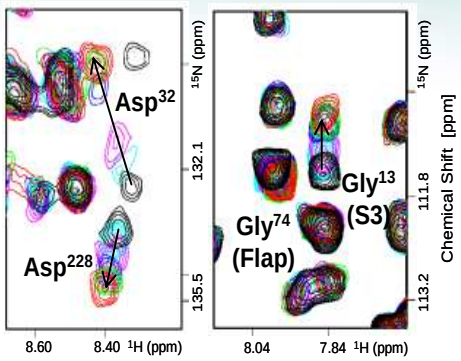
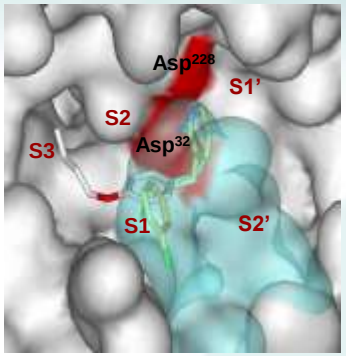
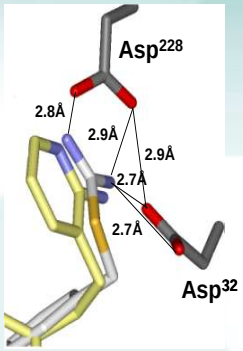
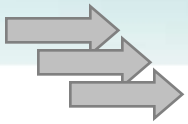








Clinical Candidates

From Weak NMR-detected Fragment Hits to Clinical Candidates for BACE-1

Daniel F. Wyss, Ph.D.
Merck Research Laboratories
Cambridge/Kenilworth

Fragment-Based Lead Discovery 2010 Conference
October 10-13, 2010, Philadelphia, PA

FBDD at legacy Schering-Plough, now MRL

- 10+ years experience in FBDD

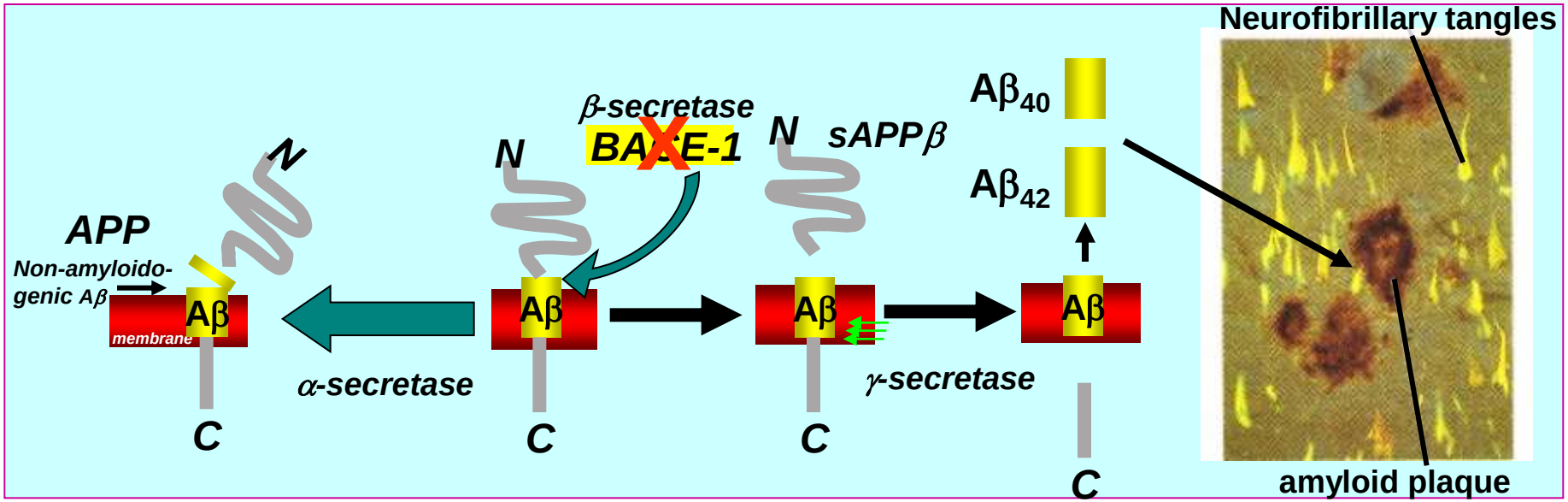
  General FBDD references:

  - *Curr Opin Drug Discov 5 (2002) 630*
  - *Modern Magn Reson 2 (2006) 1401*
  - *Frontiers Drug Des Discov 3 (2007) 171*
  - *Methods in Enzymology (in press)*

  - In-house FBDD approach based on NMR-based screening (SbN) of customized fragment libraries and structure-assisted (x-ray & NMR) fragment hit-to-lead optimization chemistry
  - Strategically applied to early & must win targets, and those struggling for leads
  - Fragment hit progression in 73% of SbN projects (for 75% of those structural data on fragment/protein complex available), yielding valid lead series in ~30%
  - BACE-1 clinical candidates are a direct result of FBDD

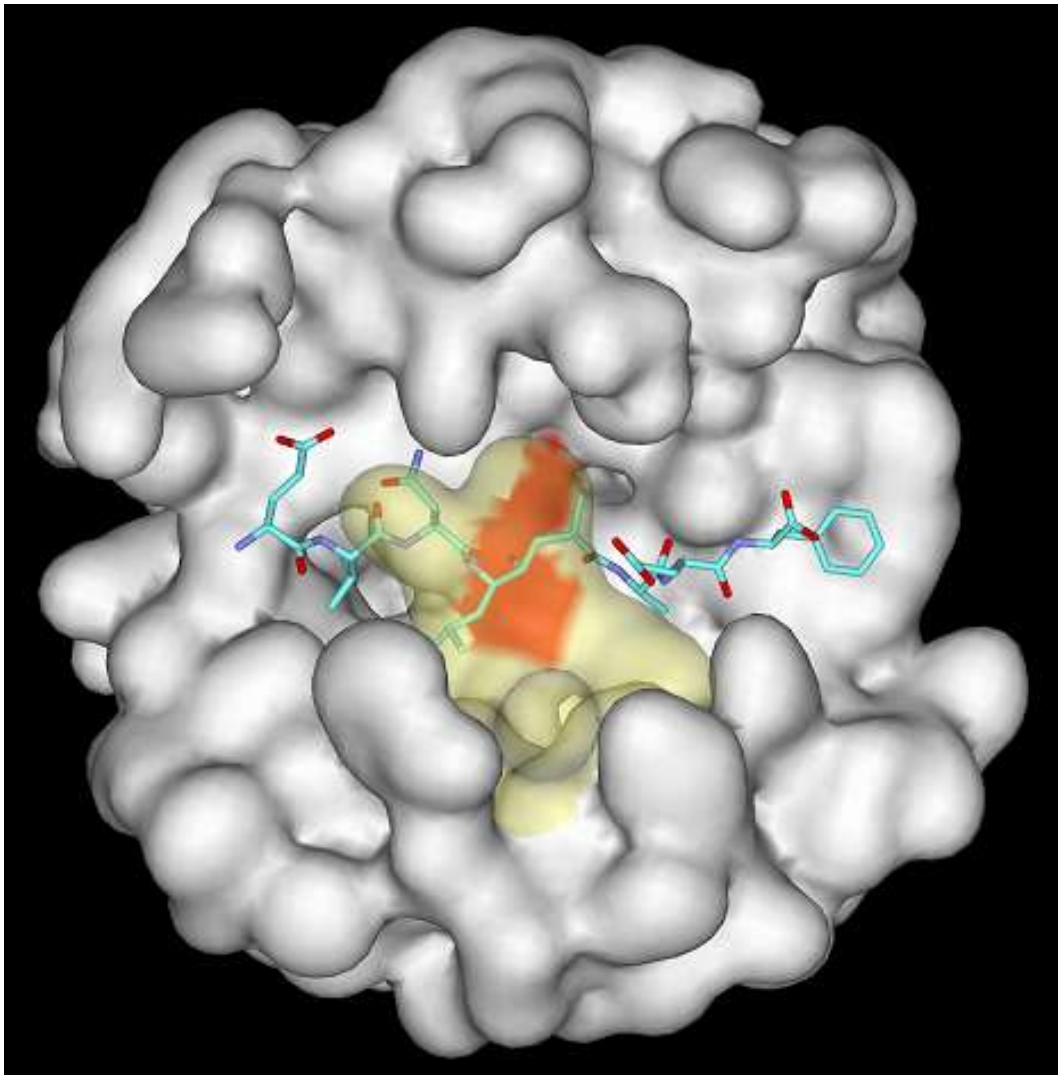
- Globalization of FBDD

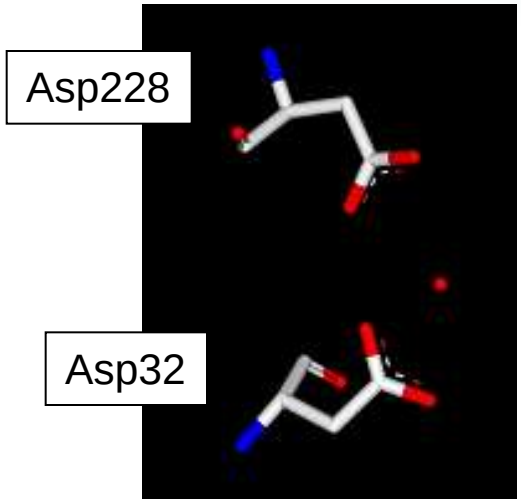
BACE-1 (β-secretase) as a AD target



- Amyloid hypothesis of AD supported by:
  - Genetics (FAD mutations increase Aβ), neuropathology (amyloid plaques) and clinical data (Aβ vaccine)
- BACE-1: therapeutic target for the treatment of Alzheimer's disease
  - Inhibition of BACE-1 should reduce Aβ production, have disease modifying effect on AD
- Historically highly challenging in obtaining CNS active BACE-1 inhibitors

BACE-1 characteristics



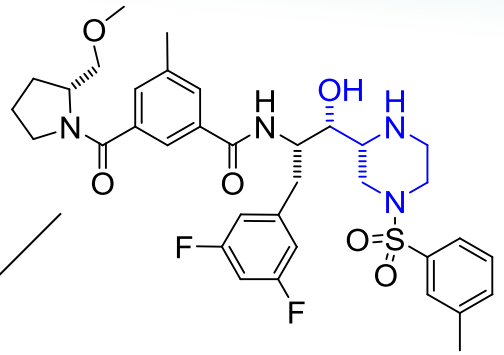


- Membrane-anchored aspartyl protease
- Expressed in endosomes within neurons
- Optimum pH ~5
- Extended substrate binding cleft – partially covered by flap

Tang et al., Science 290 (2000) 150
Ghosh et al., JACS 122 (2000) 3522

BACE-1 inhibitors – Design challenges

- CNS target
  - Brain penetration required
  - Intracellular localization – FU important
- Extended substrate binding cleft
  - Relatively open, hydrophilic active site
- Traditional aspartyl protease inhibitor scaffold
  - Poor pharmacokinetics, poor brain penetration, Pgp substrate
  - Did not reduce rat brain or CSF Aβ upon oral dosing



BACE1 K_i = 0.8 nM

cell $A\beta_{40}$ IC_{50} = 9 nM

- Molecular properties of inhibitor must enable effective concentrations to reach BACE-1 active site
  - New chemotypes required

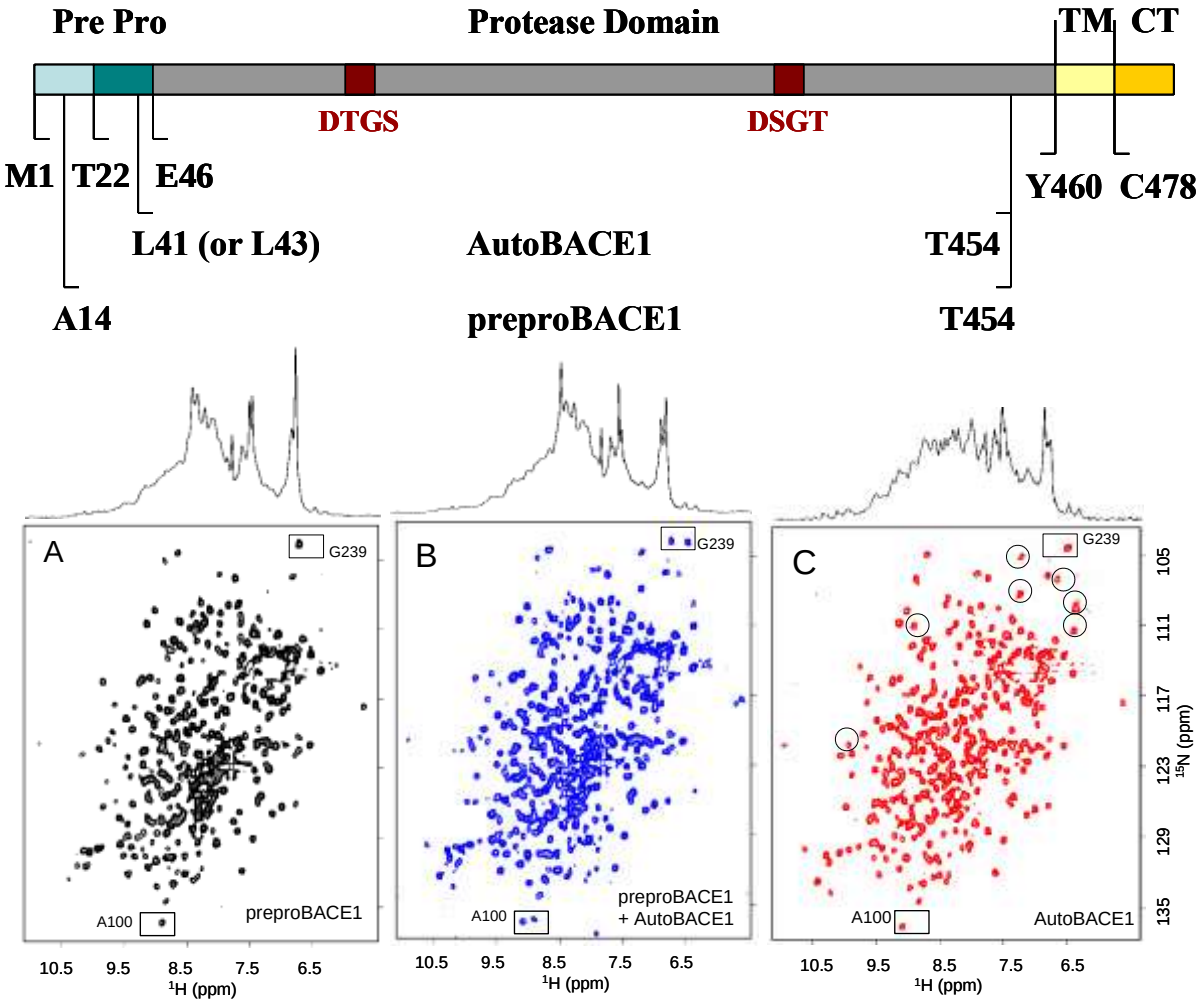
▶ High Throughput Screening methods

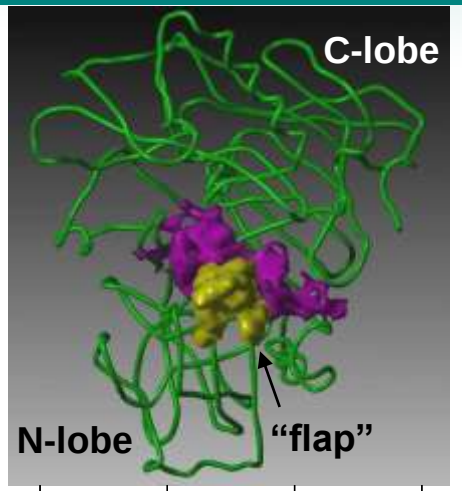
- No suitable leads

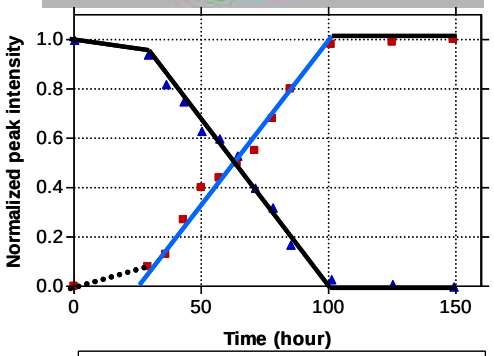
▶ SbN / FBDD

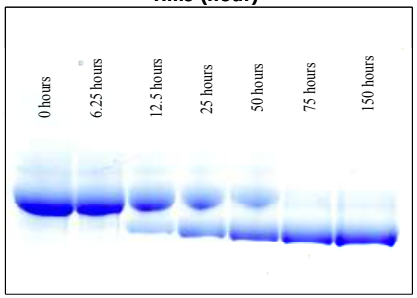
- Novel chemotypes yield clinical candidates

Large scale production of BACE-1 for FBDD





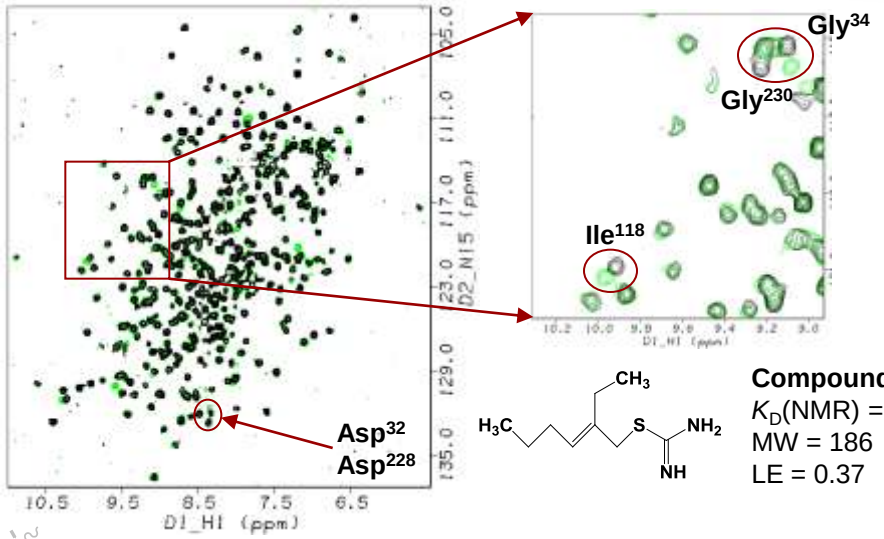




Biochemistry 44 (2005) 16594

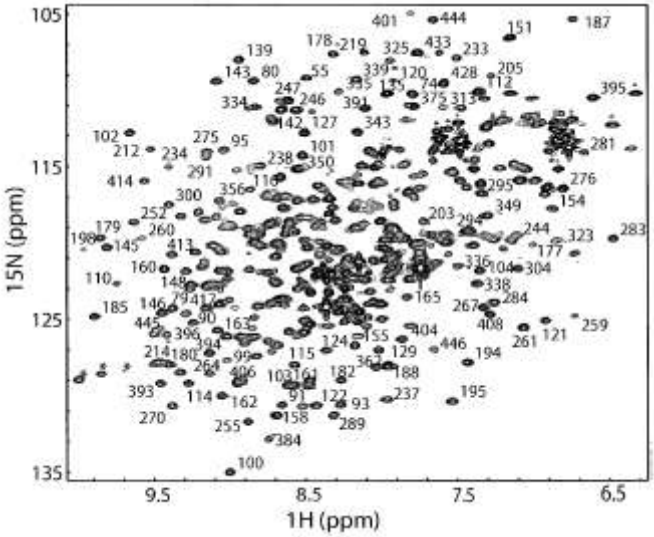
Fragment-based NMR screening

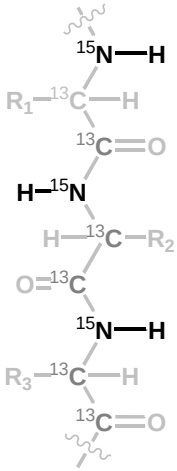
- HSQC-based SbN
  - Active site hits by CSP
  - Initially: active site map
  - Later: full assignments
  - >10k fragments in clusters of 12
  - Hit follow-up by NMR / x-ray
  - 9 distinct active site chemotypes identified (K_D[NMR] ~30μM-3mM)



Compound 1
K_D(NMR) = 550 μM
MW = 186
LE = 0.37

J Biomol NMR 29 (2004) 425

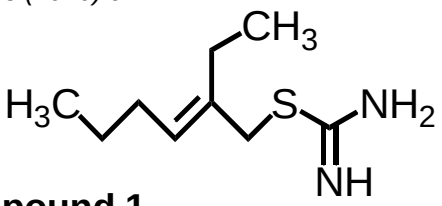




Sequence-specific NMR resonance assignments allowed more detailed binding evaluation of SbN hits

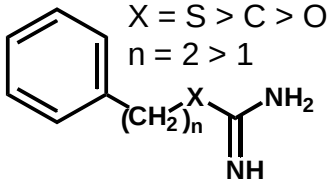
Isothiourea SbN fragment hit optimization

J Med Chem 53 (2010) 942

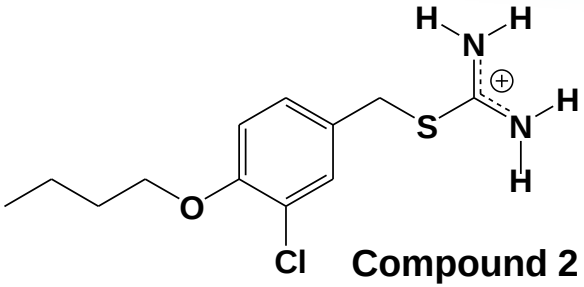


Compound 1

K_D(NMR) = 550 μM
MW = 186
LE = 0.37

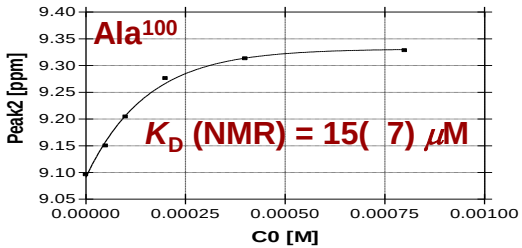


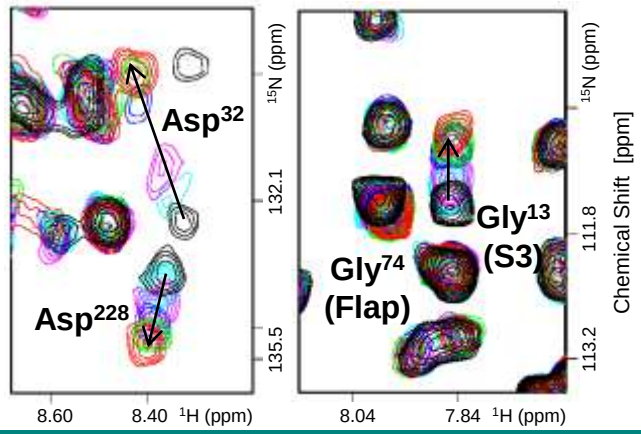
204 analogs

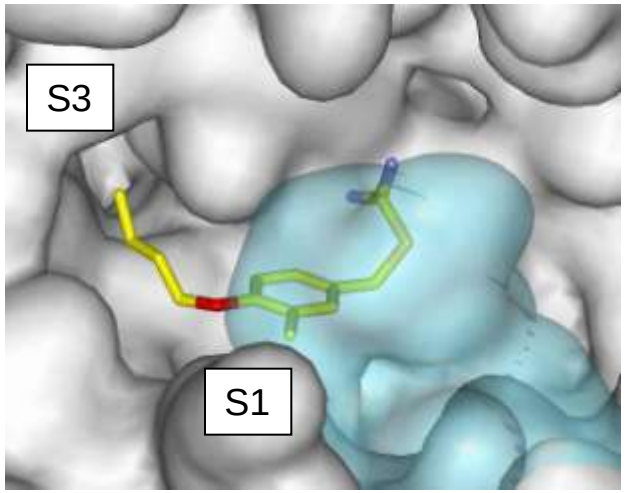


Compound 2

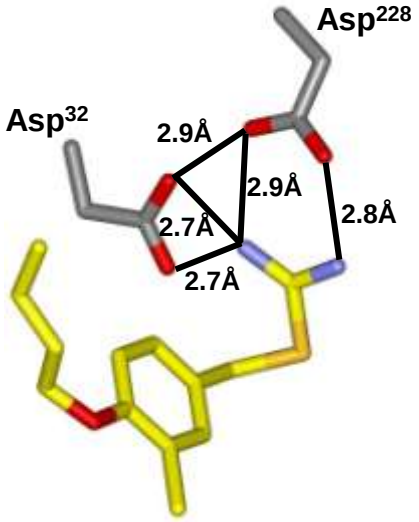
K_D(NMR) = 15 μM
IC_{50}(HTRF) ~ 210 μM
MW = 273
LE = 0.39







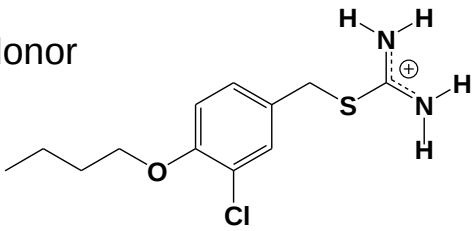
Crystal structure of **compound 2**



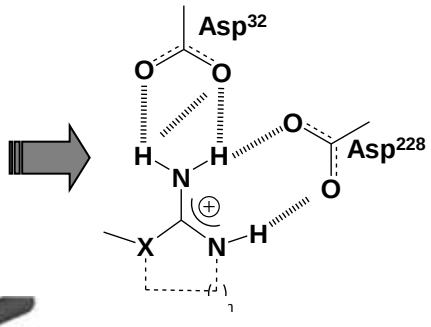
Search for heterocyclic isothiourea isosteres

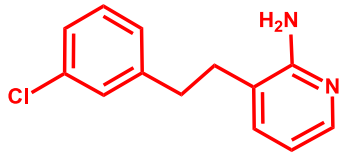
Goals:

- Pharmaceutically attractive
- Maintain H-bond interaction network
- pKa range ~ 6-10
- Limit # of H-bond donor

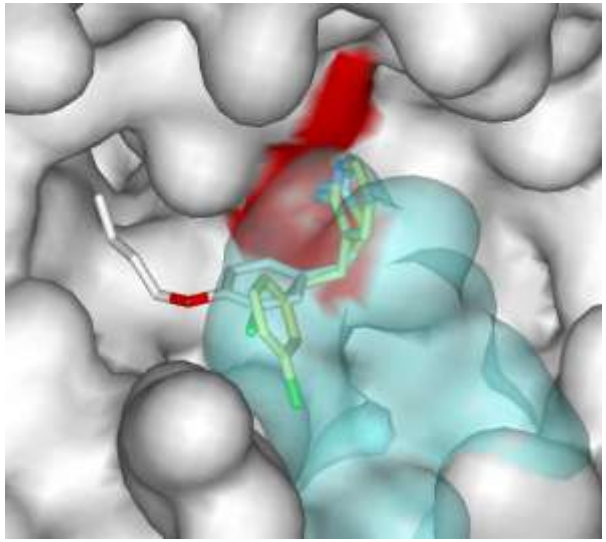


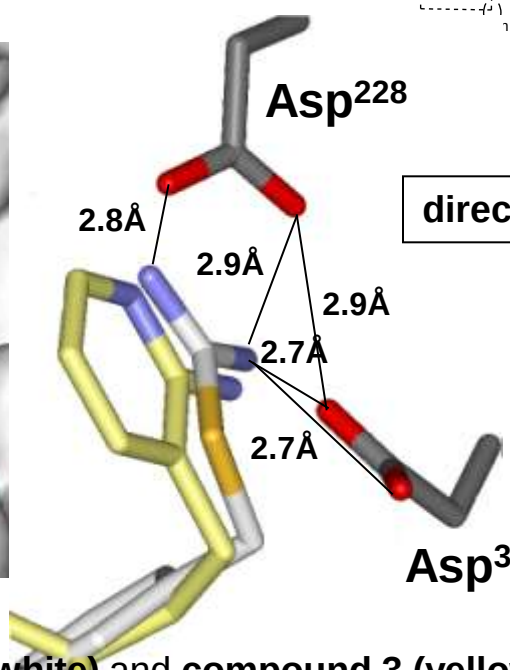
Compound 2
K_D(NMR) = 15 μM
pKa ~ 9
LE = 0.39





2-aminopyridines
Compound 3
K_D(NMR) = 32 μM
pKa ~ 7.2
CLogP = 3.4
MW = 233
LE = 0.38





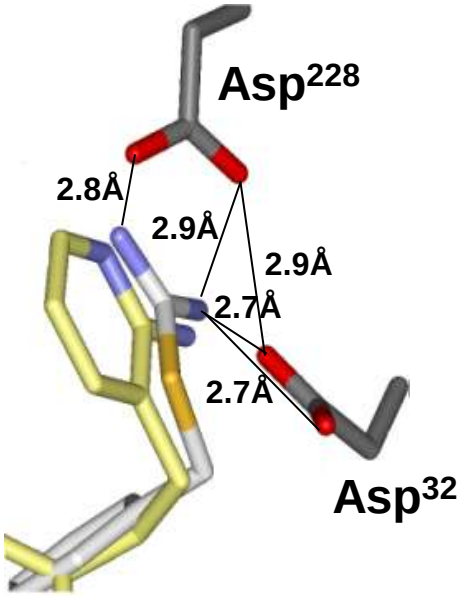
directed NMR screens

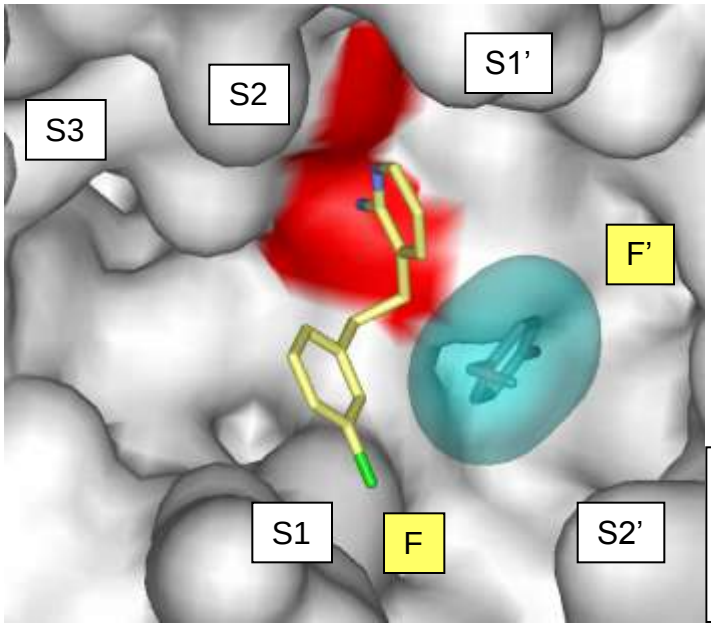
2-amino-pyridines
(exploratory chemistry)
2-amino-imidazoles
2-amino-benzimidazoles
2-amino-triazines
benzoamidines

crystal structures of **compound 2 (white)** and **compound 3 (yellow)**

2-Aminopyridine series

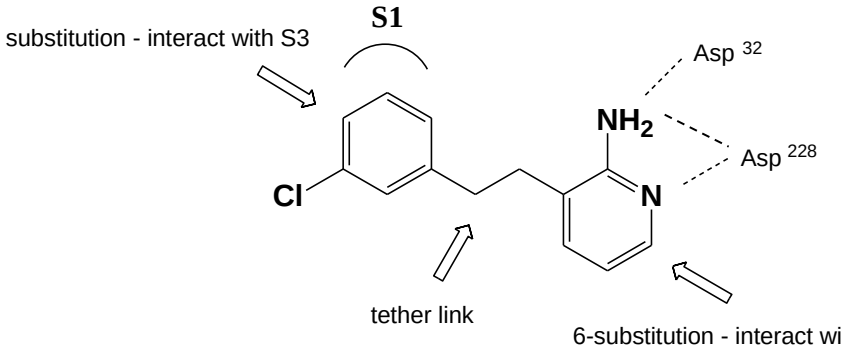
- Directed screen of thirty-one 2-amino-pyridines





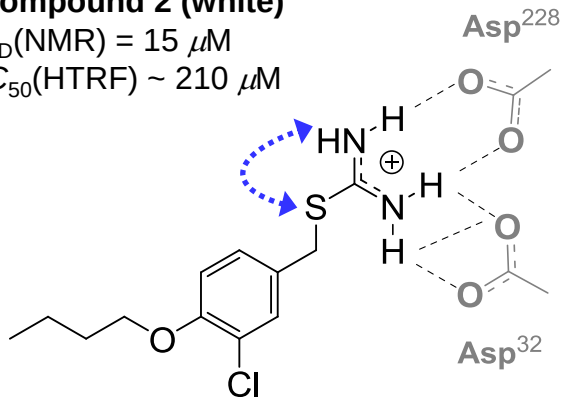
Compound 3 (yellow)
K_D (NMR) = 32 μM
low inhibition in assay
X-ray structure solved

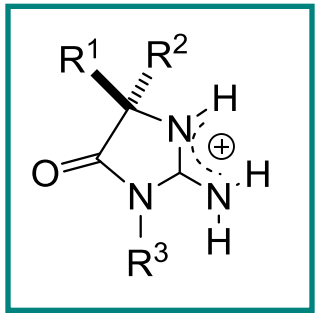
- Structure-assisted synthesis of extended 2-amino-pyridines



Structure-based design of iminohydantoins

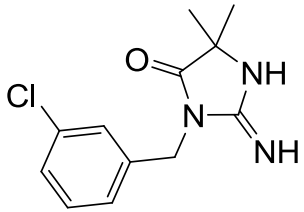
Compound 2 (white)
K_D(NMR) = 15 μM
IC_{50}(HTRF) ~ 210 μM



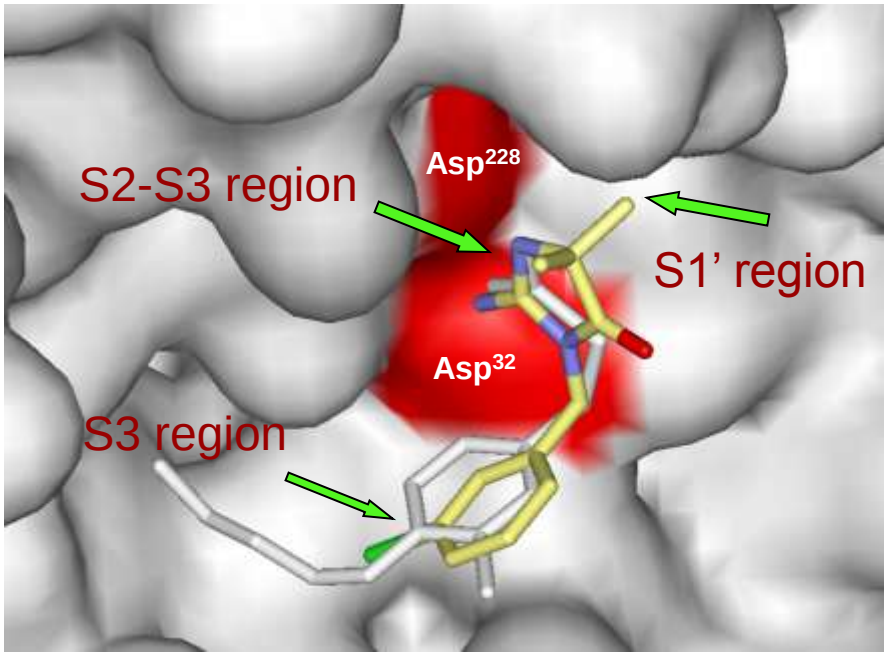


pK_a ~7-8
Rapoport, J Org Chem 33 (1968) 552

J Med Chem 53 (2010) 951



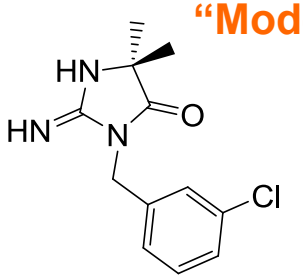
Iminohydantoin
Compound 4 (yellow)
K_D(NMR) = 200 μM
IC_{50}(HTRF) > 500 μM
pKa = 7.2
logP = 1.3
MW = 251.7
LE = 0.30



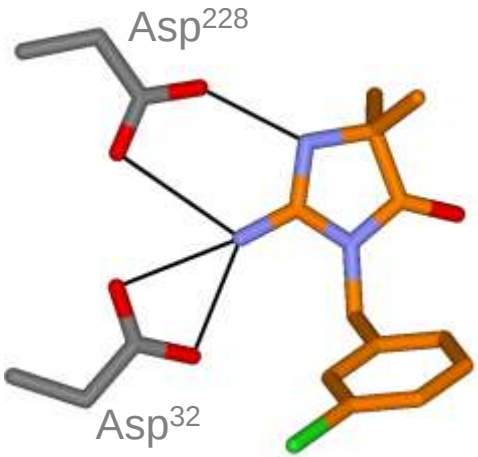
- Exploratory Med Chem designs N^1- and N^3-substituted iminohydantoins
- SbN demonstrates active site binding of N^3-substituted iminohydantoins
- Novel isostere for isothiourea SbN hit:
  - improved chemical stability
  - pKa compatible with CNS penetration
  - chemically modifiable
  - proprietary lead series

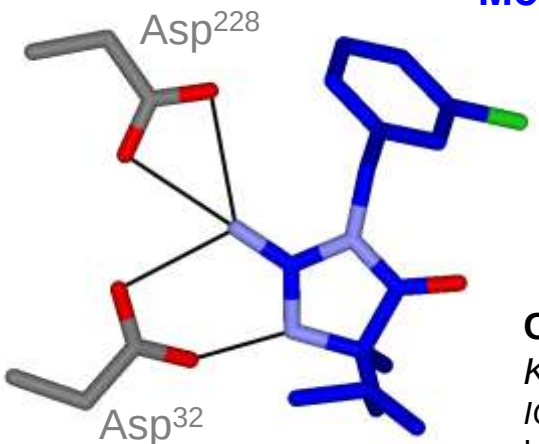
2nd binding mode of iminohydantoin core

"Mode A"

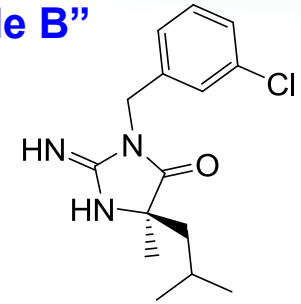


Compound 4 (orange)
K_D(NMR) = 200 μM
LE = 0.30

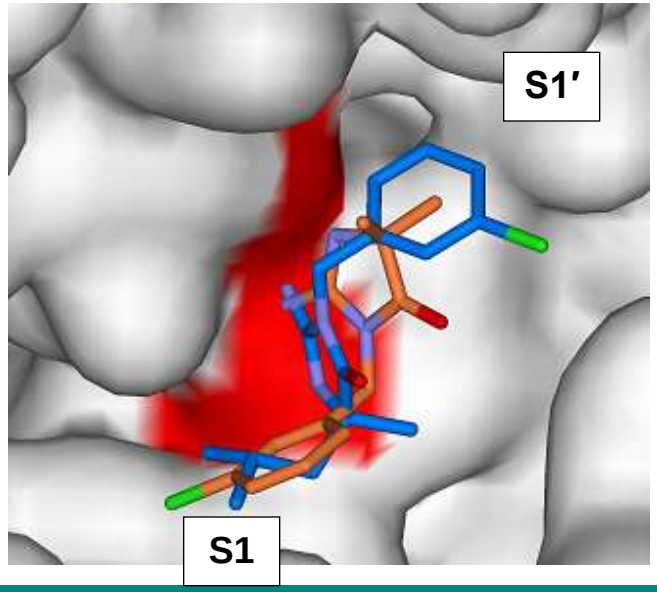




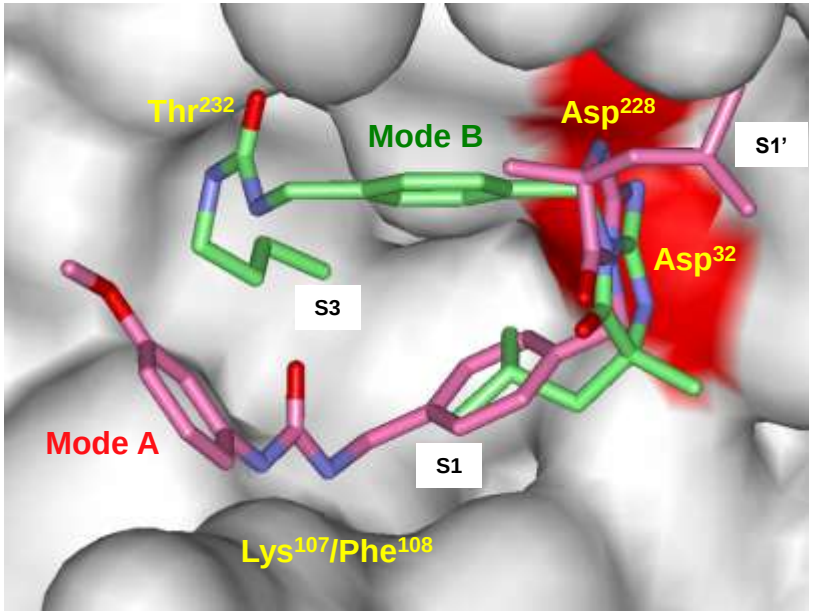
"Mode B"

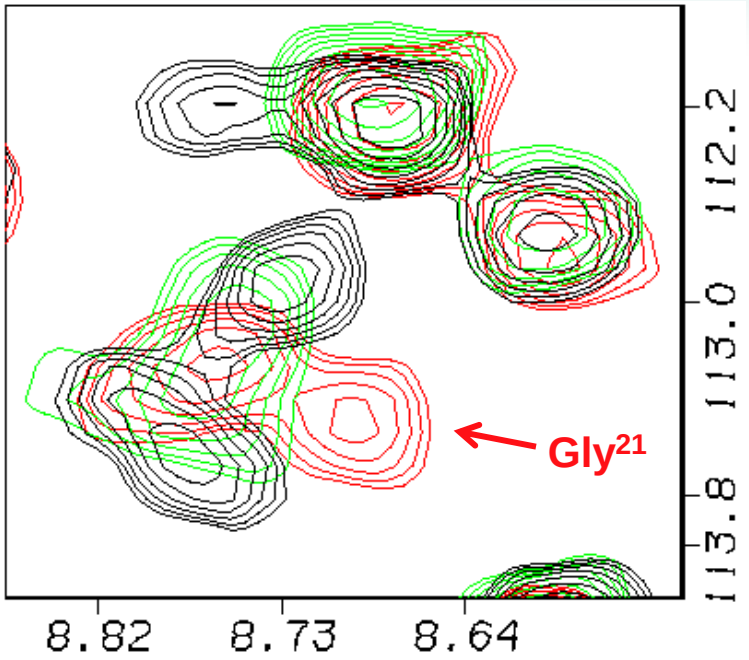


Compound 5 (blue)
K_D(NMR) = 120 μM
IC_{50}(HTRF) ~ 300 μM
LE = 0.27

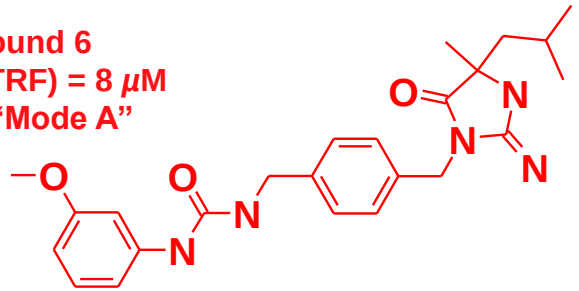


Iminohydantoin fragment-hit-to-lead optimization

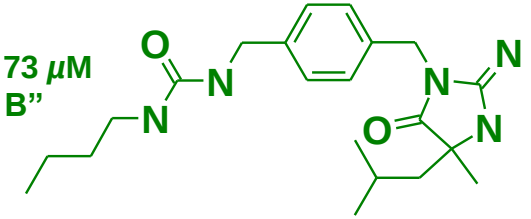




Compound 6
***IC*$_{50}$(HTRF) = 8 μM**
NMR: "Mode A"

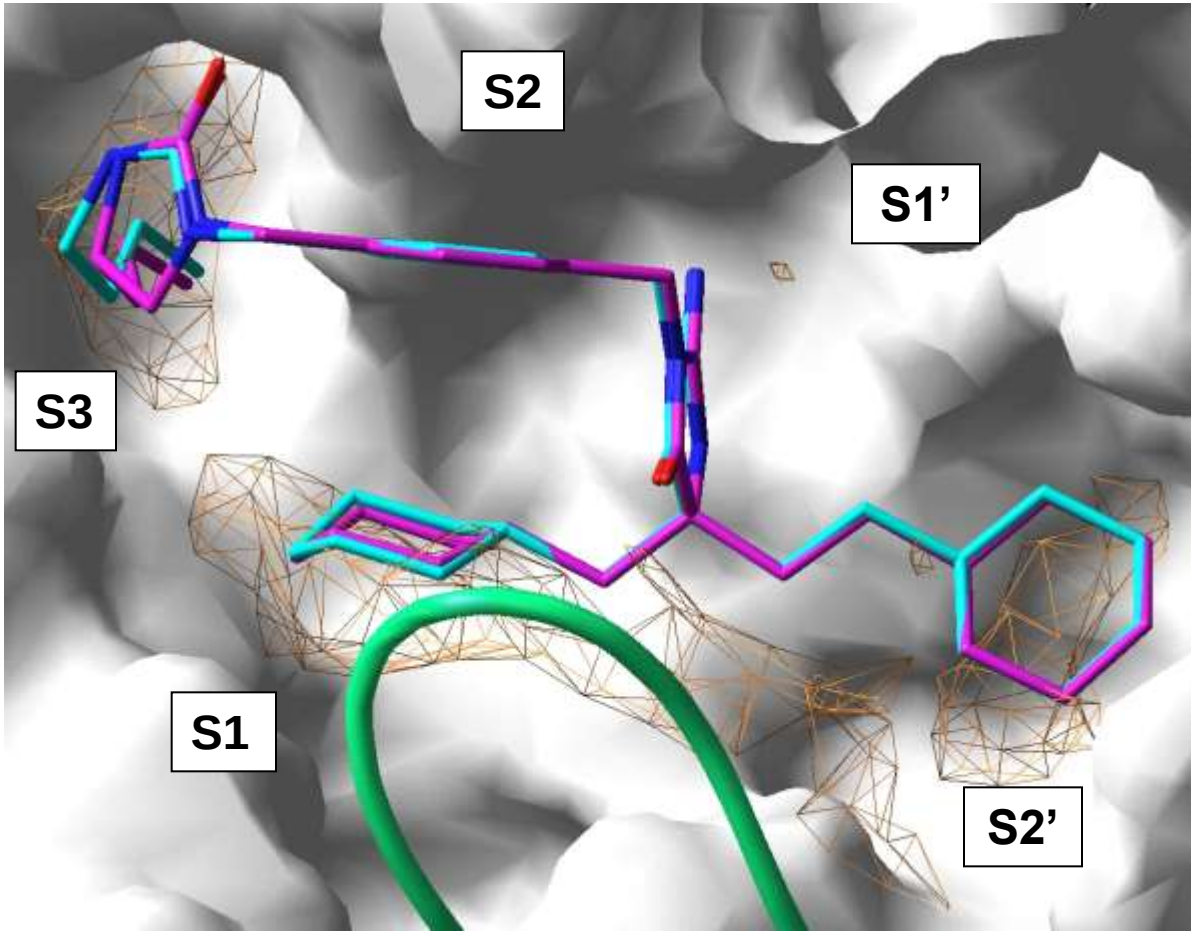


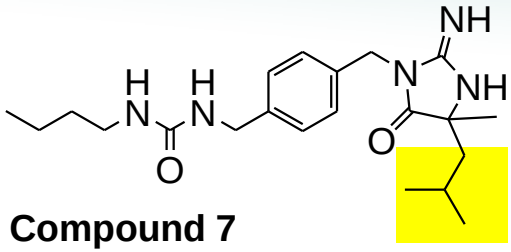
Compound 7
***IC*$_{50}$(HTRF) = 73 μM**
NMR: "Mode B"



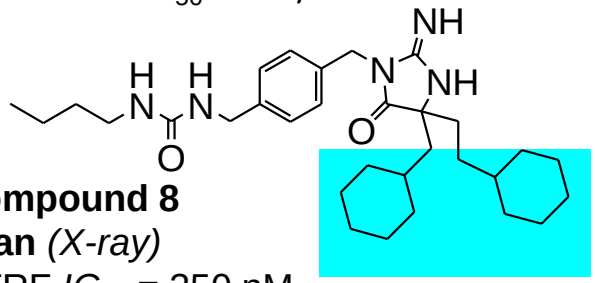
- **Fragment hit-to-lead chemistry complicated by different binding modes**
  - Orientation of iminohydantoin active site core influenced by substituents
  - Structural information helpful to explain SAR
  - "Mode B" preferred as H2L optimization evolved

Iminohydantoin design validation

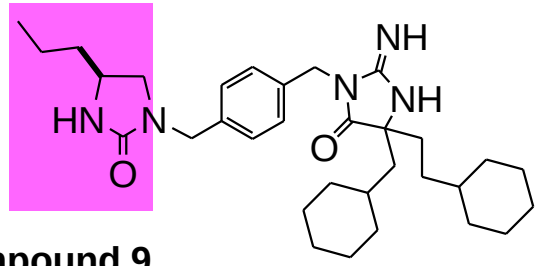




Compound 7
yellow *(X-ray)*
HTRF IC_{50} = 73 μM



Compound 8
cyan *(X-ray)*
HTRF IC_{50} = 350 nM

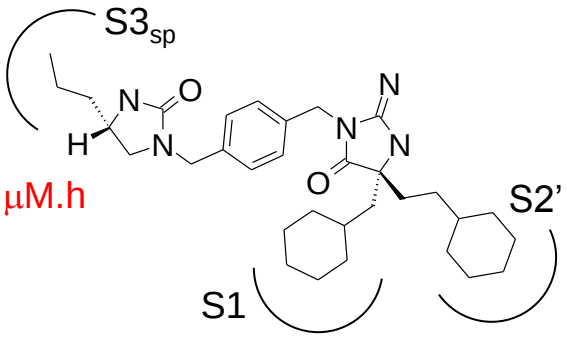


Compound 9
magenta *(X-ray)*
HTRF IC_{50} = 90 nM

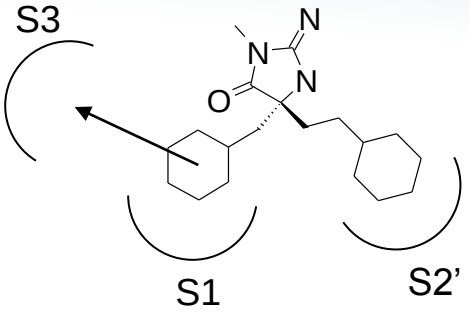
▶ **"Mode B" combinations yield <μM leads**

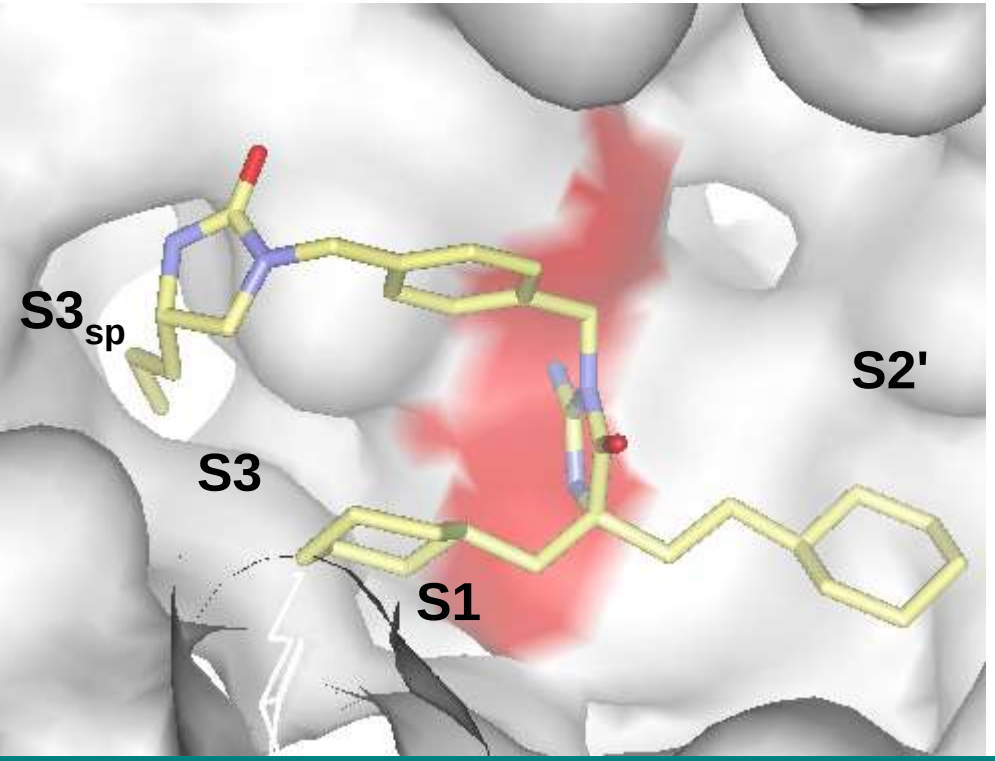
Development strategy

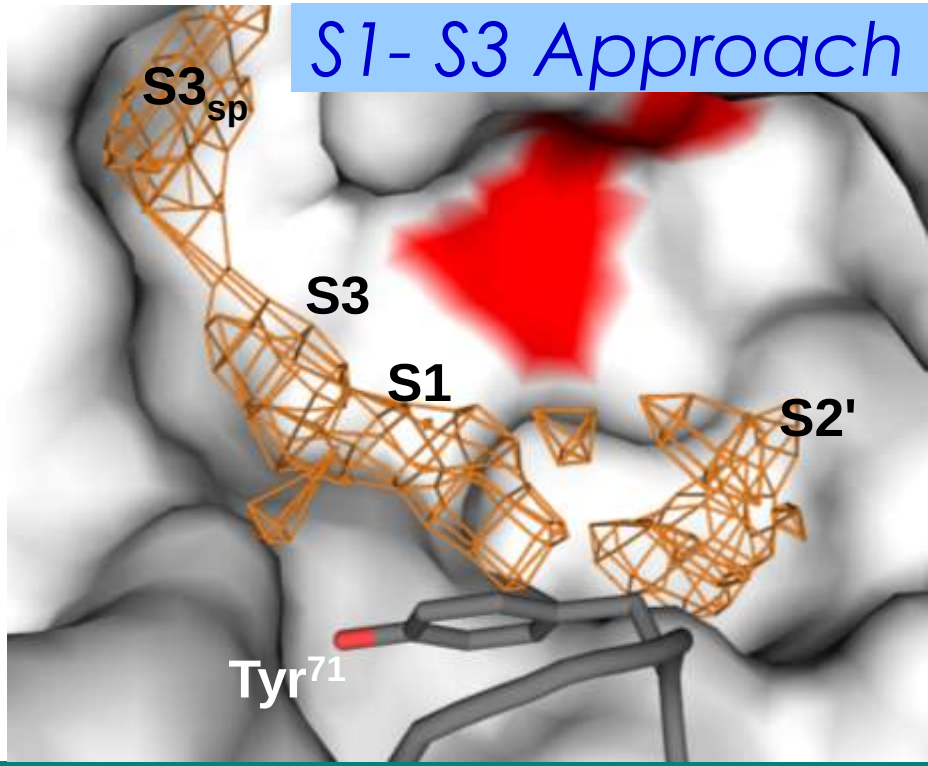
Compound 10
BACE-1 K_i = 67 nM
cell IC_{50} = 695 nM
CatD/BACE-1 = 0.5
rapid rat AUC = 0.58 μM.h
MW = 536
cLogP = 7.5
LE = 0.25



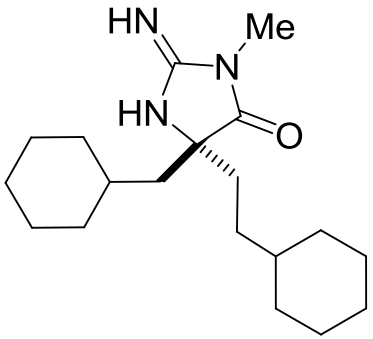
N-Me iminohydantoin
BACE-1 K_i = 650 nM
cell IC_{50} = 2600 nM
CatD/BACE-1 = 1
rapid rat AUC = 1.03 μM.h
MW = 319
cLogP = 5.4
LE = 0.36







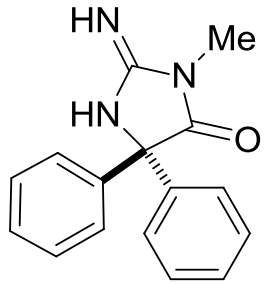
Iminohydantoin hit-to-lead



N-Me iminohydantoin
BACE-1 K_i = 650 nM
cell IC_{50} = 2600 nM
CatD/BACE-1 = 1
rapid rat AUC = 1.03 μM.h

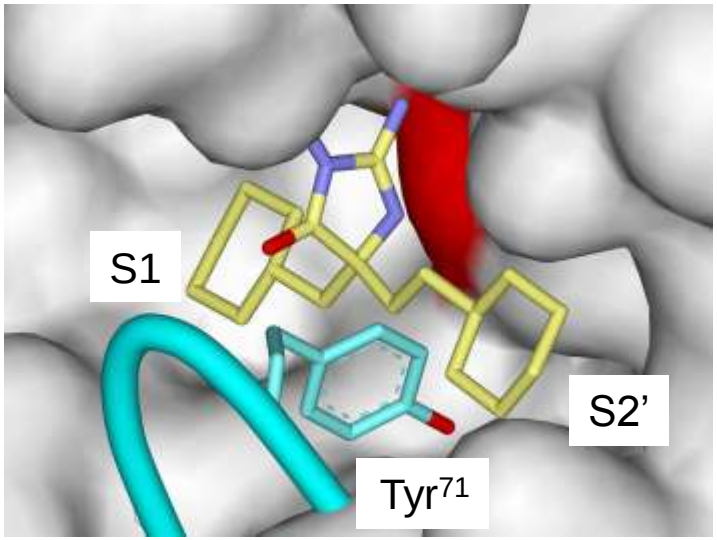
MW = 319
cLogP = 5.4
LE = 0.36

rotatable bonds

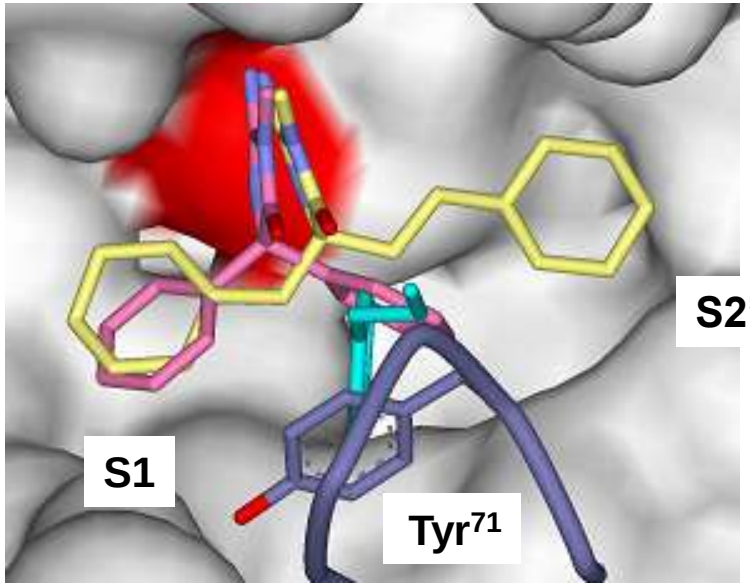


N-Me iminohydantoin
BACE-1 K_i = 3.7 μM
cell IC_{50} = 13 μM
CatD/BACE-1 > 20
rapid rat AUC = 5 μM.h

MW = 265
cLogP = 2.5
LE = 0.37

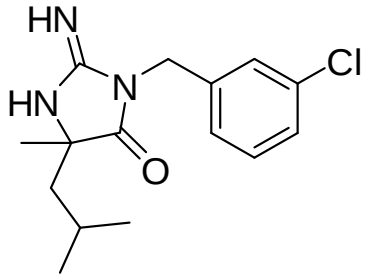


"Closed" flap
(peptidomimetic inhibitor conformation)



J Med Chem 53 (2010) 951

Iminohydantoin fragment-hit-to-lead optimization



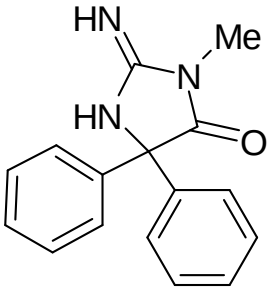
Iminohydantoin hit
BACE1 IC_{50} ~ 300 μM
LE = 0.24

Design:
- **Inhibitor docking**

Synthesis:
- **directed, library**

Assay:
- **bioNMR, X-ray, biochemical assay**

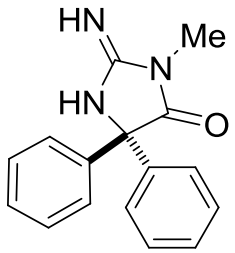
LEAD



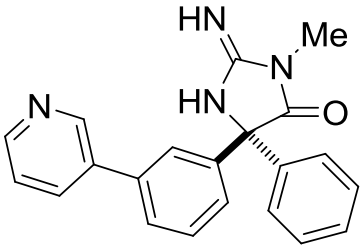
Iminohydanotin lead
BACE1 K_i = 3.7 μM
cell IC_{50} = 13 μM
CatD/BACE-1 >20
rat $AUC_{10\ mpk}$ = 5 μM.h
brain / plasma = 1.8
MW = 265; CLogP = 2.5
LE = 0.37

- ✓cellular activity
- ✓CatD selectivity
- ✓good PK
- ✓brain penetrant
- ✓low MW, ideal cLogP

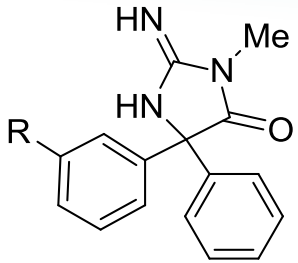
Iminohydantoins – S1-S3 Occupancy



BACE1 K_i: 3.7 μM
cell Aβ40 IC_{50}: 13 μM
CatD/BACE1: >20
LE: 0.37

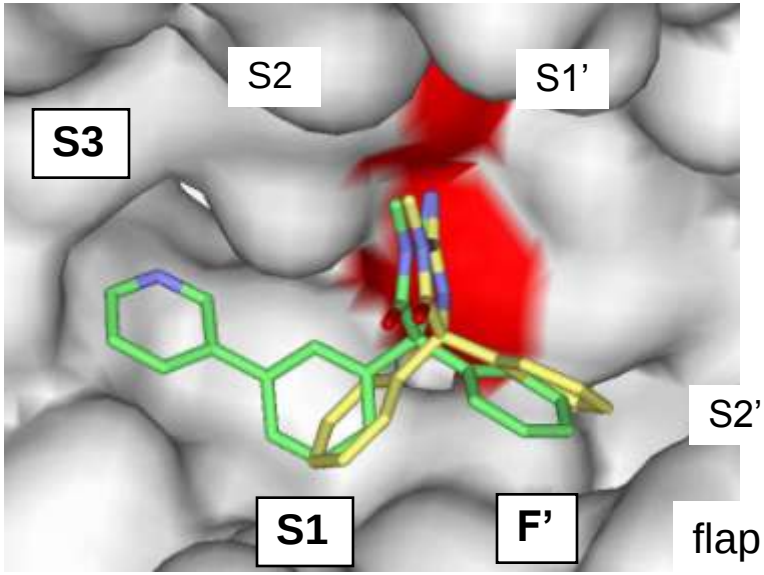


BACE1 K_i: 109 nM
cell Aβ40 IC_{50}: 633 nM
CatD/BACE1: 120
LE: 0.37



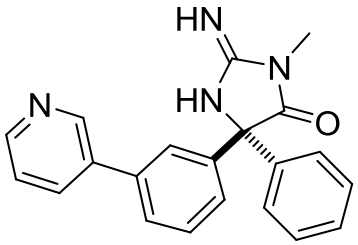
+/-

R	K_i μM
Ph	3.25
3-MePh	0.55
3-CNPh	0.37
3-ClPh	0.30
3-MeOPh	0.19
4-MeOPh	3.80
3-Py	0.53
4-Py	3.80



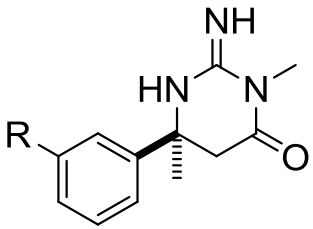
flap absent

Iminohydantoins vs iminopyrimidinones: Design

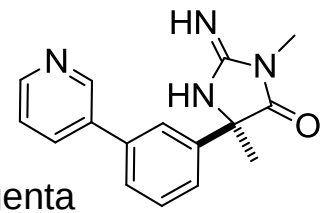


BACE1 K_i: 109 nM

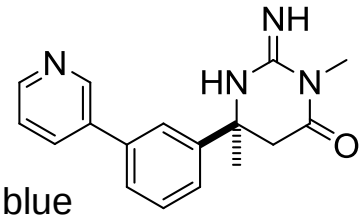
Ring enlargement



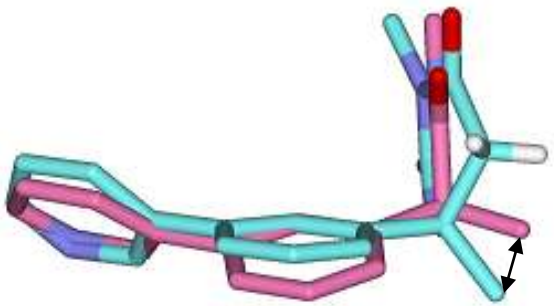
iminopyrimidinone

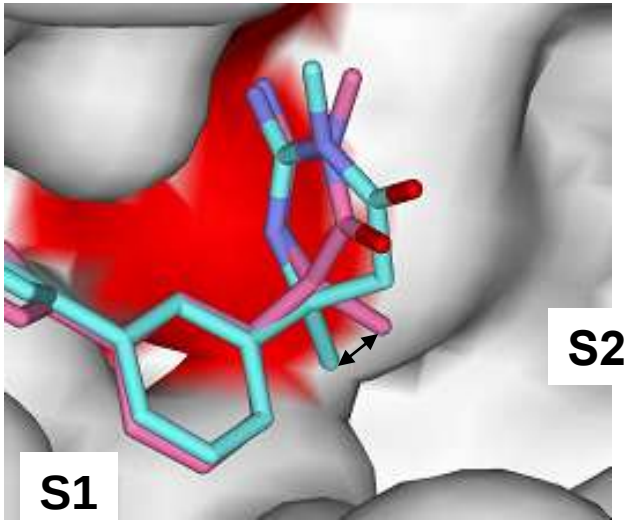


magenta

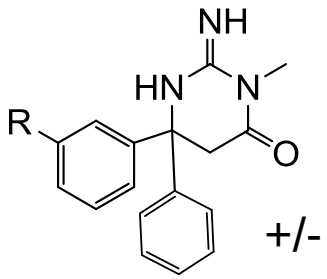


blue

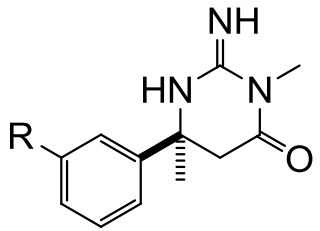




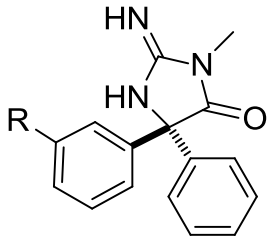
Iminohydantoins vs iminopyrimidinones: SAR



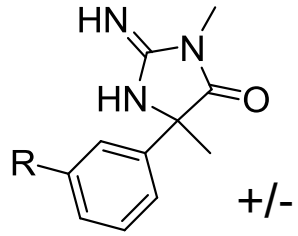
R	K_i μM	cell Aβ40 IC_{50} μM
3-Py	56	nd
3-MeOPh	26	nd



R	K_i μM	cell Aβ40 IC_{50} μM
3-Py	0.88	0.51
3-MeOPh	0.27	0.84

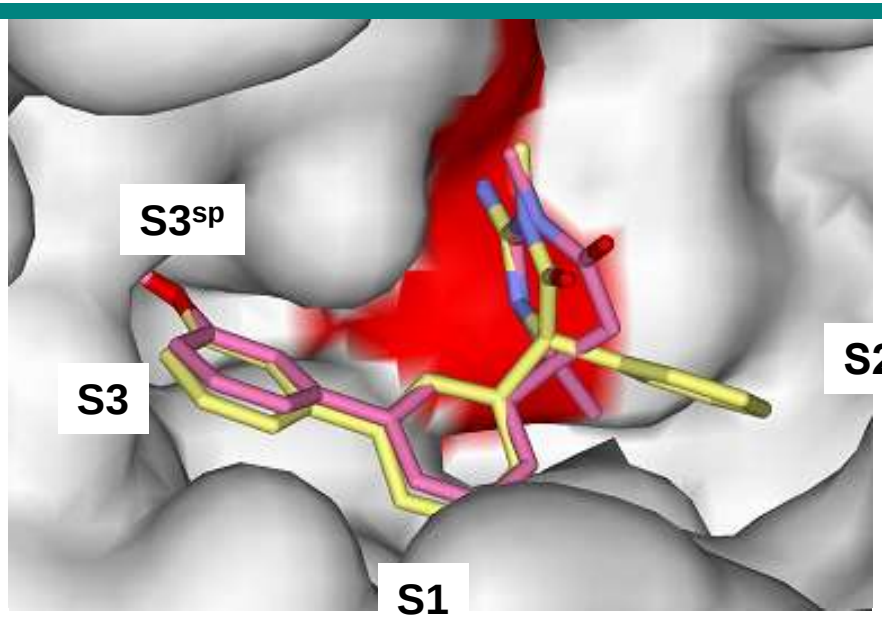


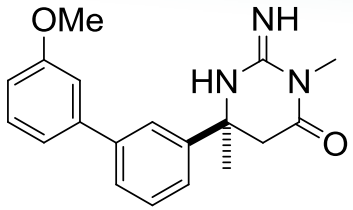
R	K_i μM	cell Aβ40 IC_{50} μM
3-Py	0.11	0.63
3-MeOPh	0.079	2.25



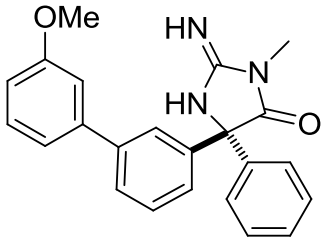
R	K_i μM	cell Aβ40 IC_{50} μM
3-Py	0.86	1.3
3-MeOPh	0.78	3.1

Iminopyrimidinones: X-ray and binding conformation



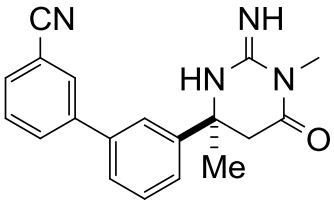


K_i: 270 nM
magenta - X-ray

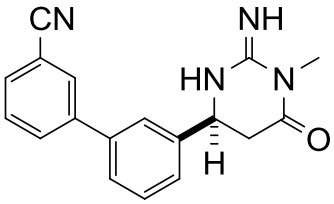


K_i: 79 nM
yellow - X-ray

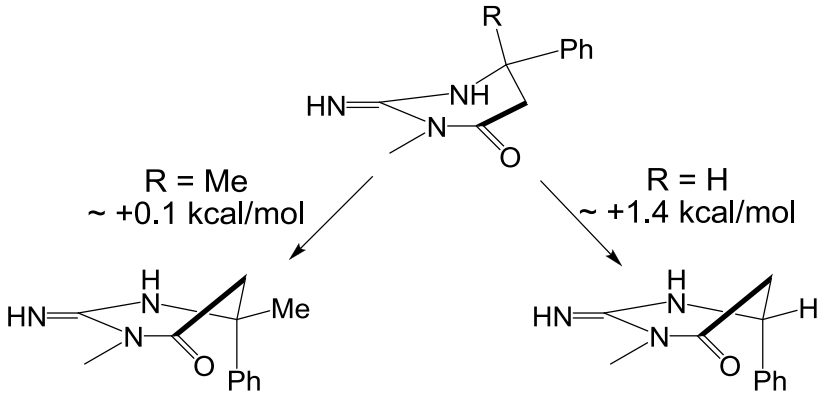
BACE1 – inhibitor X-ray co-crystal structures



K_i: 340 nM

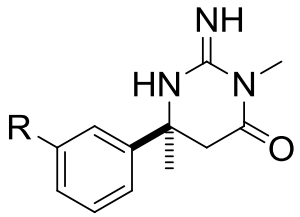


K_i: 2,430 nM

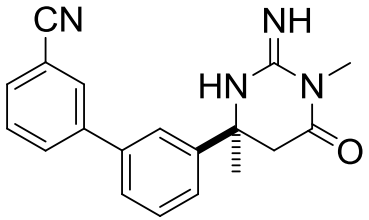


see Edwards et al J. Med. Chem., 2007, *50*, 5912

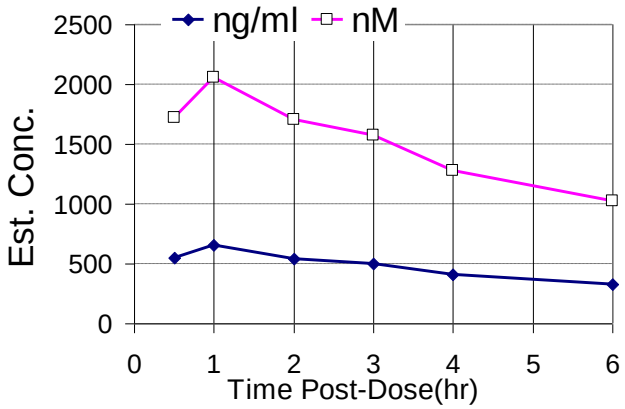
Iminopyrimidinone SAR



R	K_i μM	cell Aβ40 IC_{50} μM	CatD/ BACE1
H	7.4	6.5	>20
Ph	1.3	nd	nd
3-Py	0.88	0.51	67
3-MeOPh	0.27	0.84	61
3-CNPh	0.34	0.42	79
3-ClPh	0.21	2.3	90
3,5-diClPh	0.15	4.1	154

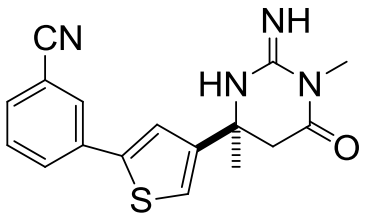


rat: 10 mg/kg PO (20% HPβCD)



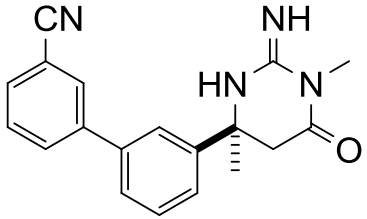
plasma $AUC_{0\text{-}6h}$: 8,623 nM.h
brain / plasma @ 6h: 0.4

P1 thienyl - improved BACE1 affinity



BACE1 K_i: 214 nM
Cell IC_{50}: 400 nM
CatD/B1: nd
rat AUC_{10}: 0.89 μM.h

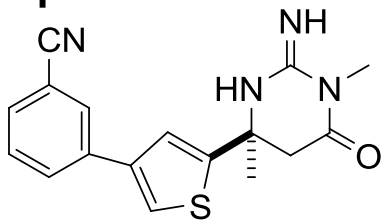
1.5x



BACE1 K_i: 340 nM (LE 0.37)
cell IC_{50}: 420 nM
CatD/B1: 79
rat AUC_{10}: 8.6 μM.h

6x

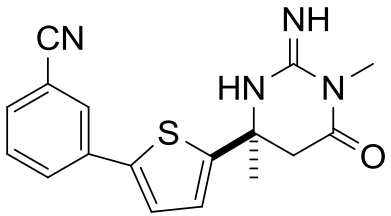
Compound 11



BACE1 K_i: 57 nM (LE 0.43)
Cell IC_{50}: 68 nM (Aβ40)
14 nM (Aβ42)
CatD/B1: 68
rat $AUC_{10mpk, 0-6h}$: 1.6 μM; C_{6h} <loq

Brain/plasma: 1
Caco2 perm: 174 nm/s (ratio: 2.6)
plasma fu: 12% (hmn), 15% (rat)
ClogP: 3.2

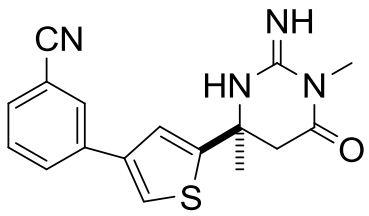
4x



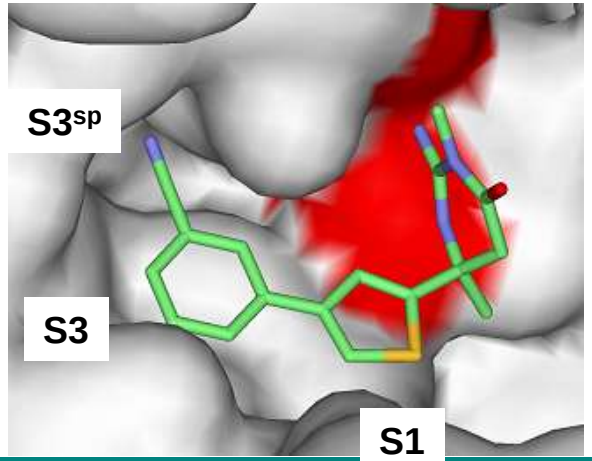
BACE1 K_i: 88 nM
Cell IC_{50}: 116 nM
CatD/B1: 26
rat AUC_{10}: 0.19 μM.h

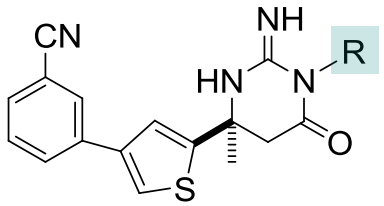
3,5-Substituted thienyl analogues – selected SAR

Compound 11

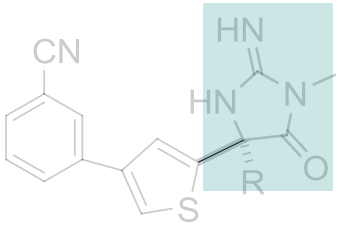


BACE1 K_i: 57 nM (LE 0.43)
Cell IC_{50}: 68 nM
cLogP: 3.2

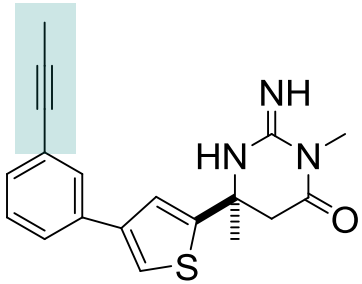




R = H K_i: 2,560 nM
R = Et K_i: 207 nM

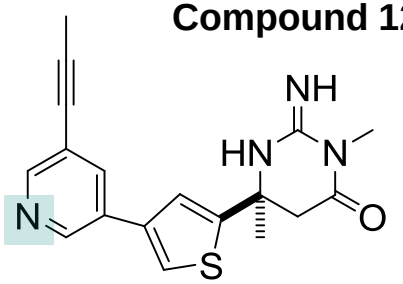


R = Me K_i: 5,820 nM
R = Ph K_i: 1,410 nM



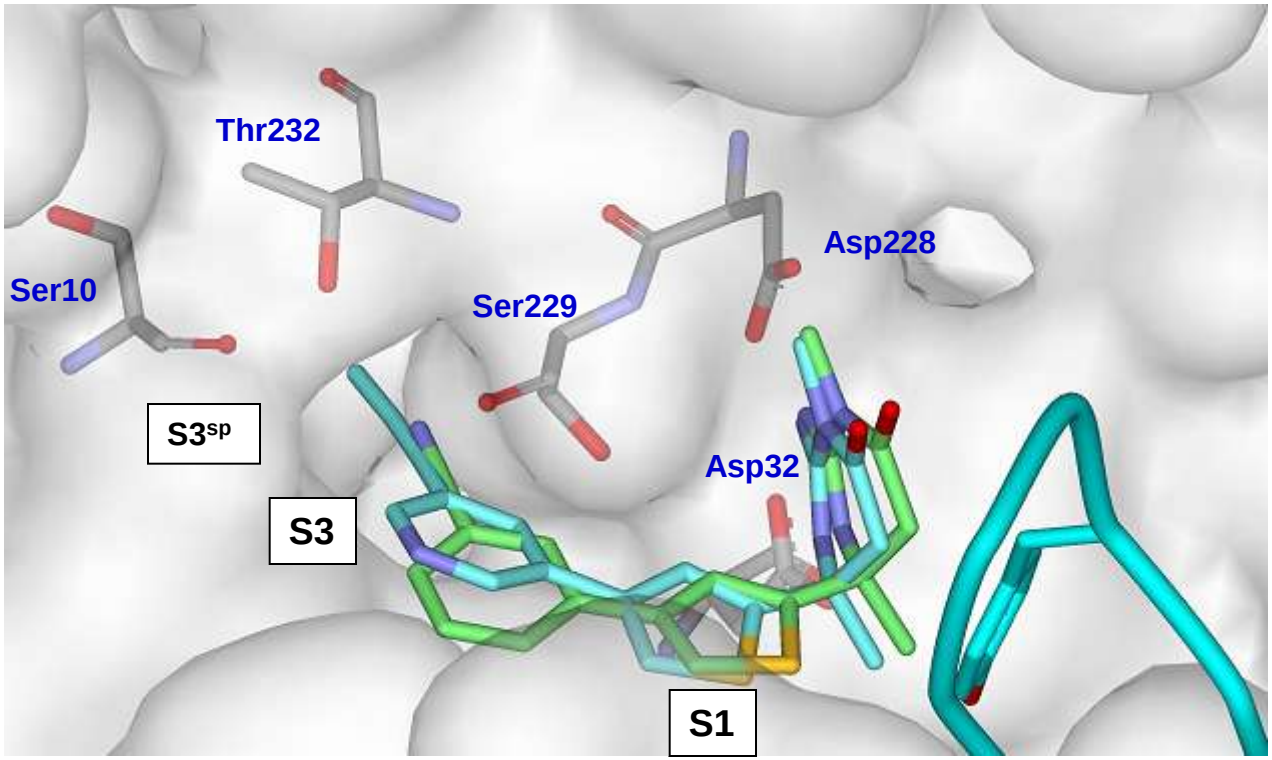
BACE1 K_i: 14 nM
Cell IC_{50}: 196 nM
cLogP: 3.7

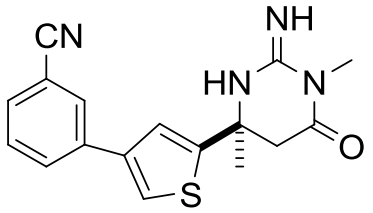
Compound 12



BACE1 K_i: 7 nM (LE 0.46)
Cell IC_{50}: 13 nM
cLogP: 2.2

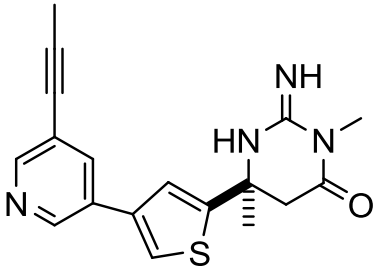
BACE1 co-crystal structures





K_i: 57 nM (green)

Compound 11

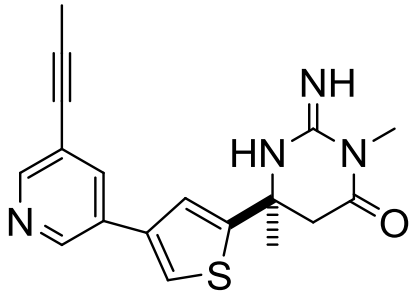


K_i: 7 nM (cyan)

Compound 12

Compound 12 - profile

BACE1 K_i nM	7
cell IC_{50} nM Aβ40	13
Aβ42	9
sAPPβ	44
BACE2/B1	0.1
CatD/B1	188
CatE/B1	708
Renin/B1	260
Pepsin/B1	>10,000



Hepatocyte Cl_{int} (μL/min/M cell):
hmn 2.2; rat 3.6; dog 7.5; mky 19.9
Caco2 perm: 144 nm/s; ratio: 3.5
Plasma protein binding: 89% (hmn), 95% (rat)

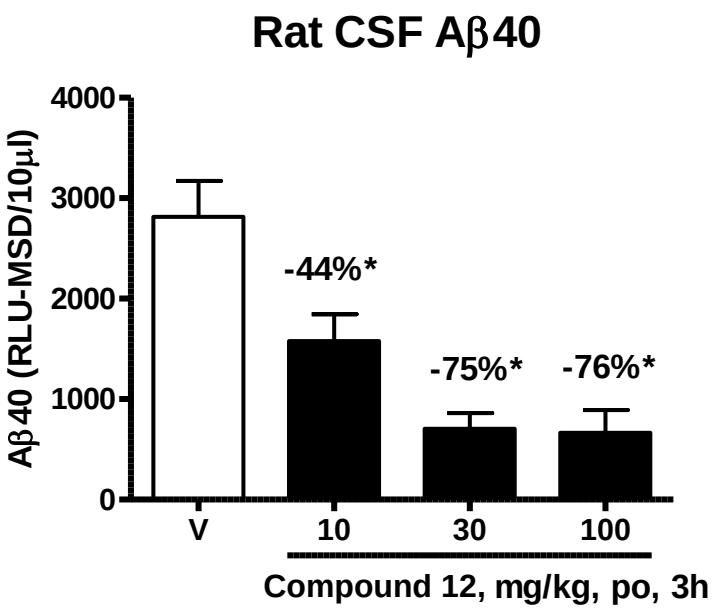
CYP IC_{50}: 3A4, 2D6, 2C9 > 20 μM
hERG VC: 34%I @ 1 μM

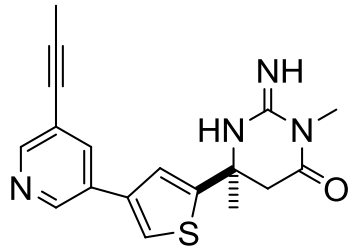
rat PK parameters

dose mg/kg	po $AUC_{0\text{-}24h}$ μM.h	%F	po C_{max} μM	Cl ml/min/kg	Vd_{ss} L/kg	iv $T_{1/2}$ h	b/p
10 po / 3 iv	20	90	3.9	24	3.5	1.6	0.3

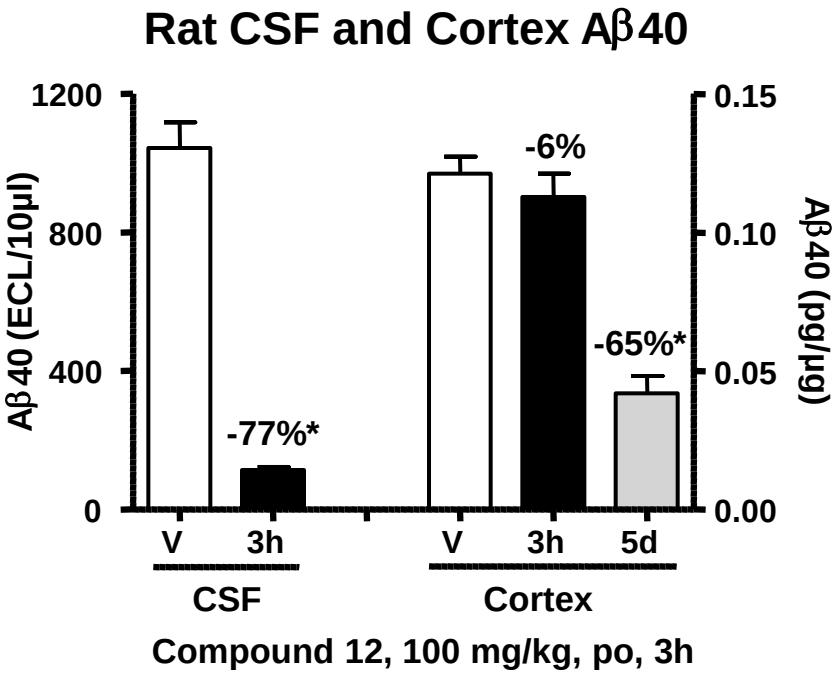
Compound 12 reduces CSF and Cortex Aβ40 in rats

Compound 12: acute dose-response





Compound 12: 100 mg/kg PO acute vs 5 day BID



	plasma µM	brain/ plasma	CSF/ plasma
acute (3h)	6.4	0.25	<0.01
5-day bid (3h)	9.9	0.32	<0.01

Summary

- Fragment-based NMR screening / X-ray crystallography enabled
  - discovery of a novel amidine binding motif to the active-site Asp's of BACE-1
  - design and validation of novel iminoheterocycle Asp protease inhibitor core
- Lead optimization guided by structure-based design afforded unique, low MW, high affinity, selective iminopyrimidinone BACE-1 inhibitors
  - hydrophobic interactions optimized in S1, S3, $S3^{sp}$
  - conformational restriction / few rotatable bonds contribute to high LE
- Iminoheterocyclic BACE-1 inhibitors possess desirable properties as potential therapeutic agents to test amyloid hypothesis
  - high oral bioavailability
  - CNS penetration
  - robust reduction of CSF and brain Aβ in rats and primates

Acknowledgments

Medicinal Chemistry
John Caldwell
Jared Cumming
Michael Czarniecki
James Durkin
William Greenlee
Ulrich Iserloh
Guoqing Li
Robert Mazzola
Brian McKittrick
Jeffrey Misiaszek
Terry Nechuta
Jianping Pan
Elizabeth Smith
Andrew Stamford
George Sun
Lingyan Wang
Yusheng Wu
Zhaoning Zhu

Synthetic Chemistry
Jianshe Kong
Tao Meng
Jesse Wong
Mark Liang
Yan Jin
Teresa Andreani

Structural Chemistry
Brian Beyer
Hung Le
Vincent Madison
Tony Mannarino
Joseph Myers Jr.
Peter Orth
Paul Reichert
Mary Senior
Corey Strickland
Shane Taremi
Johannes Voigt
Wenyan Wang
Yu-Sen Wang
Daniel Wyss

Pharmacopeia
Suresh Babu
Paul Gaspari
Helen Gu
Tao Guo
Rachael Hunter
Thuy Le
Jianming Ma
Michelle Morris
Gang Qian
Kurt Saionz
Dawitte Tadesse

***In vitro* and *In vivo* Biology**:
Carina Bleickardt
Joseph Chen
Xia Chen
Mary Cohen-Williams
Robert Del Vecchio
Michael Grzelak
Donald Guthrie
Mario Guzzi
Robert Hodgson
John Hunter
Lynn Hyde
Tatiana Kazdoba
Matthew Kennedy
Reshma Kuvelkar
Prescott Leach
Sherry Lu
Nansie McHugh
Cynthia Morgan
Deborah Mullins
Eric Parker
Lixin Song
Giuseppe Terracina
Geoff Varty
Lili Zhang
Qi Zhang

Drug Metabolism&Pharmacokinetics
Kathleen Amatulli
Ryan Anstatt
Lisa Broske
Shiying Chen
Inhou Chu
Kathy Cox
Leonard Favreau,
Yi Han
James Jean
Walter Korfmacher
Jing Lan
Cheng Li
TongTong Liu
Richard Morrison
Cymbelene Nardo
Dan Prelusky
Tony Soares
Ann Thomas
Hui Wan
Qiao Zhou

Pharmaceutical Sciences
Irina Kazakevich
Prudence Bradley