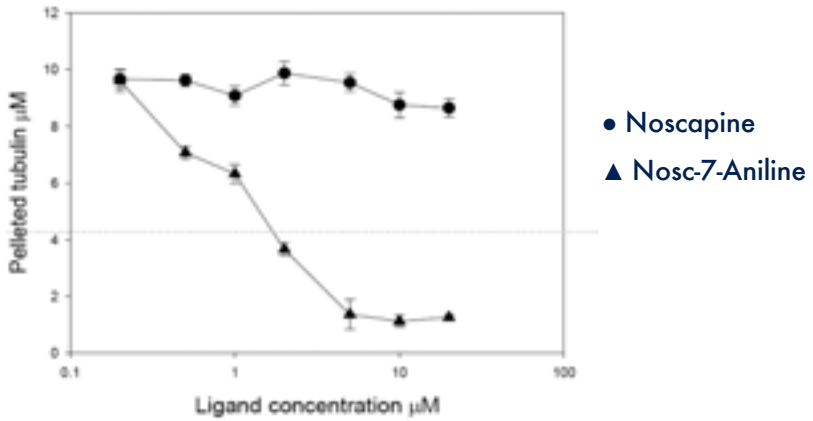
HCC cell lines exposed to various agents for 72 hrs, viability assessed by Cell Titer Glo (N=3). IC_{50} values determined using Prism4 and expressed in nM.

Chemical & Biochemical Characterization





Inhibition of Tubulin Assembly in-vitro



- Noscapine
- Nosc-7-Aniline

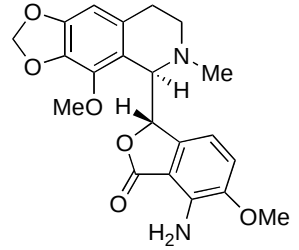
Kd= 8.7 μM; EC_{50} = 1.2 ± 0.3 μM

(Tubulin concentration 15 μM)

Chemical & Biochemical Characterization

Nocodazole Fluorescence Competition Experiments

Vinblastine:	*Vinca-binding site on Tubulin*
Pironectin:	*Covalently binds K352 α tubulin*
Podophyllotoxin:	*Colchicine binding site*
Isohomohalichondrin B:	*Inhibits GTP from binding Tubulin*
Nocodazole:	*Nocodazole binding site*



Noscapine-7-Aniline

Chemical & Biochemical Characterization

Nocodazole Fluorescence Competition Experiments

Vinblastine:	*Vinca-binding site on Tubulin*
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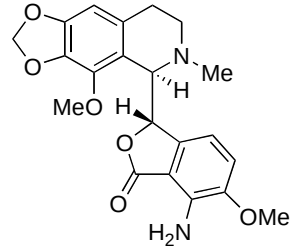
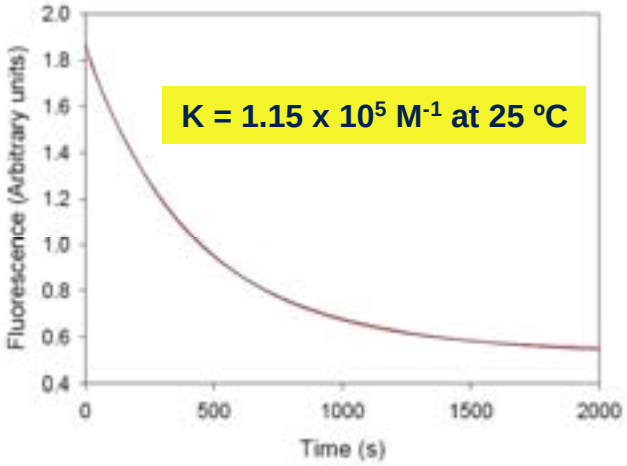
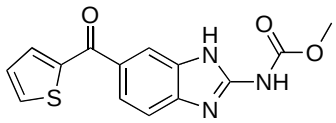


Noscapine-7-Aniline

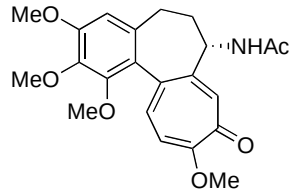


K = 1.15 x 10^5 M^{-1} at 25 ºC

Incubation of 10 μM Noscapine-7-Aniline with up to 50 μM nocodazole



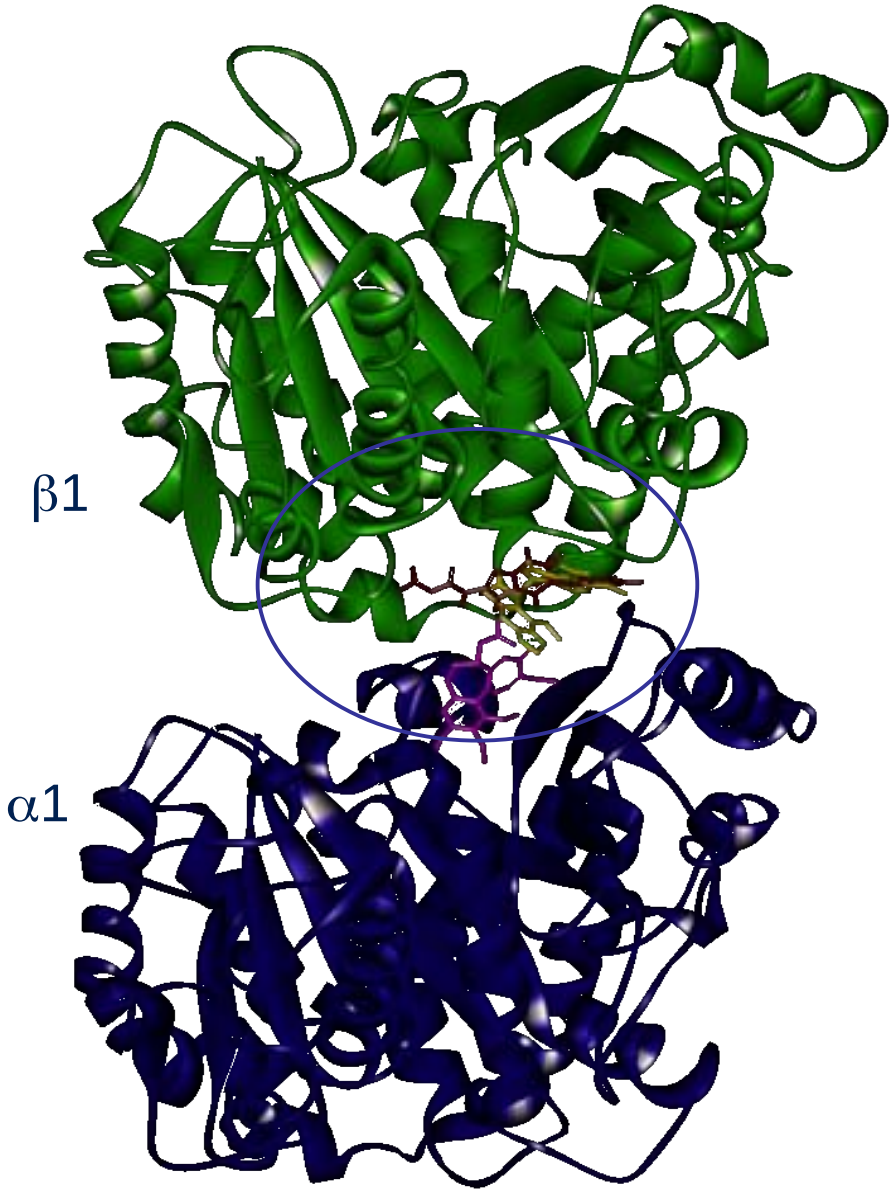
Nocodazole



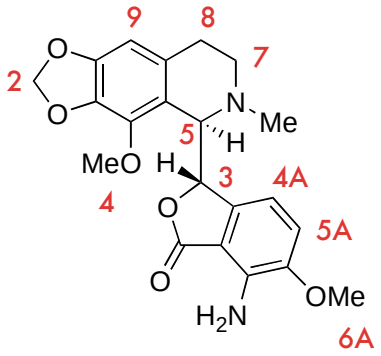
Colchicine

- Slow fluorescence decrase: Competition w/ Nocodazole
- Binding of nocodazole to tubulin affects colchicine binding
- Binding site close to colchicine between both tubulin subunits

Docking Nocodazole/aniline Derivatives (GLIDE software)

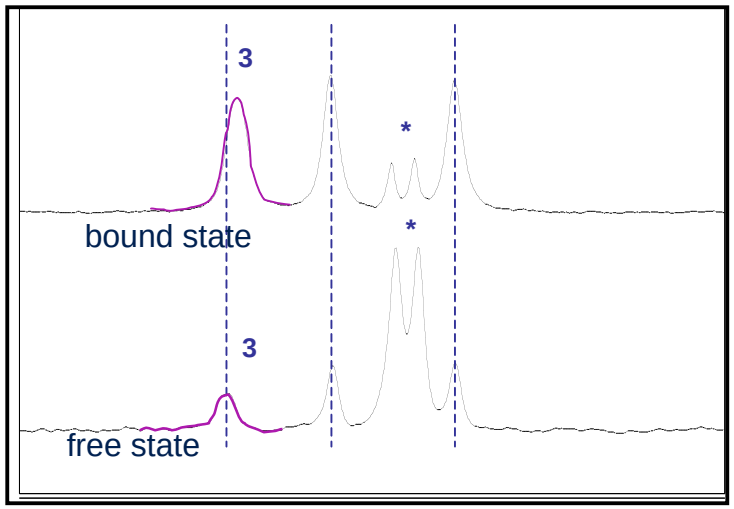


Chemical & Biochemical Characterization



	δ (ppm) D_2O
H5A	7.17 (d)
H4A	6.33 (d)
H9	6.66 (s)
H3	6.0
H2	5.96 and 5.90
H5	4.96
OMe6A	3.89
OMe 4	3.67
H7	3.09 and 2.99
NMe	2.79
H8	2.95 and 2.74

STD-NMR Experiments with Tubulin



10 mM sodium phosphate, 0.1 mM GTP, D_2O

* Signal from the GTP added to buffer for protein stability

H3-H5 small coupling constant (~ 2 Hz):

Gauche disposition, when bound to Tubulin

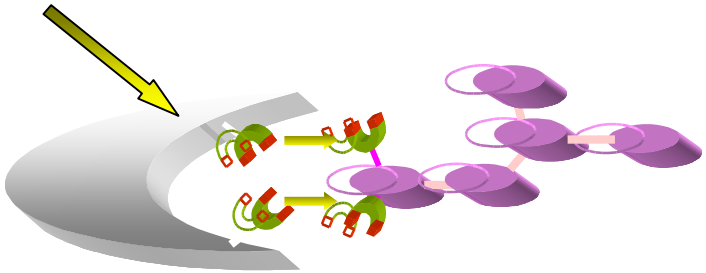
Bennani et al. J. Med. Chem., 2012, 55 (5), pp 1920–1925

Protein-Ligand NMR: STD Experiments

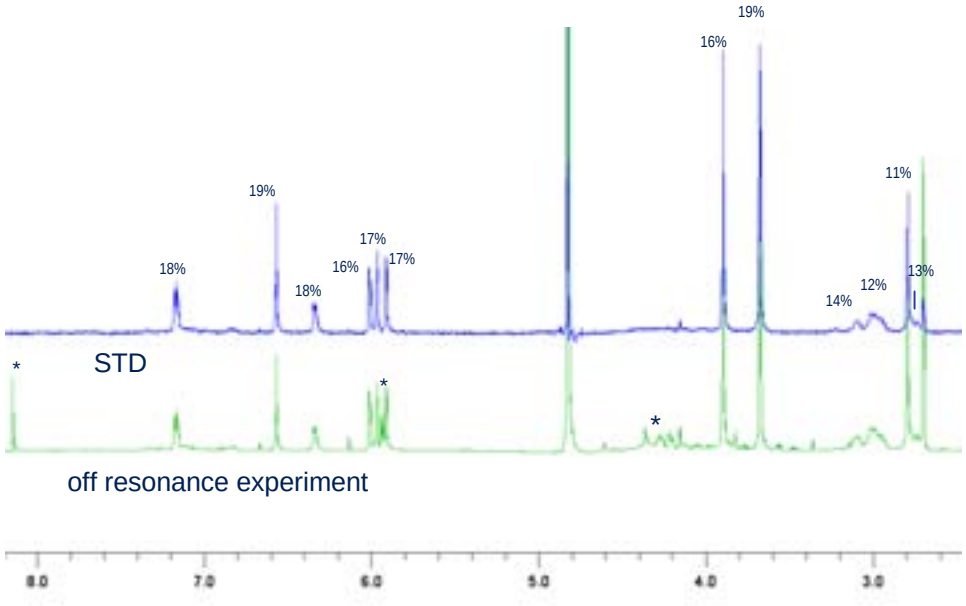
STD (Saturation Transfer Difference)

Irradiation at the aromatic or aliphatic NMR regions

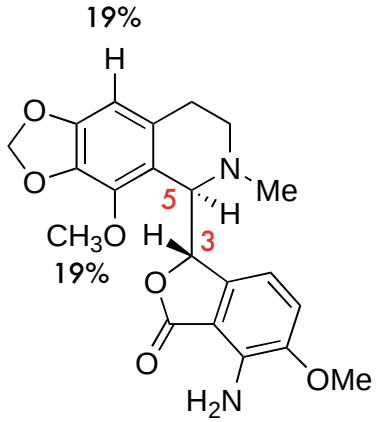
$$STD = \frac{I0 - I}{I0} \times 100$$



Saturation is transferred to the bound ligand (Fast exchange is required)



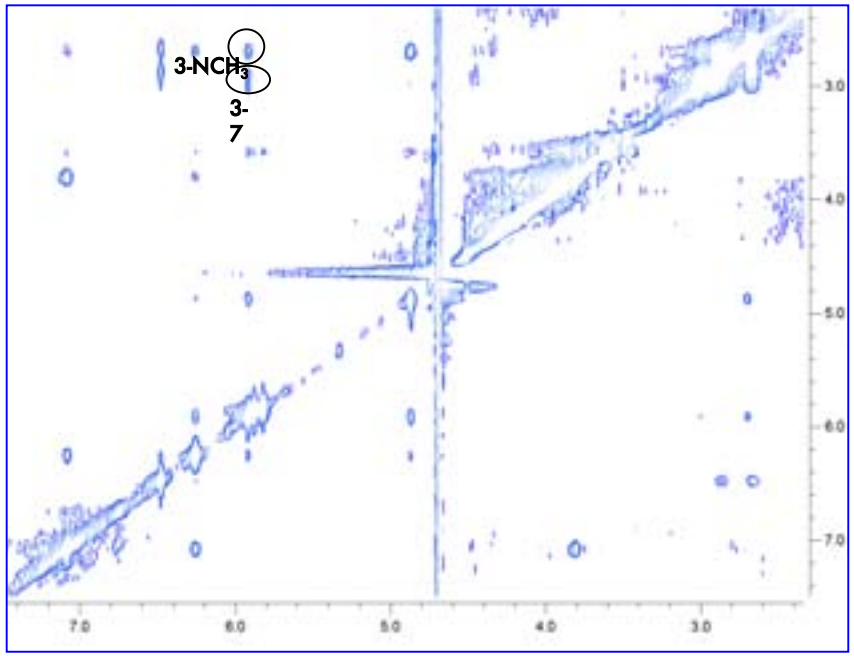
10 mM sodium phosphate, 0.1 mM GTP, pD 7.0, D_2O
Ligand-protein ratio 30:1

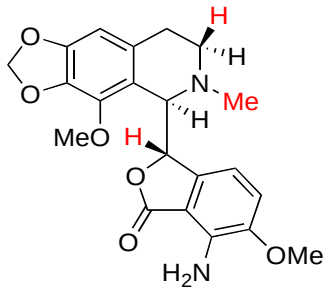


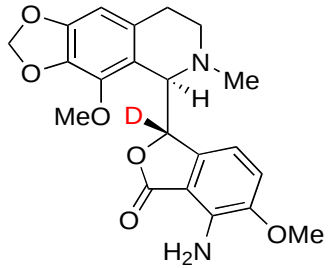
Bioactive Conformation: TR-NOESY

TR-NOE EXPERIMENTS

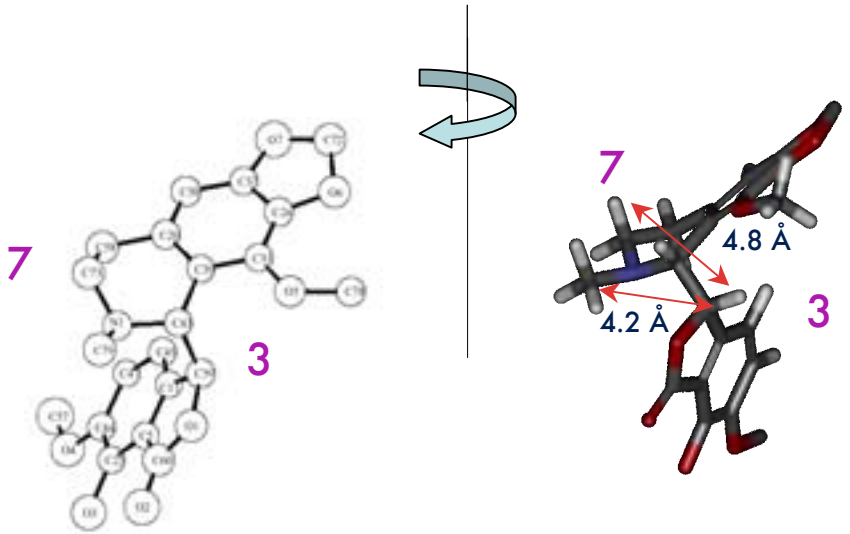
TR-NOE mixing time 100 ms, 300 µM ligand/ 10 µM tubulin







- Strong negative NOE cross peaks with tubulin
- Confirming Nosc-7-Aniline binding to tubulin



Nosc-7-Phenol X-ray Structure

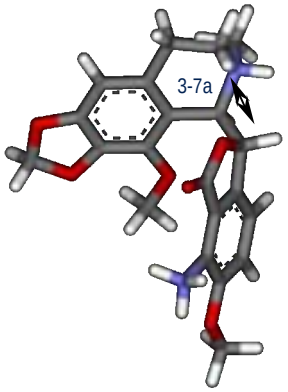
This conformation can NOT explain NOEs 3-7 and 3-NCH_3

Bioactive Conformation: NMR & Modeling

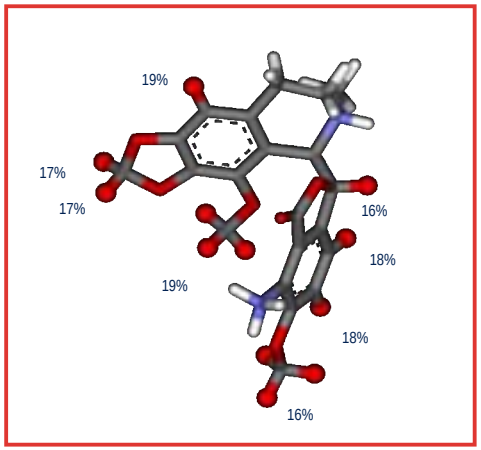
CONFORMATIONAL SEARCH MAESTRO 7.5 (SCHRODINGER SOFTWARE)

	Exp. distance estimation (Å)	Maestro conformer	X-ray
H3-H7	3.2	3.0	4.8
H3-NCH3	3.6	3.8	4.2

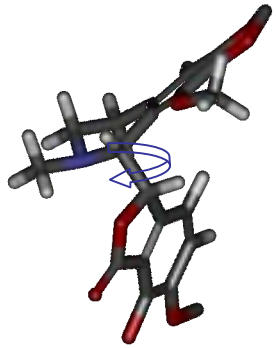
Reference distance H4A-H5A 2.4 Å



Best match of NMR-Modeling bound conformation



Higher STD effects mapped in the bound conformation



Small-molecule X-ray structure

Bennani et al. J. Med. Chem., 2012, 55 (5), pp 1920–1925

Target JAK2 for myeloproliferative neoplasms

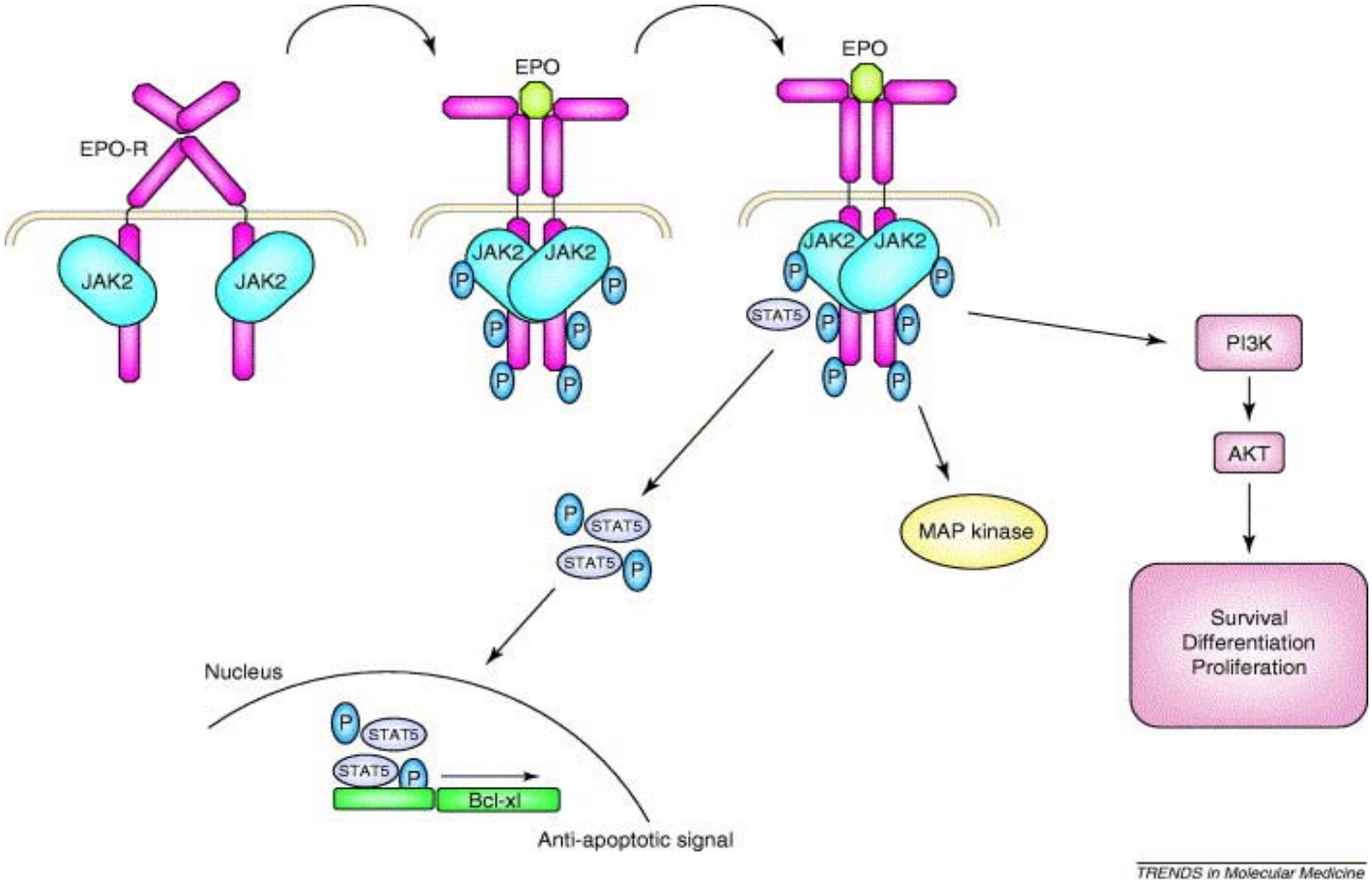
- *MPN (PV, ET, cIMF):*
  - *Polycythemia Vera (PV) (V617F, 90+%)*
  - *Essential thrombocythemia (ET) (V617F, 32-57%)*
  - *Chronic Idiopathic myelofibrosis (cIMF) (V617F, 35-51%)*
- *Annual US incidence of MPD of ~20,000 patients translates to a prevalence of 80-100,000 cases*

Current therapy: Hydroxyurea and phlebotomy

- *JAK2 is a cytoplasmic non-receptor tyrosine kinase*
- *Plays a key role in the EPO/EPOr/JAK2/STAT5 signaling cascade*
- *A causal relationship between mutant forms of JAK2 and MPNs has been established*

James C. et al; Nature, 434, 1144, 2005; Baxter, E. J. et al; Lancet, 365, 1054, 2005
Kralovics, R. et al; The NEJM, 352, 17, 1779, 2005

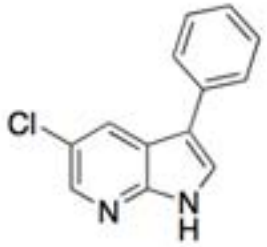
JAK2 Signaling



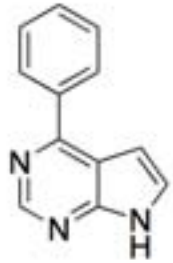
- *EPO triggers committed erythroid progenitors to undergo erythroid differentiation through JAK2/Stat5*
- *JAK2 Inhibition should benefit MPN-patients control erythroid differentiation*

Design of novel JAK2-Inhibitor chemotypes

Early Hits



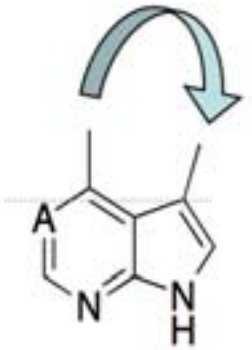
JAK2 Ki = 0.26 μM



JAK2 Ki = 0.48 μM

Overlay of putative binding orientations

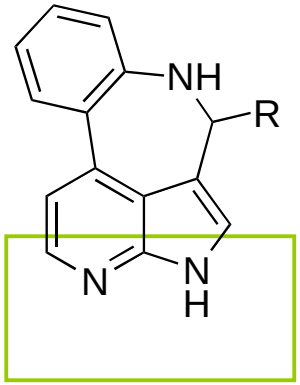
Ring Constrain?

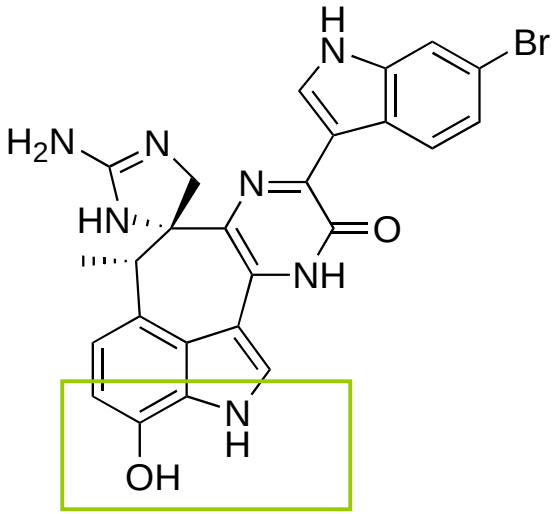


Precedent for 3,4-Fused (Aza)Indoles?

Design of a Hybrid Azaindole Lead

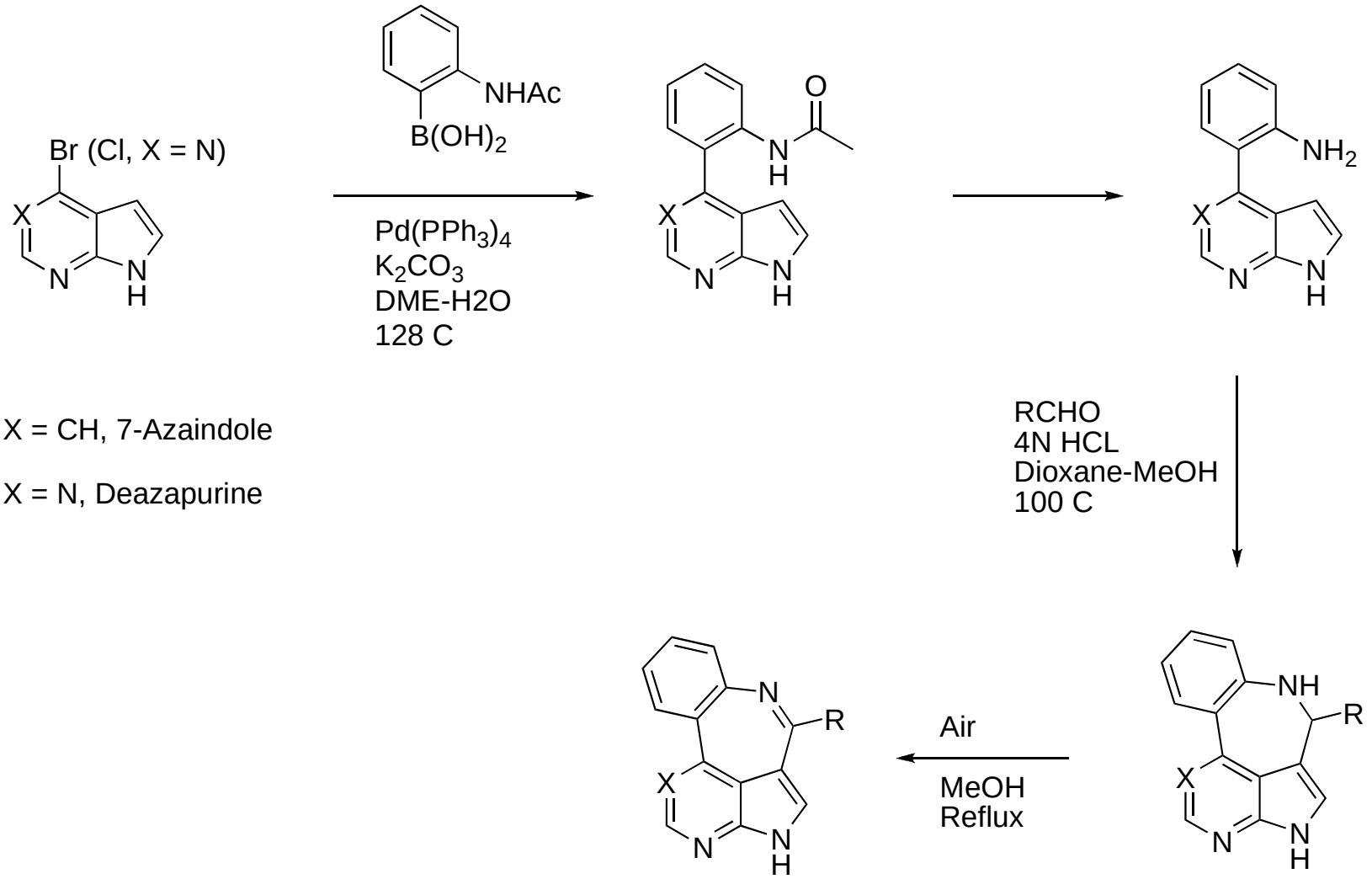
Overlay of putative binding orientations





Dragmacidin E

Entry to 3,4-Fused Azaindoles & Deazapurines?

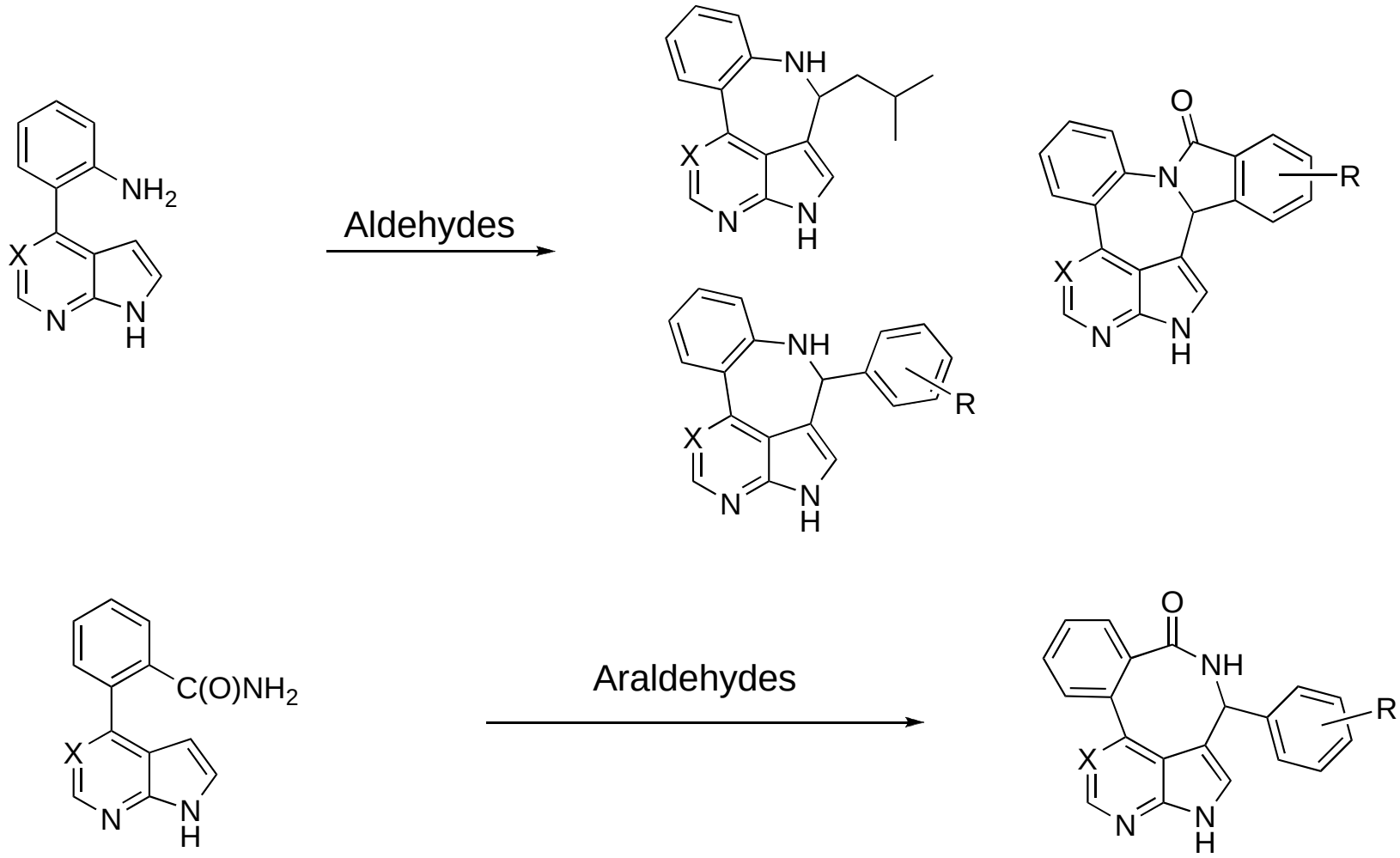


X = CH, 7-Azaindole

X = N, Deazapurine

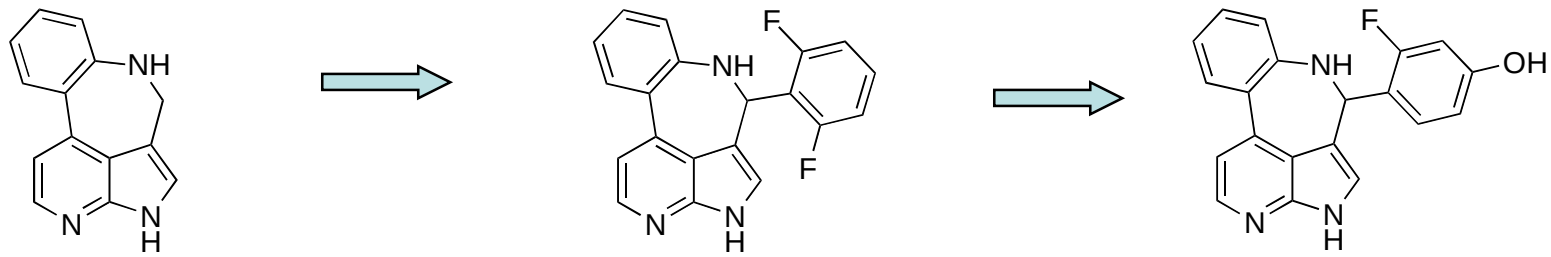
Wang, T. et al. ; Bioorg. Med. Chem,. Lett. 20, 152, 2010

Condensation chemistry is broadly applicable



Wang, T. et al. ; Bioorg. Med. Chem,. Lett. 20, 152, 2010

Lead evolution towards cellular potency



VRT-1

JAK2 Ki = 0.75 μM
JAK3 Ki = 0.83 μM

VRT-2

JAK2 Ki = 0.13 μM
JAK3 Ki = 0.29 μM

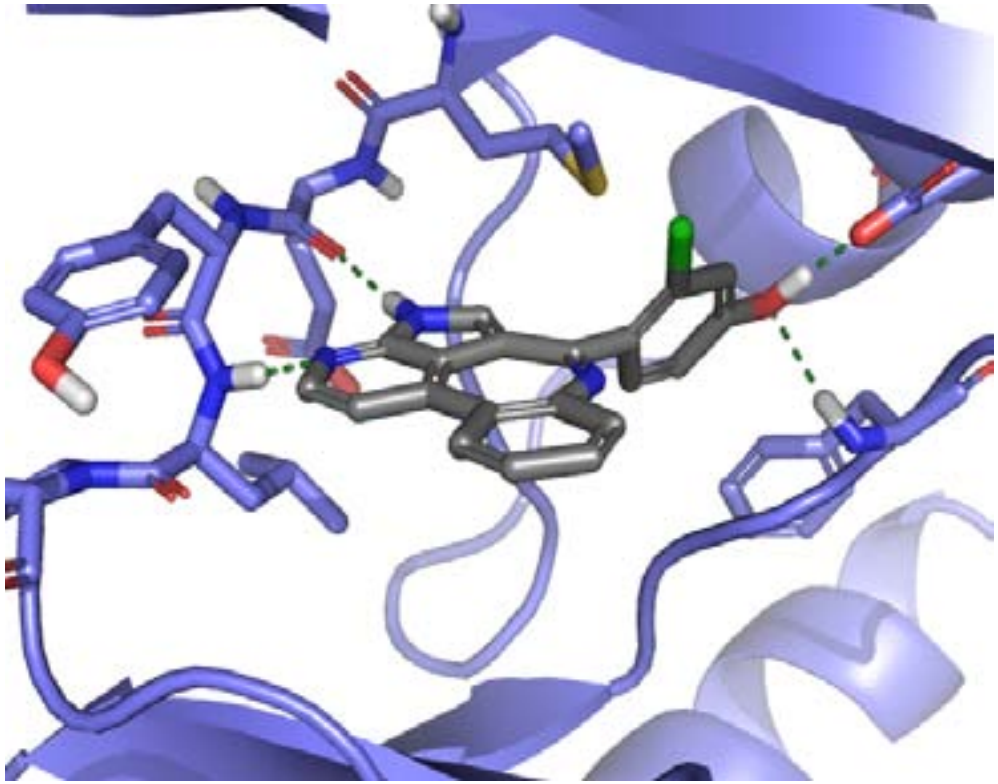
VRT-3

JAK2 Ki = 0.0006 μM
JAK3 Ki = 0.003 μM

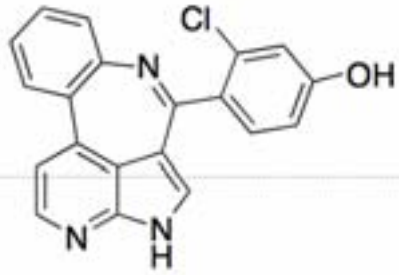
TF1-GMCSF pSTAT5 IC_{50}= 0.72 μM
HT2-IL2 pSTAT5 IC_{50} = 0.53 μ M

Ki ≤ 1 nM needed for cellular potency

What is so special about the phenol?



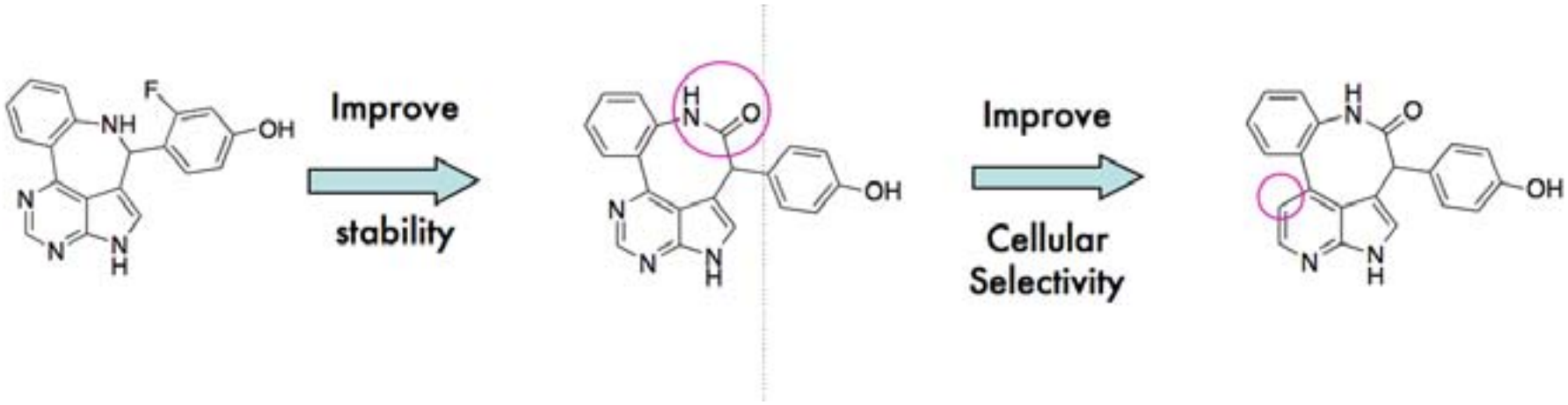
Co-complex of JAK2/VRT-4



JAK2 Ki = 0.8 nM
TF1 pSTAT5 IC_{50} = 0.16 μM

- Key H-bonds to Leu-932 and Glu-930 preserved (hinge region)
- The phenol-OH acts as both
  - *H-donor to Glu-898 carboxylate*
  - *H-acceptor from Phe-995 NH*

JAK-2 Lead evolution towards PK



VRT-5

JAK2 Ki = 0.0003 μM
JAK3 Ki = 0.001 μM

TF1 pSTAT5 = 0.14 μM
HT2 pSTAT5 = 0.07 μM

Poor microsomal stability
High Cl (>100)

VRT-6

JAK2 Ki = 0.0004 μM
JAK3 Ki = 0.002 μM

TF1 pSTAT5 = 0.13 μM
HT2 pSTAT5 = 0.17 μM

RLM = 96% remain@ 30min
Rat IV Cl = 16 mL/min/kg
T1/2 = 2.3 hr, Vss = 2.3 L/kg

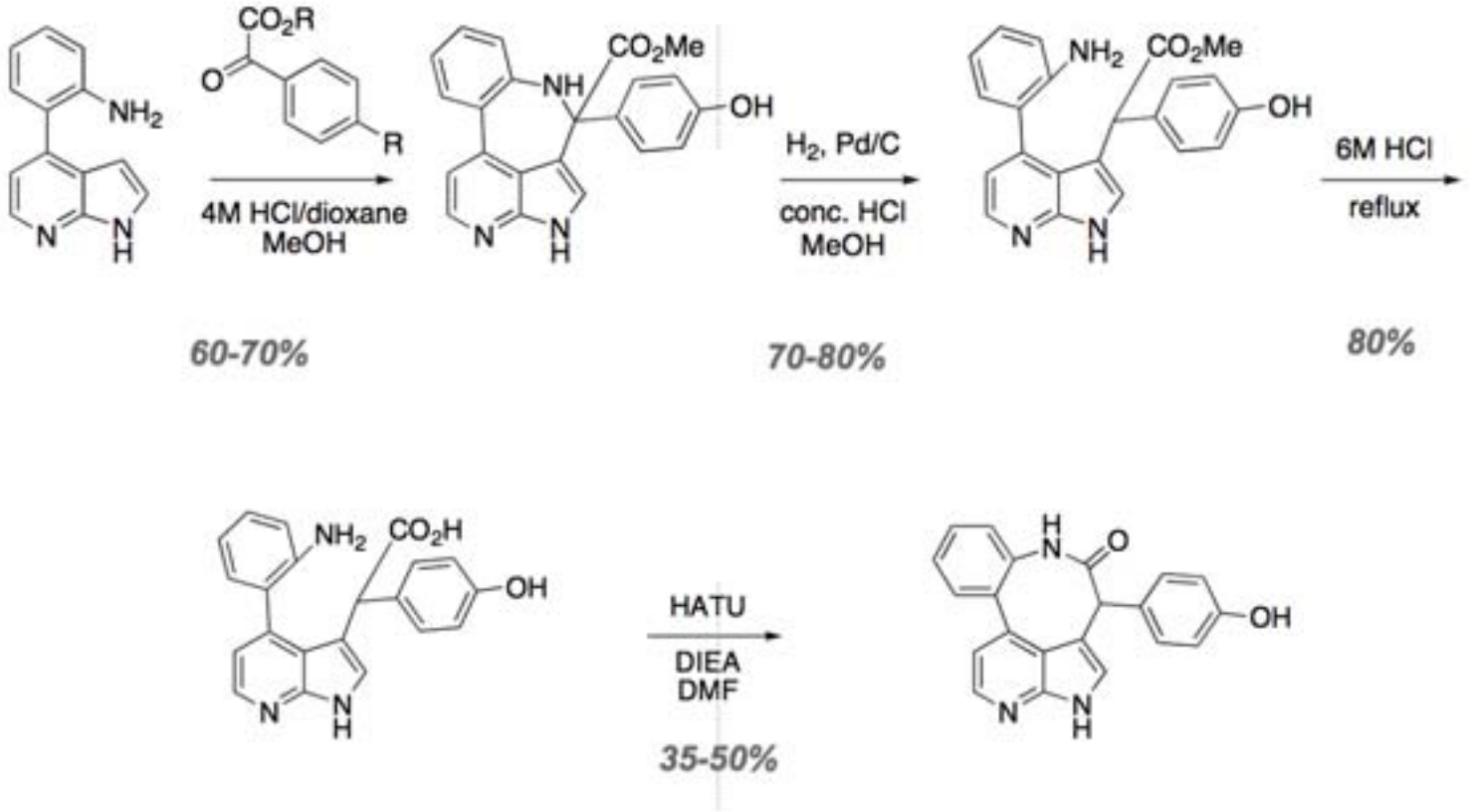
VRT-7

JAK2 Ki = 0.001 μM
JAK3 Ki = 0.006 μM

TF1 pSTAT5 = 0.26 μM
HT2 pSTAT5 = 1.5 μM

RLM = 96% remain@ 30min
Rat IV Cl = 23 mL/min/kg
T1/2 = 3.4 hr, Vss = 5.7 L/kg

Synthesis of Lead Compound

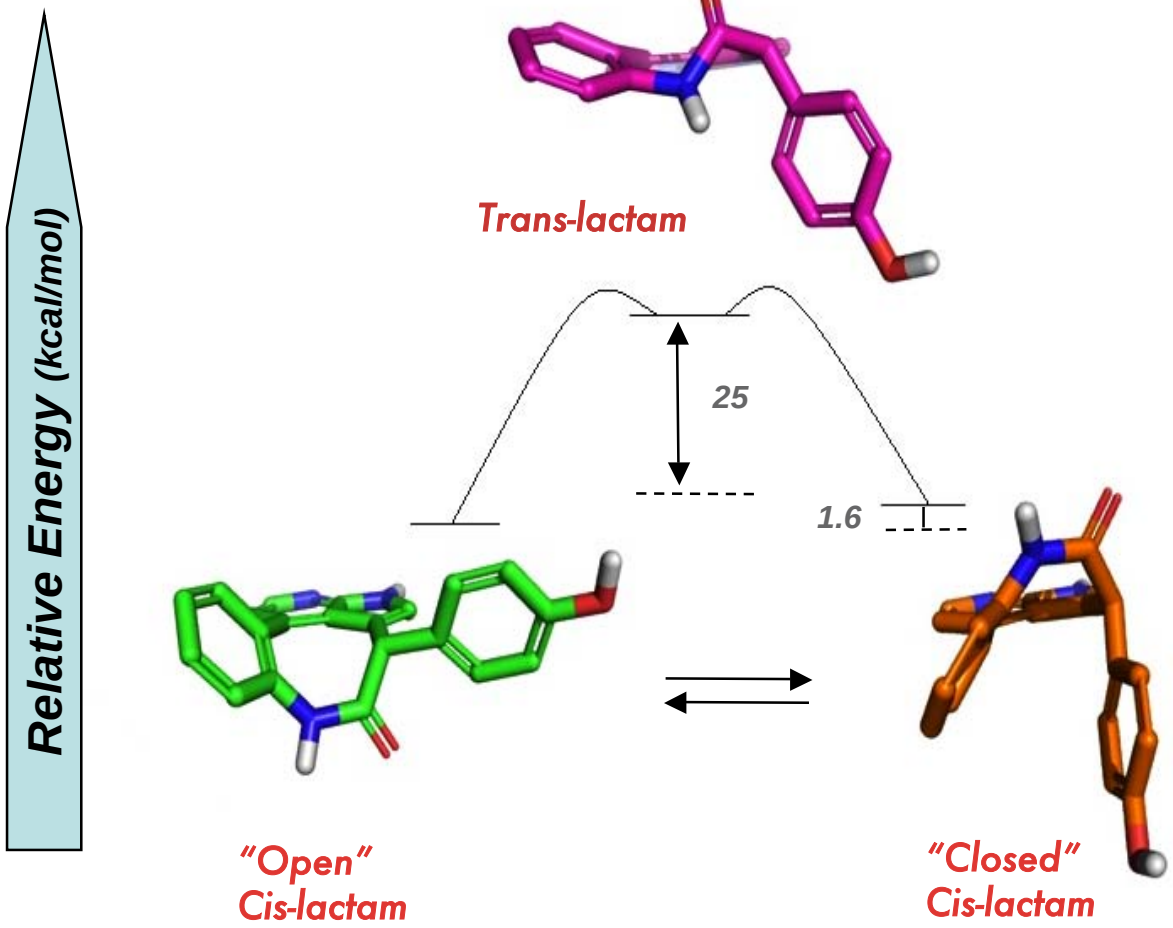


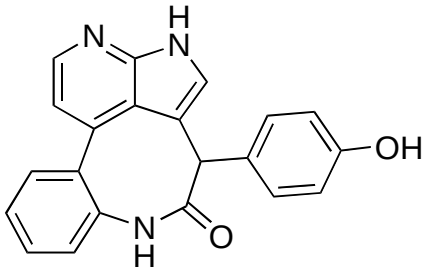
Two cyclization products with equal MW isolated in ~2:1 ratio

Isomers independently equilibrate to ~2:1 ratio products

Ledeboer, M. W. et al ; J. Med. Chem. 52, 7938, 2009

Rotational energy barrier!

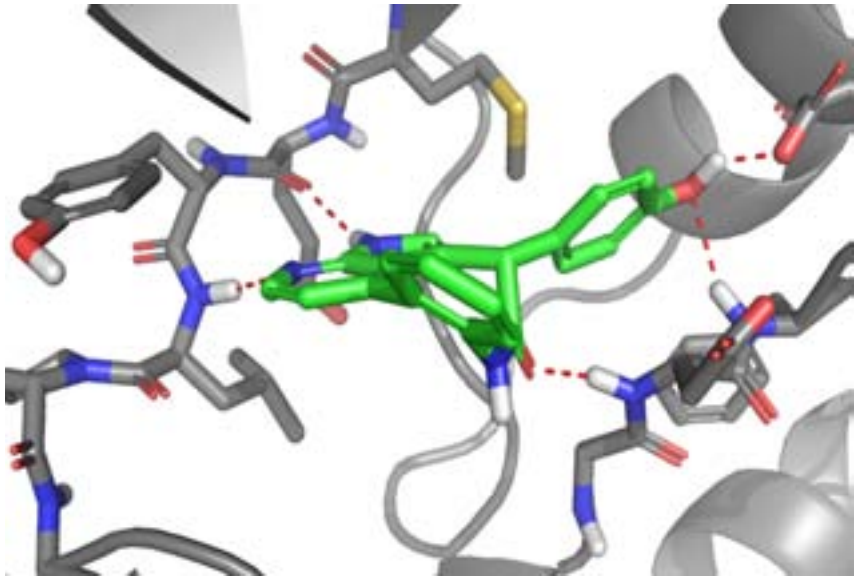


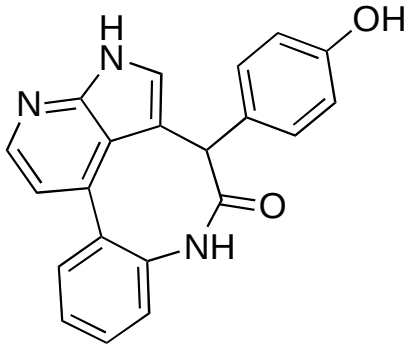


JAK2 Ki = 0.001 μM
JAK3 Ki = 0.006 μ M

- *Isomers appear equipotent*
- *Isomers independently equilibrate to ~2:1 ratio of open vs. closed forms*
- *Slow equilibration is consistent with high barrier to inter-conversion*

Lead Complexed with JAK2 (X-Ray)



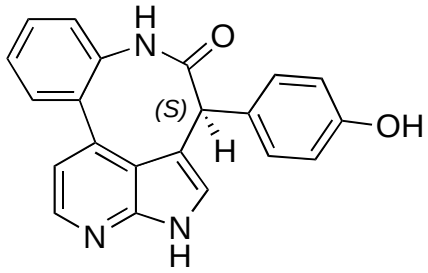


- *Bound conformation corresponds to "open" form (S-stereochemistry)*
  - *Key H-bonds to the hinge region are preserved*
  - *The phenolic-OH is involved in H-bonding to Glu-898 and Phe-995*
  - *The amide carbonyl picks up an additional H-bond to Asp-944*

Ledeboer, M. W. et al ; J. Med. Chem. 52, 7938, 2009

Tool Compound for POC

VRT-7
MW 341.36



Enzyme	Ki (μM)
JAK2	0.001
JAK3	0.006
ALK	0.017
KIT	0.19
GSK	0.34*

Cell Assay	IC50 (μM)
TF1 (GMCSF) pSTAT5	0.19-0.43
HT-2 (IL2) pSTAT5	1.1-3.2

- Good potency/target affinity
- Excellent kinase selectivity
- Modest selectivity vs JAK3
  - J3/J2= 5-10 fold on cells
  - TF1 vs HT2
- Low MW, soluble,
- Stable in microsomes
- WS1, 72 hr. CC_{50} > 20 μM
- No significant CYP inhibition

- *IC_{50}>500 nM vs. other kinases tested*

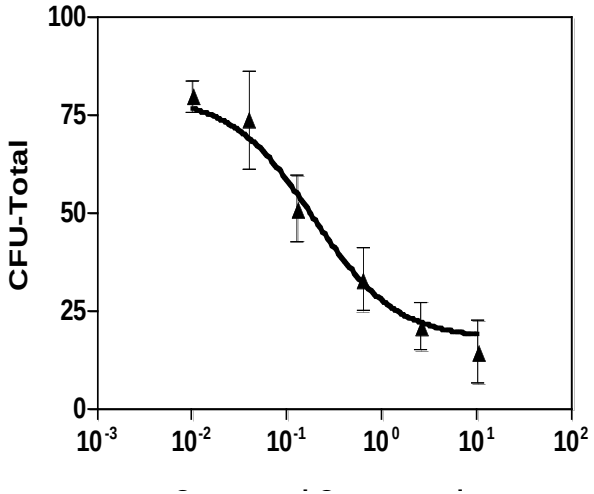
Lead Rodent Pharmacokinetics

	IV dose Rat	PO dose Rat	IV dose Mouse	PO dose Mouse
Dose (mg/Kg)	1.8	10	9	10
C_{max} (µg/mL)	0.70	1.08	7.3	1.0
T_{max} (hr)		3		2
$AUC_{0\text{-}inf}$ (µg·h/mL)	1.26	7.7	12.5	8.3
$T_{½}$ (hr)	3.4	3.6	2.7	3.2
CL (ml/min/Kg)	23.8		12.1	
V_{ss} (L/Kg)	5.7		2.3	
F (%)		100		66.1

Dose proportional PO PK in mice at 10/30/100 mg/Kg

In-vitro/In-vivo POC in Erythroid Colony Formation

PV-patients bone marrow samples: JAK2^{V617F} positive

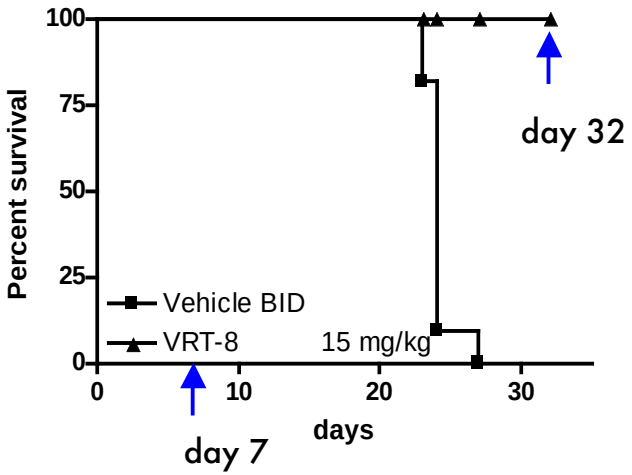


VRT-7

PV CFU-E AVG
IC_{50} = 0.87 µM

Dosing of BaF3$^{V617F-JAK2}$ (EPO-Independent) grafted mice

Effect on survival

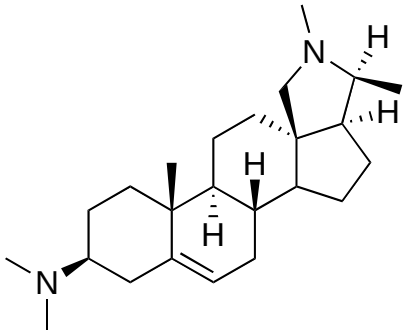


70% Reduction in spleenomegaly
Survival extended to day 32

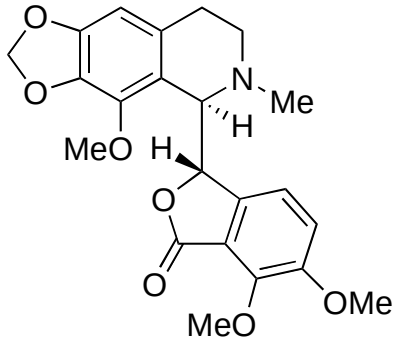
Michiels, J. J. et al; Semin. Thromb. Hemostasis, 23, 339, 1993.
Ledeboer, M. W. et al ; J. Med. Chem. 52, 7938, 2009

"3 Short Stories with 3 Natural Products"

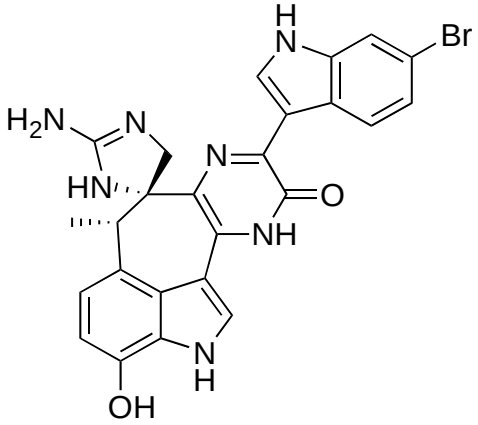
"Practicalia: One key reaction can open doors"



Conessine



Noscapine



Dragmacidin E

Three NPs lead to three new indications

Acknowledgments for Conessine Project

J. Med. Chem. **2008,** *51,* 5423–5430

The Alkaloid Conessine and Analogues as Potent Histamine H_3 Receptor Antagonists

Chen Zhao,* Minghua Sun, Youssef L. Bennani,† Sujatha M. Gopalakrishnan, David G. Witte, Thomas R. Miller, Kathleen M. Krueger, Kaitlin E. Browman, Christine Thiffault, Jill Wetter, Kennan C. Marsh, Arthur A. Hancock, Timothy A. Esbenshade, and Marlon D. Cowart

Department of Neuroscience Research, Global Pharmaceutical Research Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6123

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Design of a New Histamine H_3 Receptor Antagonist Chemotype: (3a*R*,6a*R*)-5-Alkyl-1-aryl-octahydropyrrolo[3,4-*b*]pyrroles, Synthesis, and Structure−Activity Relationships

Chen Zhao,* Minghua Sun,† Youssef L. Bennani,‡ Thomas R. Miller, David G. Witte, Timothy A. Esbenshade, Jill Wetter, Kennan C. Marsh, Arthur A. Hancock, Jorge D. Brioni, and Marlon D. Cowart

Department of Neuroscience Research, Global Pharmaceutical Research Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6123. †Present address: Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, CA 94080. ‡Present address: Vertex Pharmaceuticals, 130 Waverly Street, Cambridge, MA 01239.

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Discovery of S-Phase Arresting Agents Derived from Noscapine

James T. Anderson, Anthony E. Ting, Sherry Boozer, Kurt R. Brunden, Joel Danzig, Tom Dent, John J. Harrington, Steven M. Murphy, Rob Perry, Amy Raber, Stephen E. Rundlett, Jianmin Wang, Nancy Wang, and Youssef L. Bennani

J. Med. Chem., **2005**, *48* (8), pp 2756-2758

Identification of Novel and Improved Antimitotic Agents Derived from Noscapine

James T. Anderson, Anthony E. Ting, Sherry Boozer, Kurt R. Brunden, Chris Crumrine, Joel Danzig, Tom Dent, Laurel Faga, John J. Harrington, William F. Hodnick, Steven M. Murphy, Gary Pawlowski, Robert Perry, Amy Raber, Stephen E. Rundlett, Alain Stricker-Krongrad, Jianmin Wang, and Youssef L. Bennani

J. Med. Chem., **2005**, *48* (23), pp 7096-7098

From Vertex

Chemistry

Wenxin Xu
Tiansheng Wang

Cell Biology

Brenda Eustace
Russ Hoover

Biochemistry

Azin Nezami
Kirk Tanner

From Universidad Complutense Madrid

Structural Chemistry

Maria Angeles Canales Mayordomo

Biochemistry

Fernando Diaz

Janus Kinase-2 Inhibiton

Chemistry

Tiansheng Wang
John Duffy
Summer Halas
Jian Wang
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Ravi Ramachandran
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Martyn Botfield

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Stephanie Krogmeier

Research management

Mark Namchuk
Scott Raybuck
Raymond Winquist

Project management

Olga Futer

Strategic Alliances

Guido Buchbinder

Mosaic Laboratories LLC

Lisa M. Dauffenbach

Questions are welcome!