KAT II Inhibitors; a Novel Approach for the Treatment of Schizophrenia

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Schizophrenia – an unmet clinical need

- **Schizophrenia remains an area of high unmet clinical need**
  - Affects around 24 million people worldwide
  - 9th leading cause of disability
  - Average onset of disease in 20s
  - Estimated to cost >$60 billion/year in USA

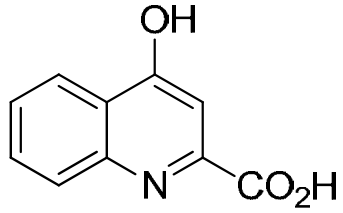
- **Causes of schizophrenia remain unclear**
  - Some genetic links discovered
  - Environmental factors thought to have an important role

- **Symptoms are many and varied**
  - Positive symptoms - hallucinations and delusions
  - Negative symptoms – emotional withdrawal, lack of energy (avolition), anhedonia
  - Cognitive effects – memory, attention, executive functioning

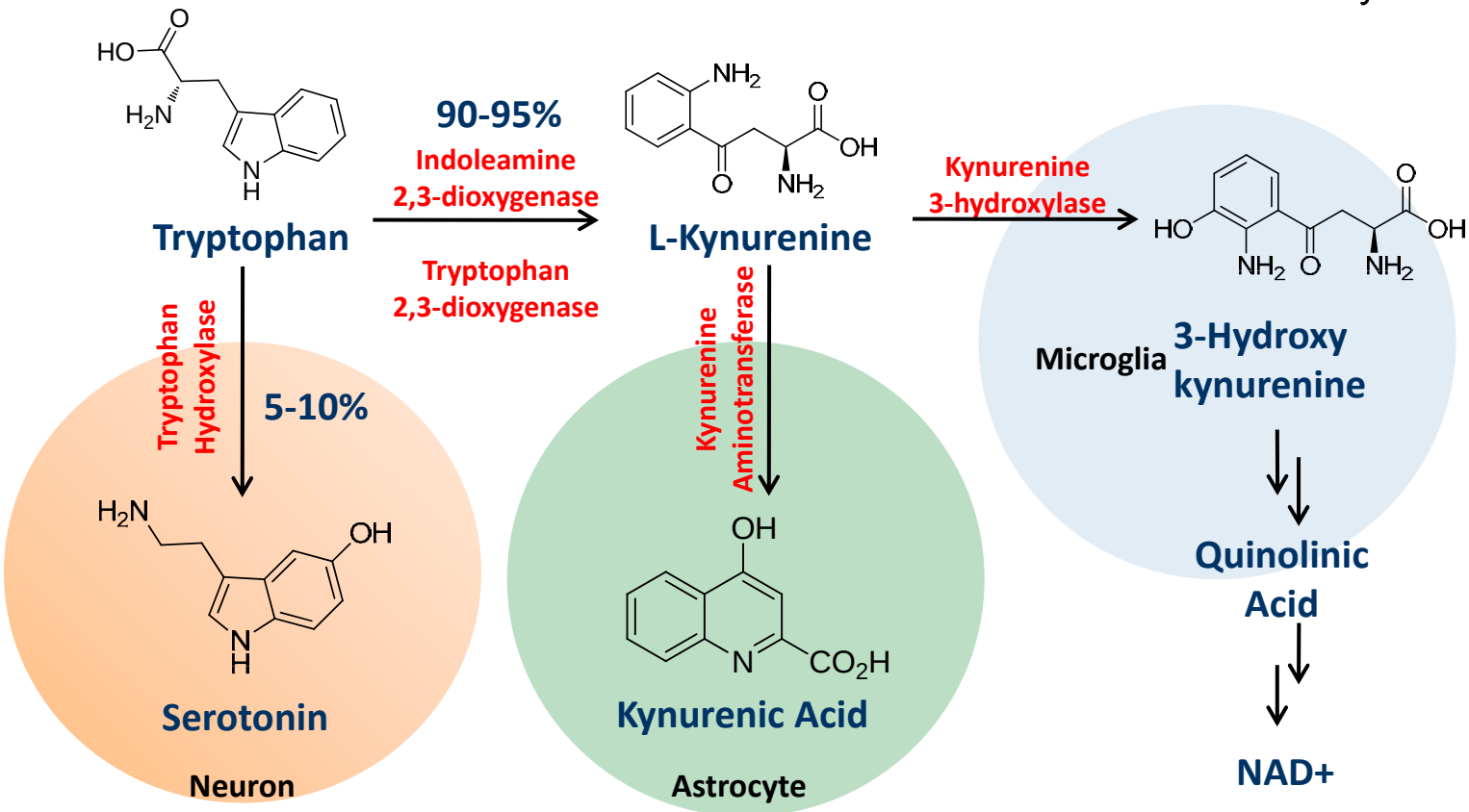
- **Standard therapies target dopamine and serotonin systems**
  - Majority of treatments treat only the positive symptoms of Schizophrenia
  - Few effective treatments available for negative symptoms or cognitive impairments

KATII and its role in Schizophrenia

Elevated levels of Kynurenic Acid (KYNA) have been found in the CSF and brain (postmortem) of schizophrenic patients



Kynurenic Acid



Review: Erhardt, S.; Olsson, S. K.; Engberg. G. *CNS Drugs* 2009, *23*, 91-101.

KATII and its role in Schizophrenia

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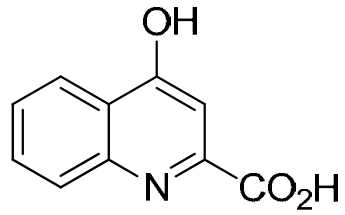
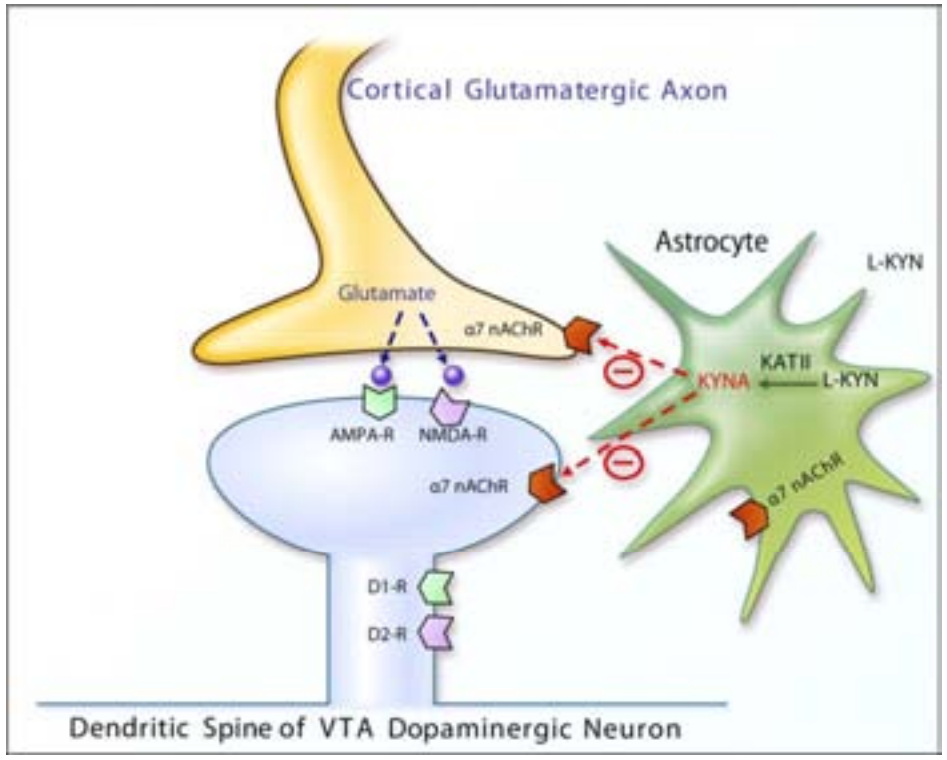


Kynurenic Acid



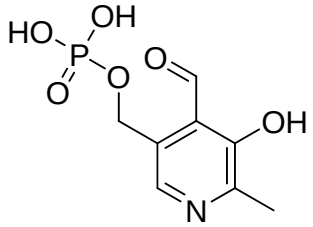
- **Altered levels of KYNA are thought to impact the glutamate system**
  - Antagonist of NMDA receptor
  - Inhibitor of nAchR receptor
- **Elevating KYNA levels in rodents affects sensory gating, attention and cognition.**

Reducing KYNA levels in schizophrenics may combat negative and cognitive symptoms of schizophrenia

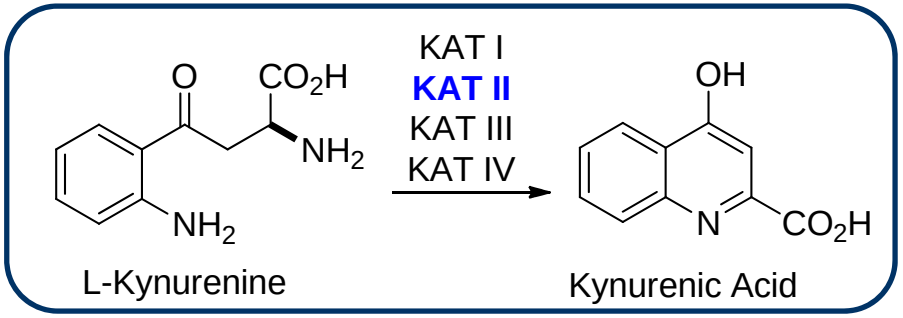
Rogers, S. W. *et al. Physiol Rev 89: 73–120, 2009*

KATII and its role in Schizophrenia

- **Kynurenine Aminotransferase is responsible KYNA biosynthesis**
  - Converts L-Kynurenine to Kynurenic acid (KYNA)
  - Requires the co-factor pyridoxyl phosphate (PLP)
- **KAT II is the prominent isoform in the brain**
  - KYNA is not brain penetrant



pyridoxal-5-phosphate

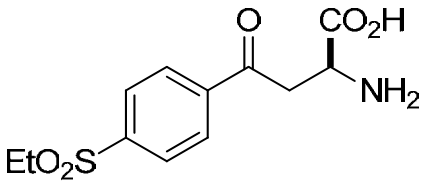


A brain-penetrant KATII inhibitor would be a useful tool in further studying the affects of KYNA in the CNS and validating KATII as a target for schizophrenia

Review: Erhardt, S.; Olsson, S. K.; Engberg. G. *CNS Drugs* 2009, *23*, 91-101.

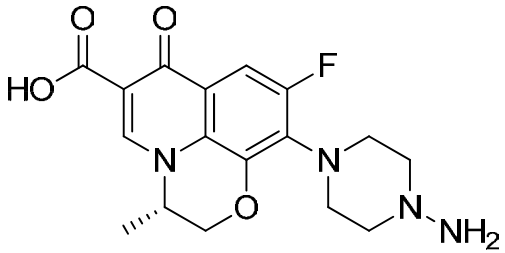
Literature compounds provide support for KATII as a target

- **Two 'tool' compounds have been reported**
  - Not brain penetrant
  - Poor activity against rat KATII
  - Central administration has allowed some study of KATII inhibition *in vivo*



(*S*)-ESBA

hKAT II IC_{50} = 1 mM
rKAT II IC_{50} = 6 μM



BFF-122

hKAT II IC_{50} ~ 1 μM
rKAT II IC_{50} ~ 1 μM

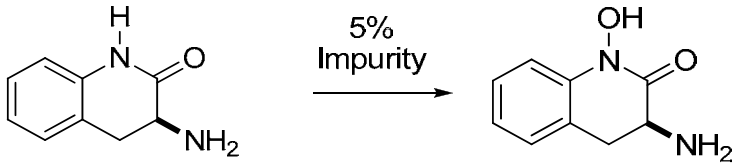
Criteria for in-house KATII inhibitor

- Potency at both human KATII (<100 nm) and rat KATII (<1 uM)
- Brain penetrant
- Drug-like physicochemical properties
- No drug-drug interactions

Schwarcz, R. *et. al ChemMedChem* **2006**, *1*, 528-531.
Rossi, F.; *et.al. J. Med. Chem*. **2010**, *53*, 5684-5689.

HTS identifies potent KATII inhibitor

- Initial HTS hit did not retest – 5% impurity identified as active component.



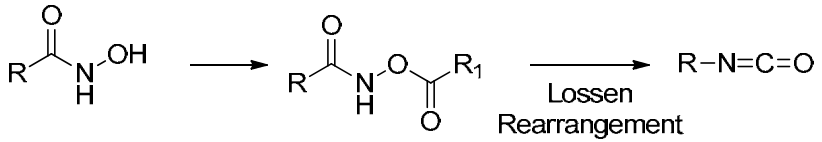
HTS Hit

PF-04859989

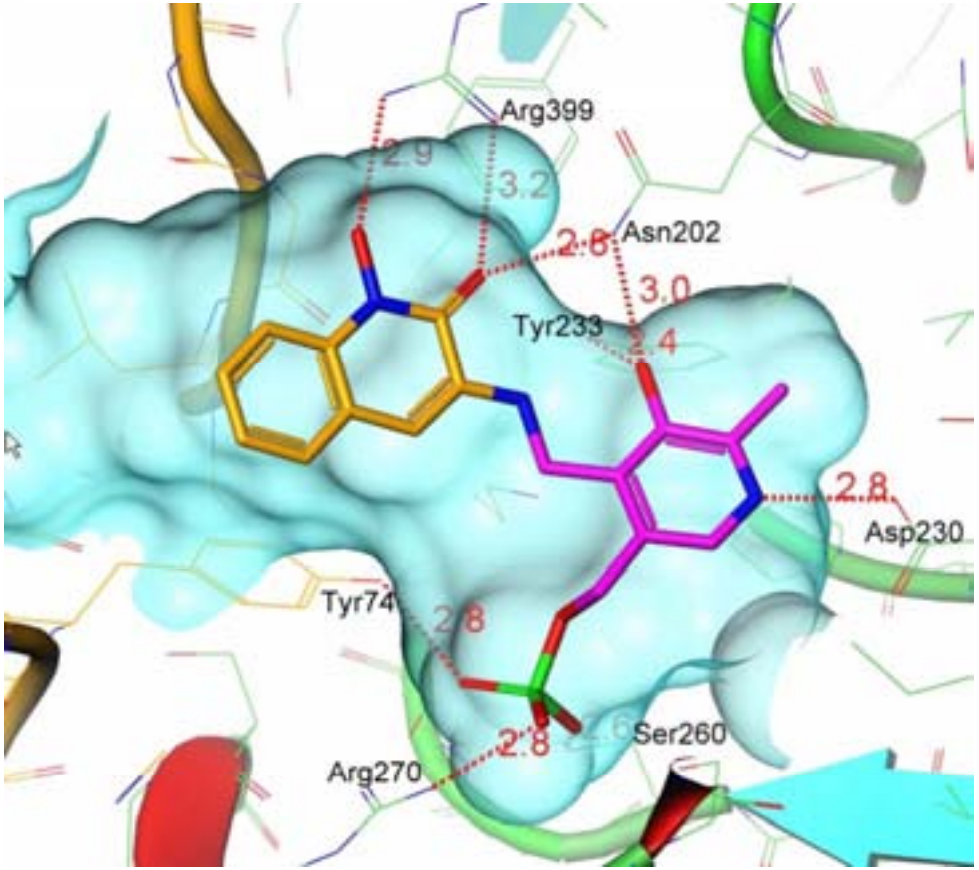
HTS sample – 96 nM
Solid retest - >10 000 nM

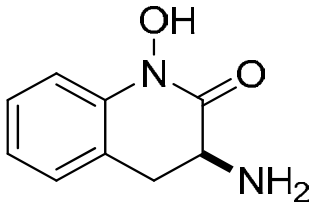
hKAT II IC_{50}=23 nM
rKAT II IC_{50} = 263nM
MW = 178; logD = -0.7
clogP = -0.1; tPSA = 66.6
LE = 0.82; LipE=8.4

- High potency, low molecular weight hit
- Physicochemical properties consistent with likelihood of brain availability
- Clean profile in CEREP panel
- Modest shift between human and rat isoforms
- Hydroxamic acid can have potential for tox issues
  - Mechanism not relevant to N-Aryl hydroxamic acid
  - IVMN and AMES negative

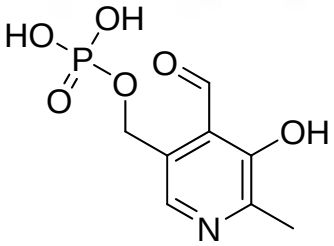


Crystal structure suggests unusual mechanism





PF-04859989



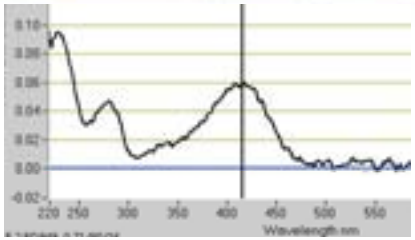
pyridoxal-5-phosphate

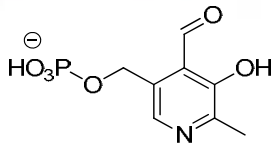
- **Early structural data demonstrated importance of hydroxamic acid**
  - Key hydrogen-bonding interactions with multiple amino acids
  - Hydroxamic acid forms an H-bond bridge with Arg 399
  - Carbonyl H-bonds with Asn 202
- **Also reveals covalent bond between inhibitor and cofactor**

Artem Evdokimov and Jay Pandit

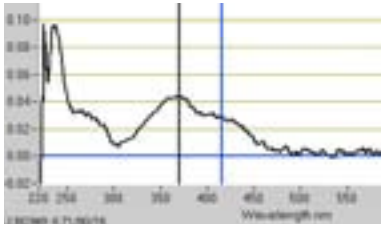
Is PF-04859989 an irreversible inhibitor of KATII?

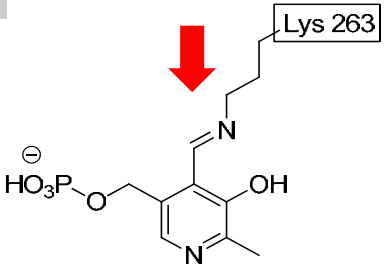
Defining irreversibility



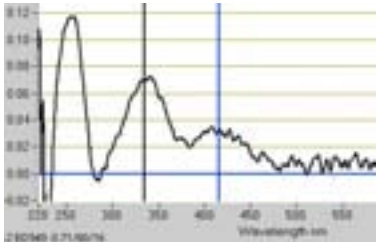


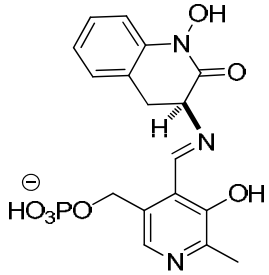
Free PLP: 415 nm





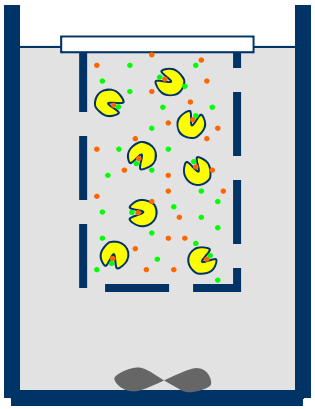
PLP- KAT II complex: 370 nm





PLP- inhibitor adduct: 330 nm

Dialysis experiment

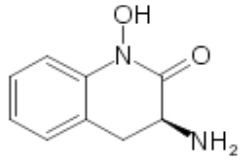


t=0

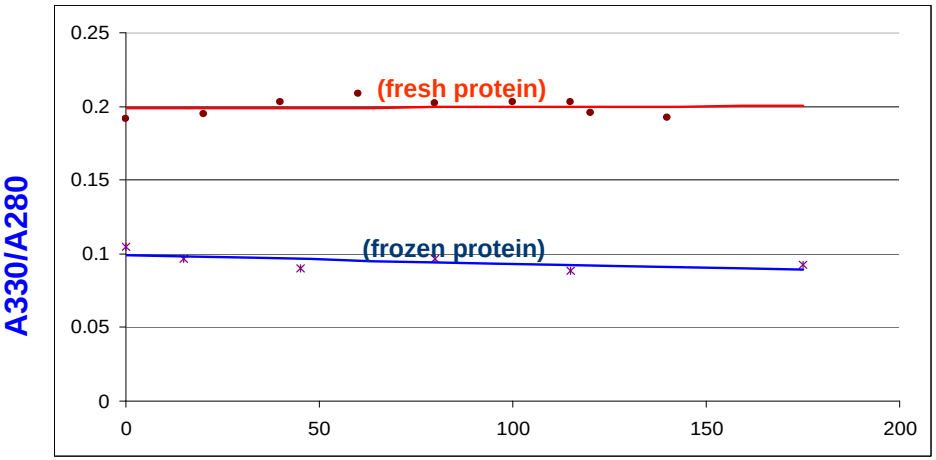
Protein — 280 nm

+ PLP — 370 nm

+inh+PLP — 330 nm

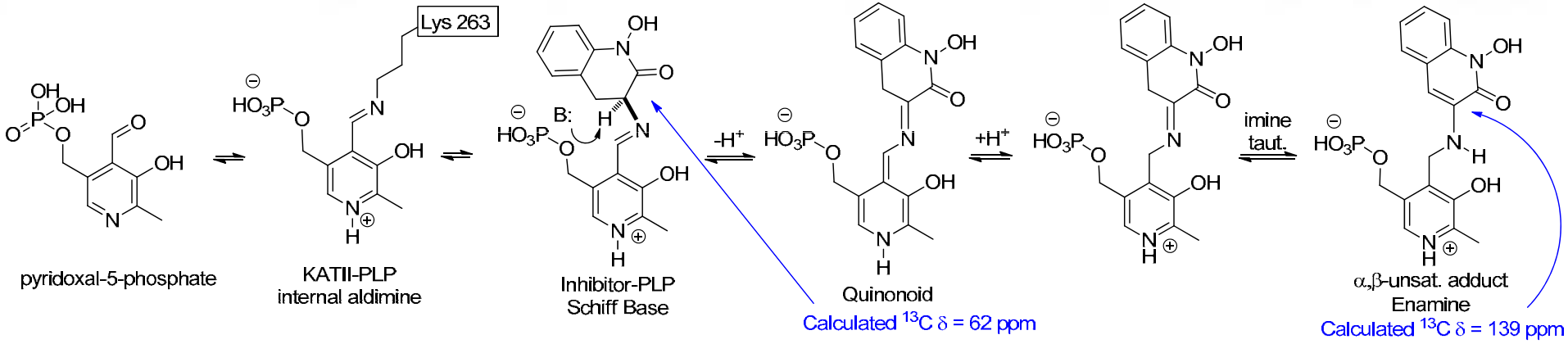


PF-04859989

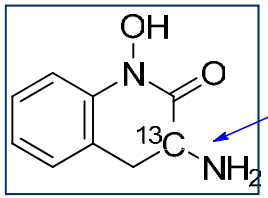


Dialysis Experiments confirm that PF-04859989 is an irreversible inhibitor of KATII

Determining the mechanism of irreversible inhibition

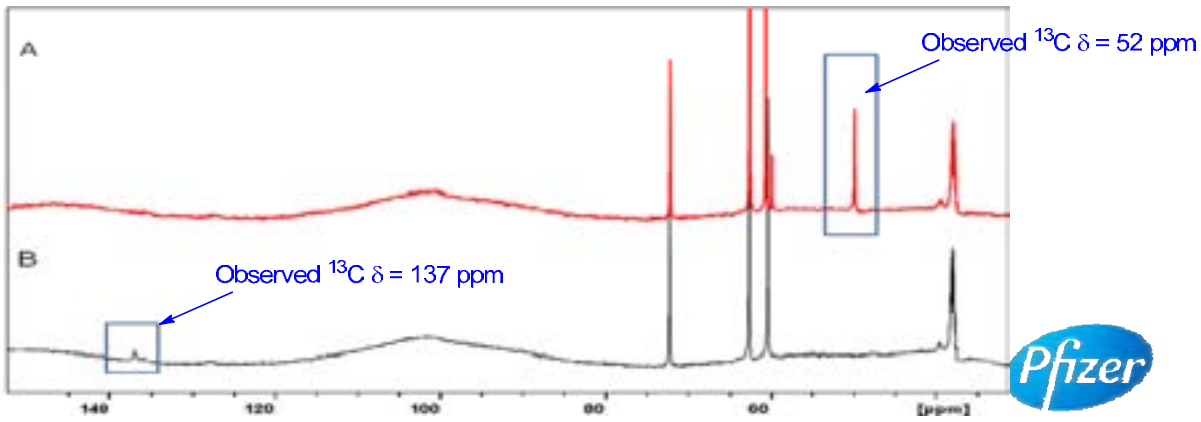


- **PLP adduct has been observed with other aminotransferase inhibitors**
  - Silverman proposed enamine formation for irreversible GABA aminotransferase inhibitor
- **X-Ray and MS cannot distinguish between covalent adducts**



A) ^{13}C-PF-04859989 + KAT II (550 uM each)

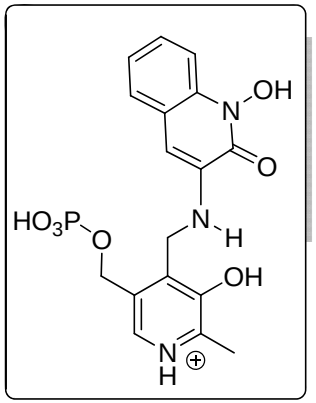
B) ^{13}C-PF-04859989 + KAT II + PLP (550 uM each)

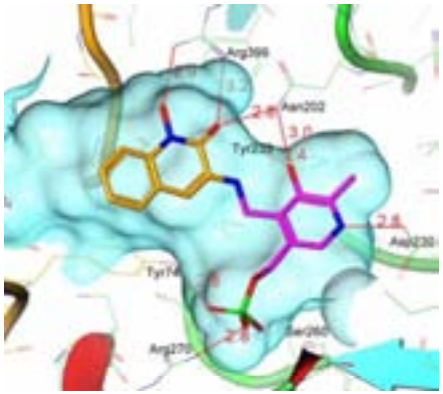


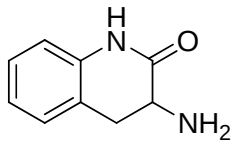
R.B. Silverman et al. *J. Am. Chem. Soc.* 1998, 120, 2256

Confirming structural requirements for irreversible inhibition

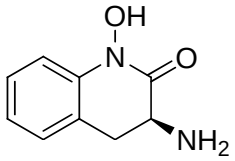
- **Small series of analogues confirmed structural requirements for KATII inhibition**



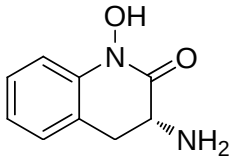




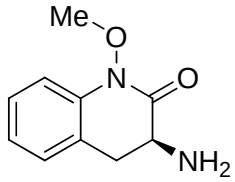
hKAT II IC_{50} - >10000 nM



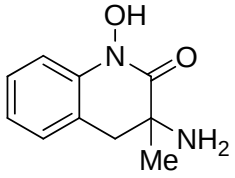
hKAT II IC_{50} - 23 nM
rKAT II IC_{50} - 263 nM



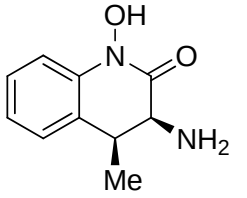
hKAT II IC_{50} - 219 nM
rKAT II IC_{50} - 1170 nM



hKAT II IC50 - >10 000 nM
rKAT II IC50 - >10 000 nM



hKAT II IC50 - >10 000 nM
rKAT II IC50 - >10 000 nM



hKAT II IC50 - 3280 nM

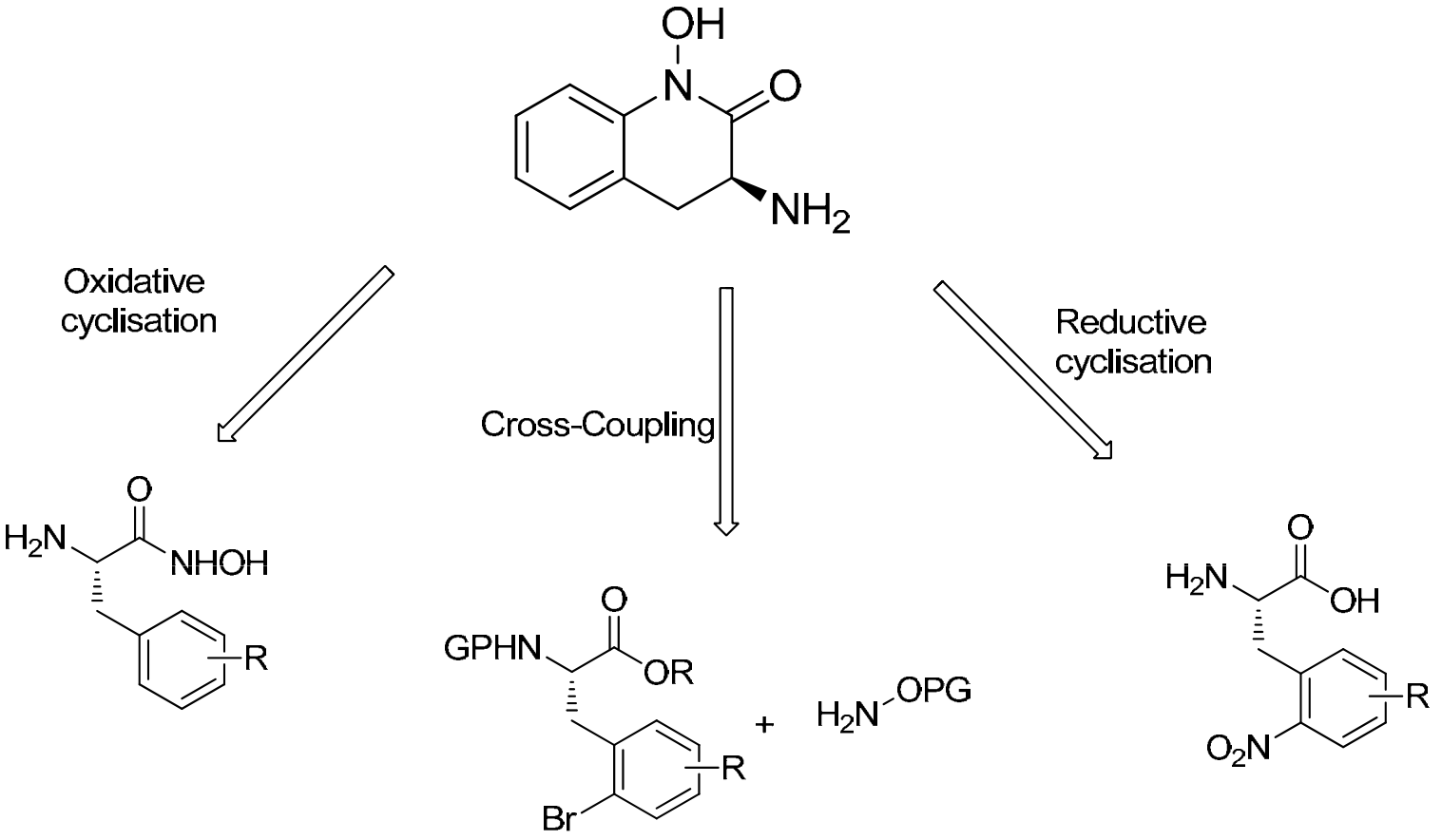
What does irreversibility mean for the project

- Irreversible inhibitors often avoided
  - Covalent modification of enzyme can lead to immune response
  - Idosynchratic tox findings
- Can have benefits in terms of PK/PD
  - Longer duration of action
  - Lower dose
- Important to understand biological system
  - Enzyme resynthesis rate
  - Enzyme occupancy

Whitty,A. *et. al. Nature Reviews Drug Discovery* 10, 307-317 (April 2011)
Duggan, M.E; *J.Med.Chem.* **2009**, *52* (5), 1231

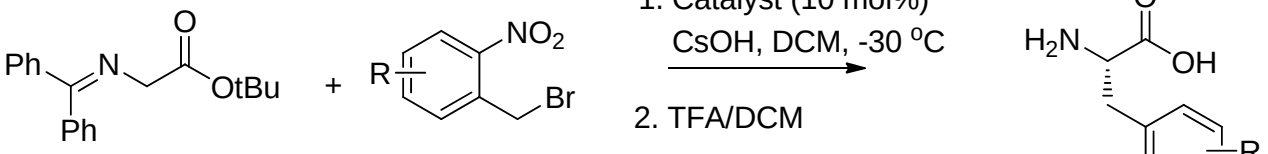
A small molecule, but synthetically challenging

- Enantioselective synthesis desired
- Not possible to oxidise lactam to hydroxamic acid

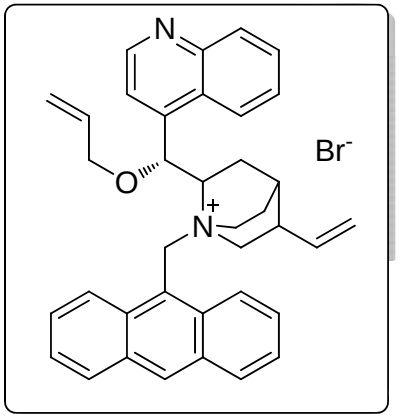


Concise synthesis of KATII inhibitors

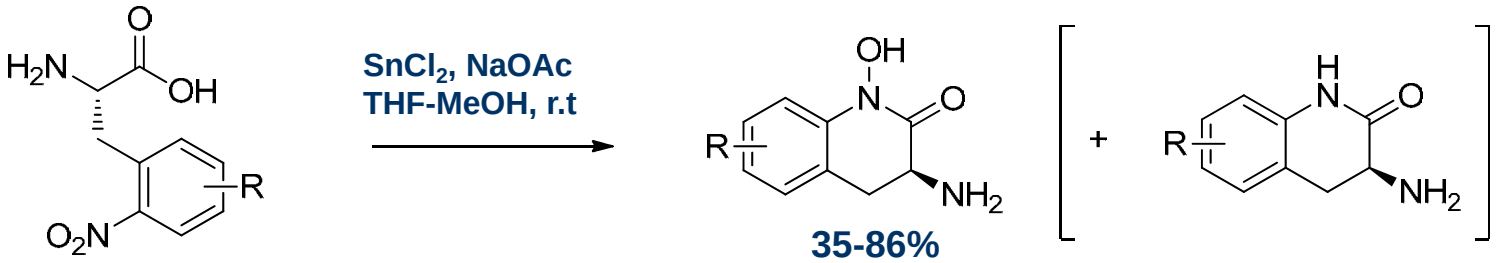
- Enantioselective synthesis using cinchonidine derived catalyst
- Allowed access to chiral compounds without chiral chromatography
- Long reaction times at cryogenic temperatures



50-92% yield,
75 - 99% ee



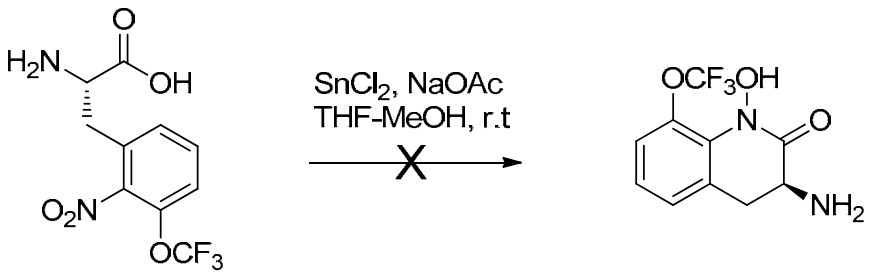
- Reductive cyclisation furnished KATII inhibitors.
- Majority of conditions led to mixtures of hydroxamic acid and lactam



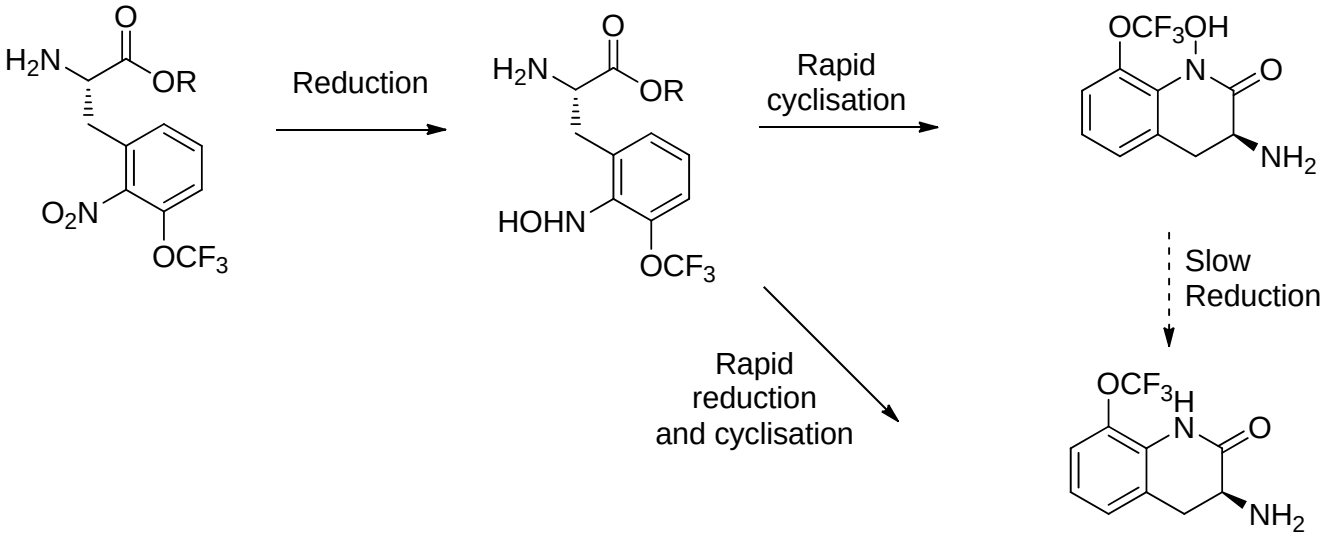
MacAllister, L. A.; *et.al. J. Org. Chem*. 2011, 76, 3484–3497

Concise synthesis of KATII inhibitors

- **$SnCl_2$ reduction ineffective when nitro group is *ortho*-substituted.**



- **Examined activated esters to enhance rate of cyclisation**



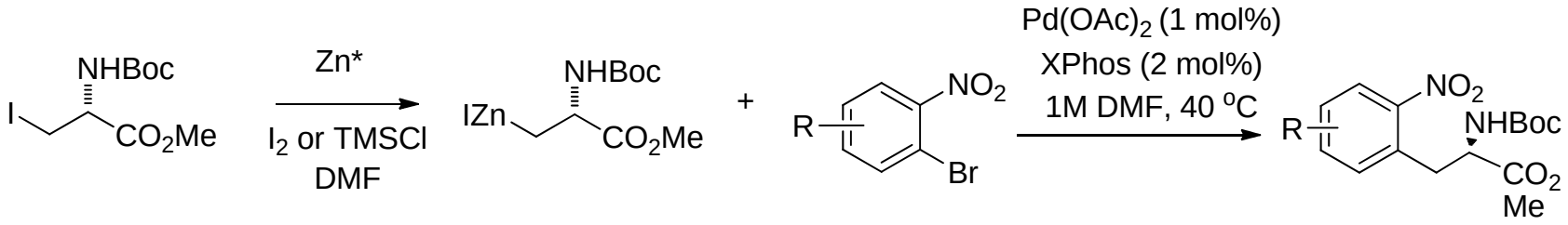
H_2, 5% Pt/C

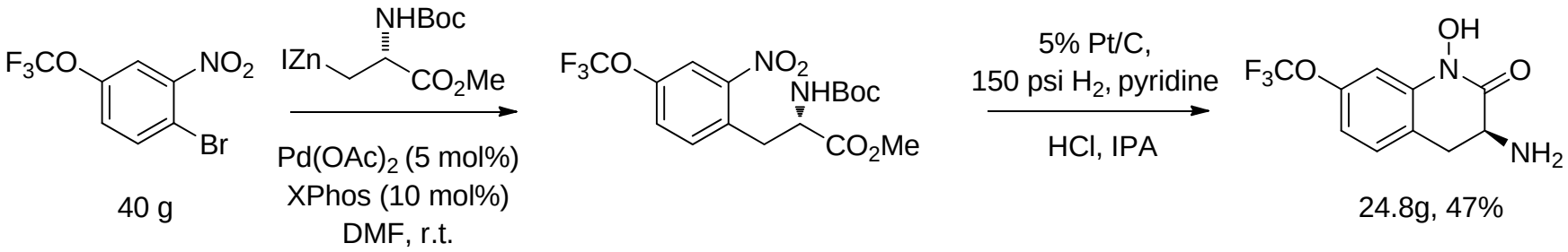
R = H – 1:2.3
= Me – 2:1
= CH_2CF_3 – 1:0

MacAllister, L. A.; *et.al. J. Org. Chem*. 2011, 76, 3484–3497

Using the Negishi reaction as alternative synthetic approach

- **Negishi reaction between iodoalanine and *o*-nitroaryl halides has provided a complimentary route**
- **Exploits chiral pool**
- **Suitable for scale-up**



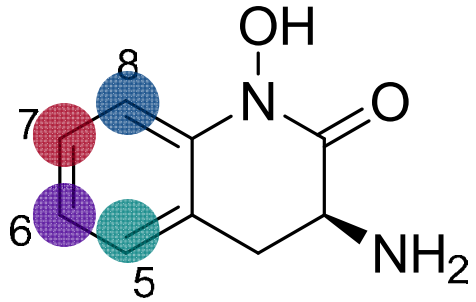


Tuttle, J. B.; *et al. Tetrahedron Lett.* (2011), doi:10.1016/j.tetlet.2011.07.083

Initial SAR – is there room to maneuver?

	hKATII	rKATII
F	40	631
OMe	572	>10 000
Cl	252	>10000
Me	1050	>10000
CF_3	174	>6810

	hKATII	rKATII
OMe	22	137
Cl	29	118
Me	37	368



PF-04859989

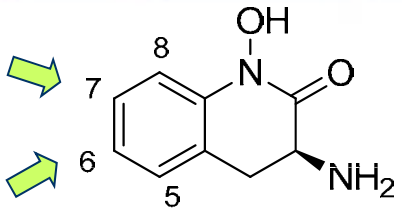
hKAT II IC50=23 nM

rKAT II IC50 = 263nM

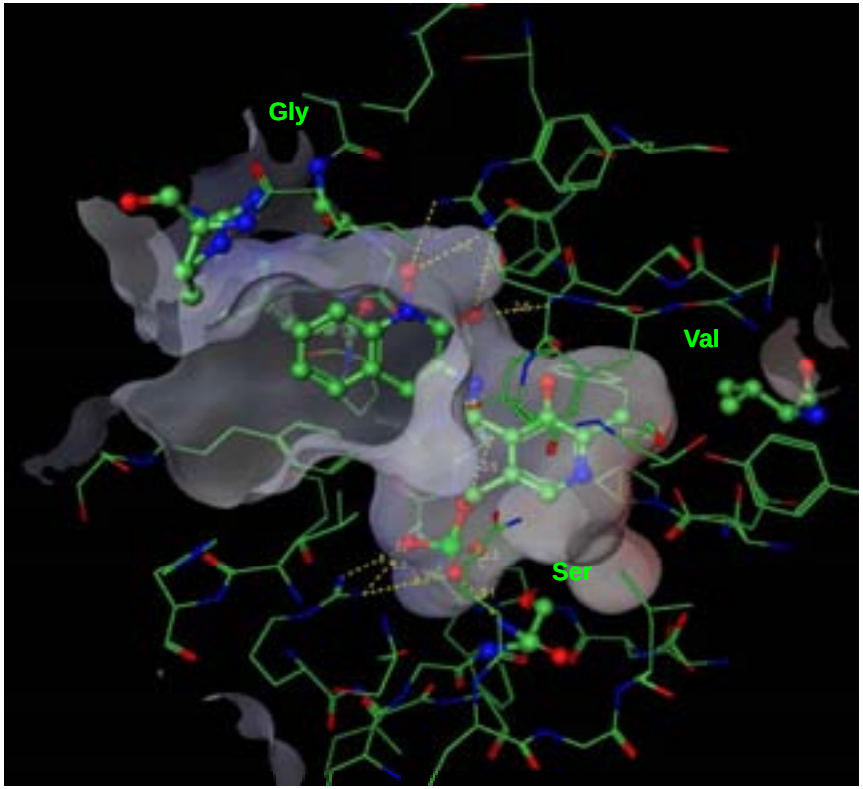
	hKATII	rKATII
Cl	36	258
Me	30	402
CF_3	29	488

	hKATII	rKATII
F	45	2060
OMe	179	>4920
Cl	349	>7970
Me	319	>10000
CF_3	>10000	>10000

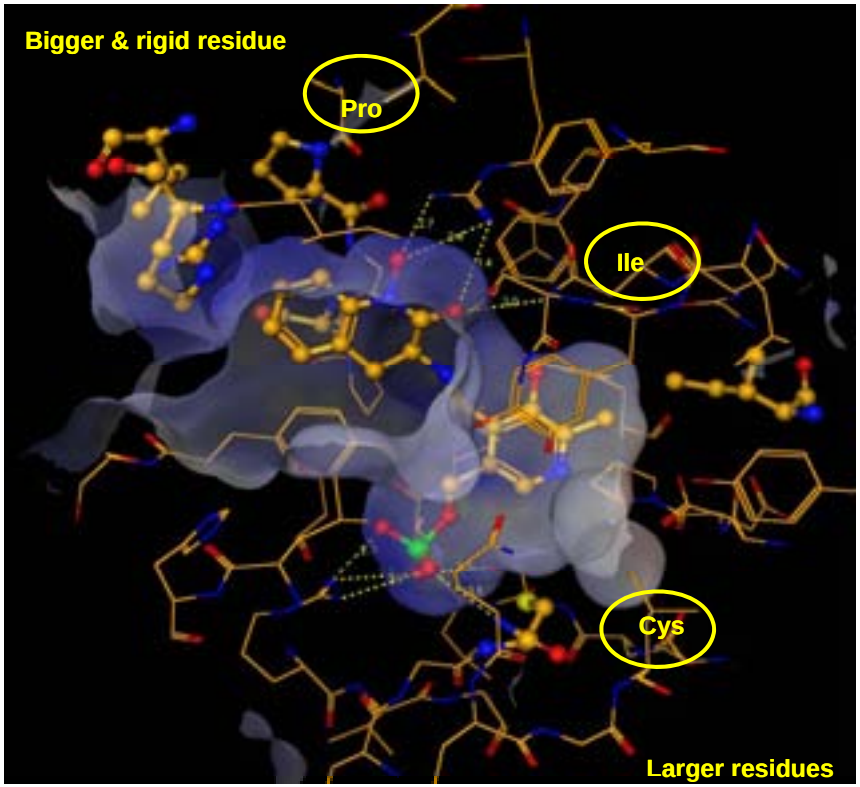
SAR observations consistent with X-ray structure



Human KAT II structure



Rat KAT II model

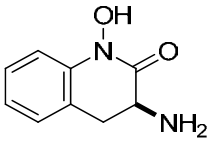
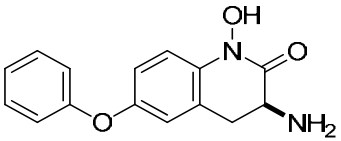
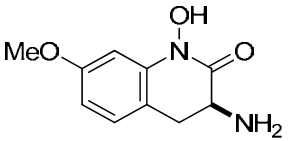
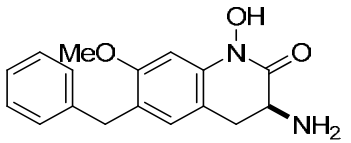


SuoBao Rong

- Comparing X-ray structure and homology model suggests rKAT II is more rigid
- Opportunities for further optimisation at positions 6 and 7

Using kinact/Ki as a more accurate measure of potency

- Further analogues explored potential space at positions 6 and 7
- Potency 'barrier' observed, hard to distinguish between analogues

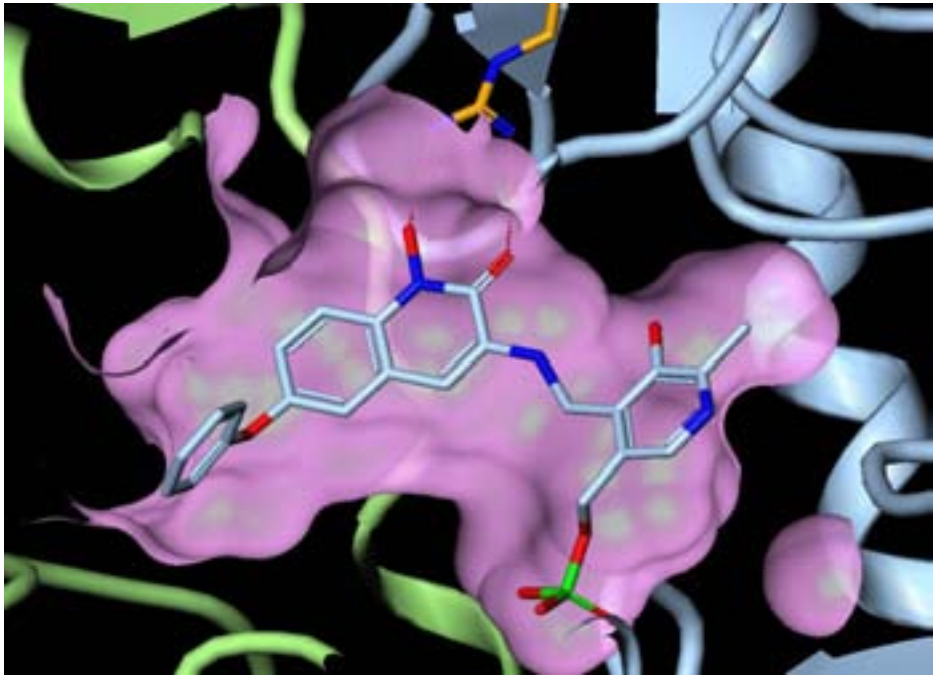
				
hKATII (nM)	23	23	22	37
hkinact/Ki ($M^{-1}s^{-1}$)	18,500	26,800	31,700	**129,000**

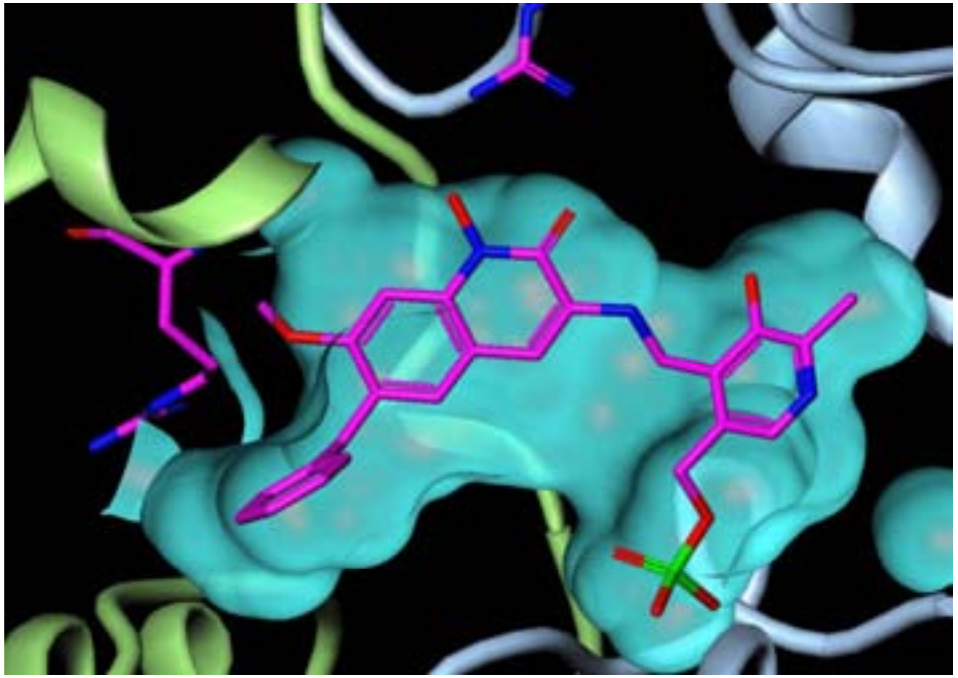
$$E + I \underset{}{\overset{Ki}{\rightleftharpoons}} EI \xrightarrow{kinact} E\text{-}I$$

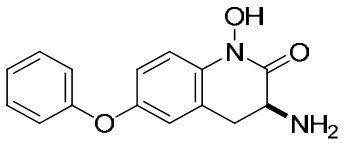
Ki: Initial binding affinity (rapidly reversible)
***k*inact: Reactivity (covalent bond formation)**
Overall potency—inhibition rate constant: *k*inact/Ki ($M^{-1}s^{-1}$)

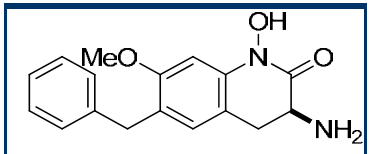
Like Ki for reversible compounds, kinact/Ki is independent of pre-incubation time, enzyme and substrate concentrations.

Structural synergies lead to significant potency enhancement





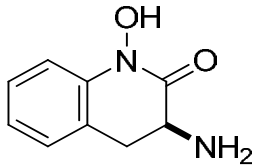
	
hKATII (nM)	23
hkinact/KI ($M^{-1}s^{-1}$)	26,800


37
129,000

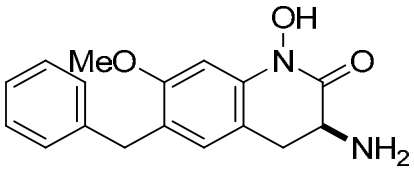
Enhanced potency is derived from a novel flexible domain α-helix interaction with Arg B20
New interactions with Arg B20 include an H-Bond with the 7-methoxy and a cation-pi interaction with the phenyl ring

Medicinal Chemistry efforts deliver a 'tool' compound and clinical candidate

- Using structure guided design and by choosing the right assay optimised potency of HTS hit whilst maintaining favourable properties
- Further optimisation led to compound nomination as a clinical candidate.



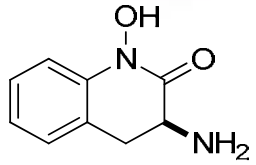
hKAT II IC_{50} = 23 nM
hKi/kinact = 26 800 $M^{-1}s^{-1}$
rKAT II IC_{50} = 263nM
MW = 178; logD = -0.7
clogP = -0.1; tPSA = 66.6
LE = 0.82; LipE=8.4



hKAT II IC_{50} = 37 nM
hKi/kinact = 120000 $M^{-1}s^{-1}$
rKAT II IC_{50} = 232 nM
MW = 298; logD = 1.4
clogP = 2.1; tPSA = 75.8
LE = 0.46; LipE=6.0

Clinical Candidate

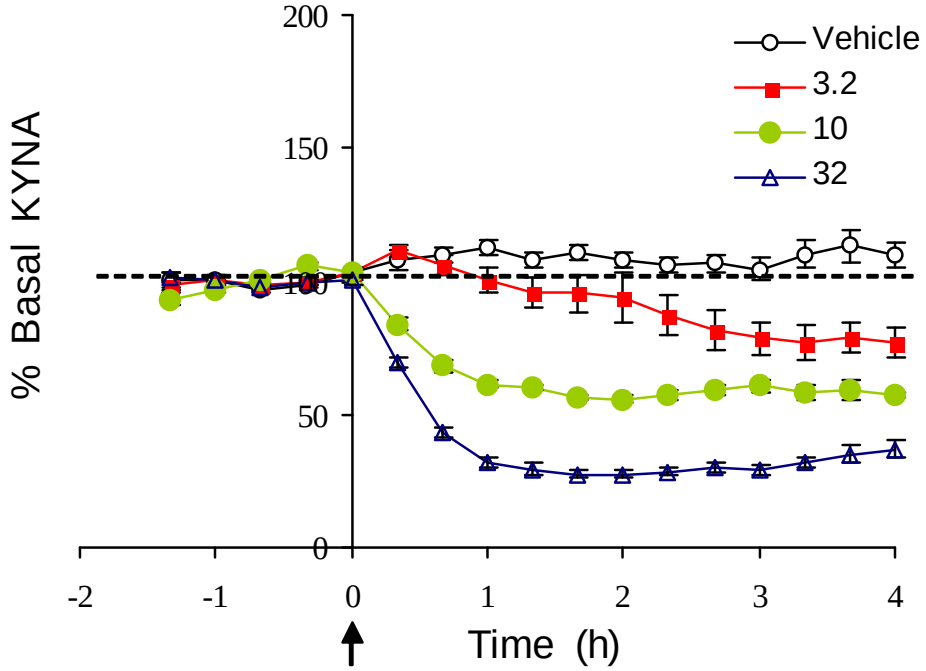
HTS hit proves to be a useful *in vivo* tool



PF-04859989
10 mg/kg s.c.

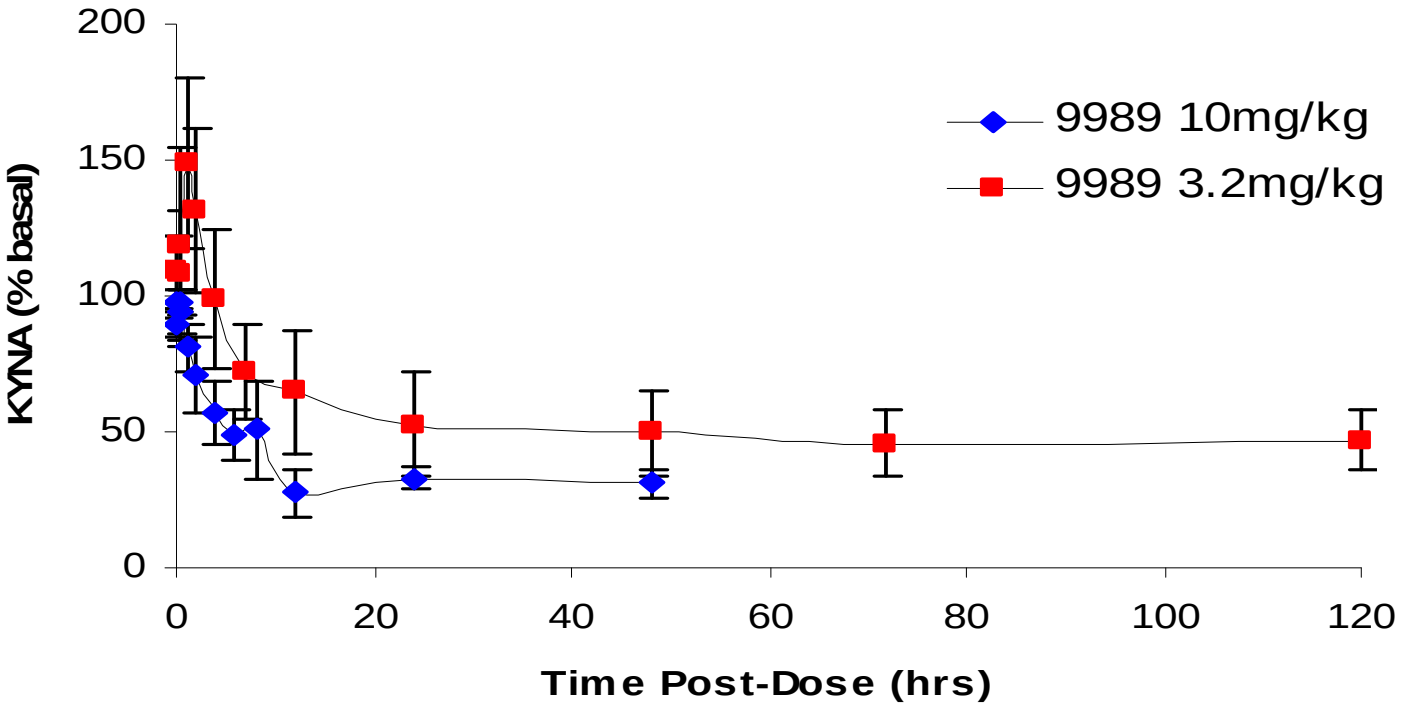
Brain fraction unbound	Plasma fraction unbound	Free plasma conc (nM)	Free brain conc (nM)	CSF conc (nM)
39.3	89.3	10200 ± 403	3760 ± 776	4060 ± 969

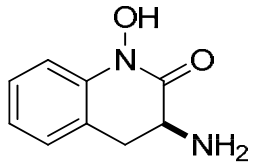
Rat prefrontal cortex microdialysis



- **PF-04859989 produces maximal KYNA reduction of ~80% @32 mpk**
- **Corresponds to complete inhibition of KATII**
- **KYNA concentration returns to baseline ~20 h postdose**

KYNA decrease also observed in primates





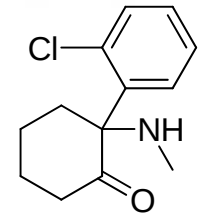
PF-04859989

- PF-04859989 effects sustained reduction of KYNA (>50% decrease, 5 d) in CSF of primates (Maccine)
- No adverse effects observed

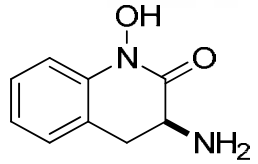
PF-04859989 Causes Dose-Dependent Reduction in Central KYNA in Rat and Monkey

Examining effects of KATII inhibitor on cognition

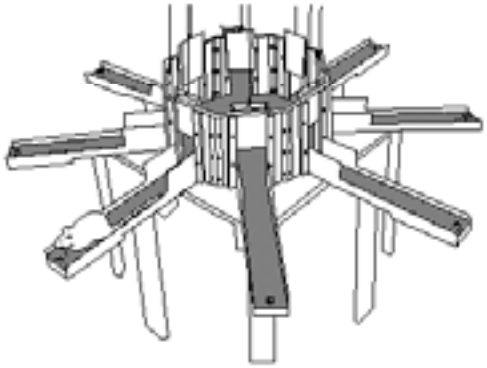
- Initial *in vivo* studies to probe cognitive effects of KATII inhibitors
- Examine effects on basal cognition
- Cause memory deficits using ketamine and look for reversal
  - Mimics cognitive impairments observed in schizophrenia

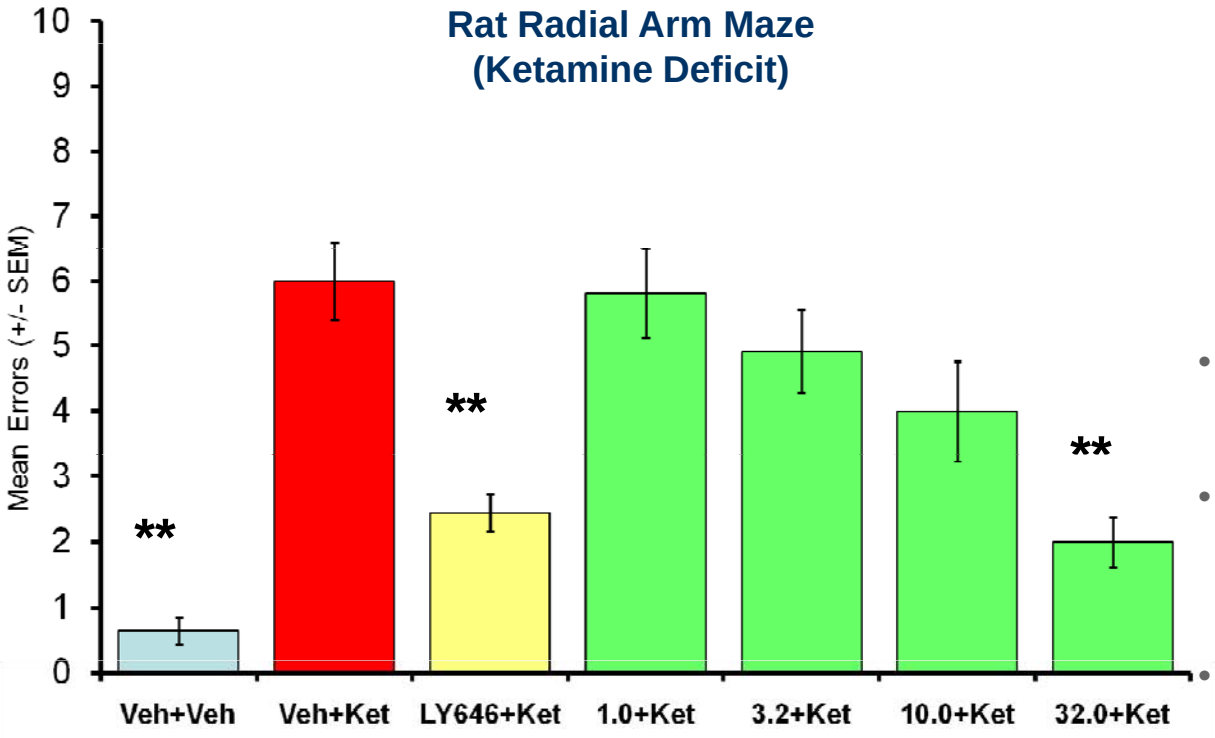


Ketamine



PF-04859989



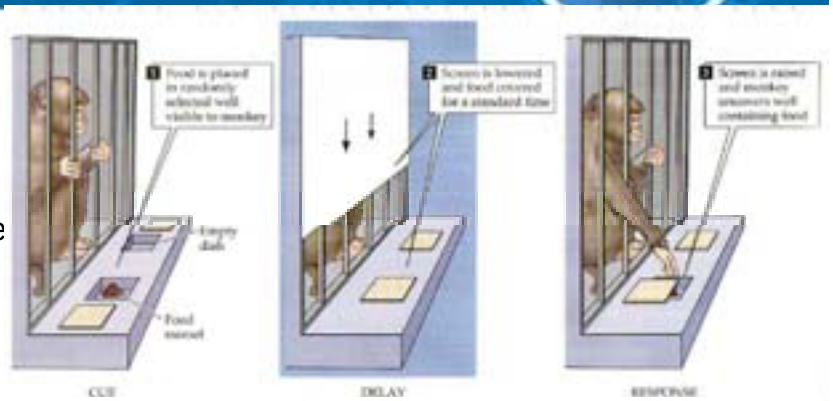


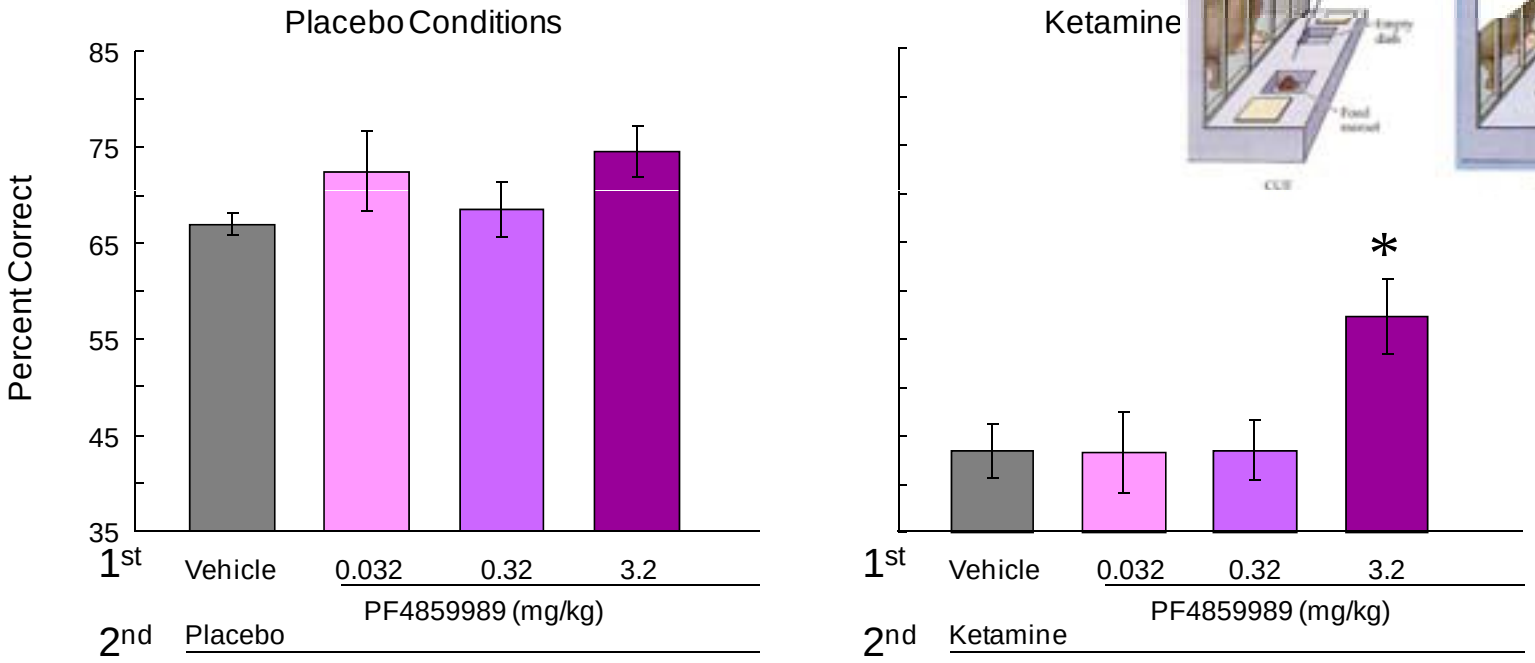
Strick et al. 2010, SFN

- Rat radial arm maze is a measure of short-term memory
- PF-04859989 reversed spatial memory deficits induced by ketamine in a dose-dependent manner
- Had no effects on basal memory

Cognitive effects in rats are confirmed in a primate model

Primate Delayed-Responding Task (Ketamine Deficit)





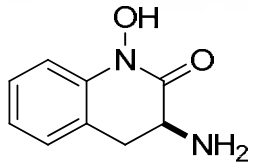
- **KAT II inhibitors reverse ketamine-induced deficits in attention/working memory in a primate delayed responding task**
- **Efficacy of PF-04859989 occurred at a lower dose in primates than in rats, which is consistent with the higher in vitro potency at the human/primate enzyme relative to rats**

Abbott et al. 2010, SFN

PF-04859989 enhances cognition in disease-relevant models

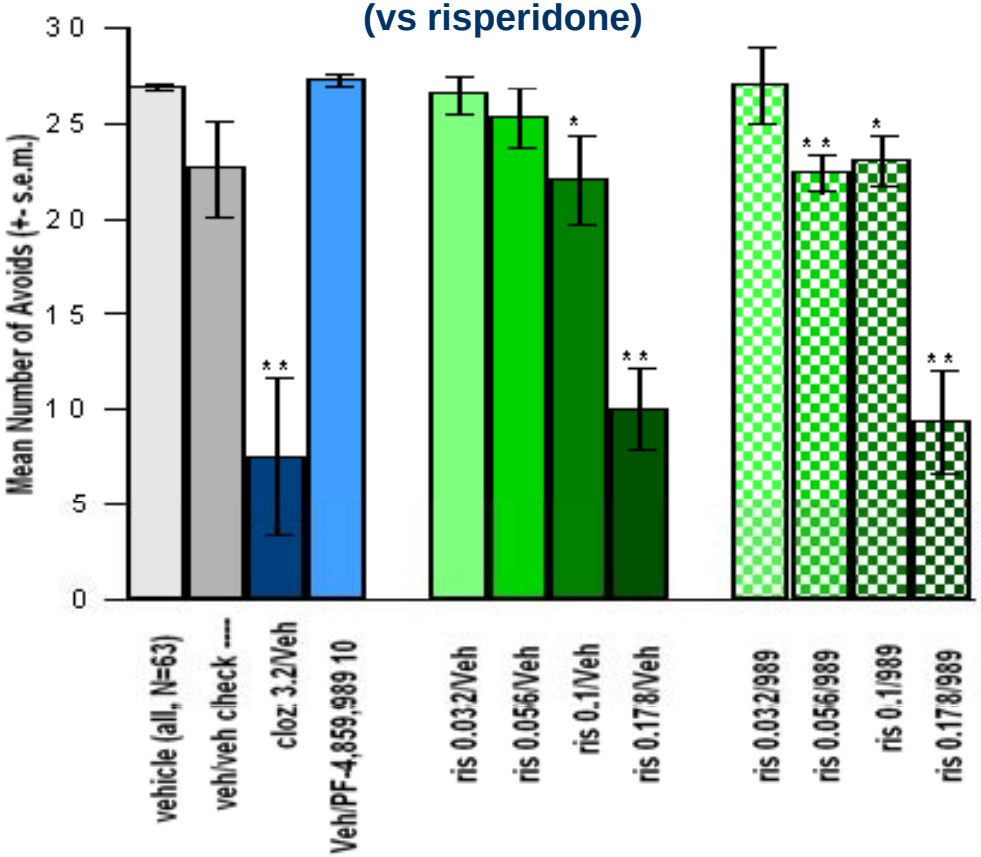
PF-04859989 does not disrupt the activity of standard antipsychotics

- A KATII inhibitor is not expected to affect positive symptoms of schizophrenia
- Important that it does not inhibit effects of antipsychotic medications



PF-04859989

Rat Conditioned Avoidance Response (vs risperidone)

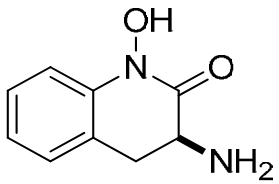


- Conditioned avoidance response is used to predict antipsychotic activity
- PF-04859989 did not affect avoidance responding when given alone
- PF-04859989 does not show activity in several other models of schizophrenia (positive symptoms)
- Does not affect the activity of other antipsychotics in these models

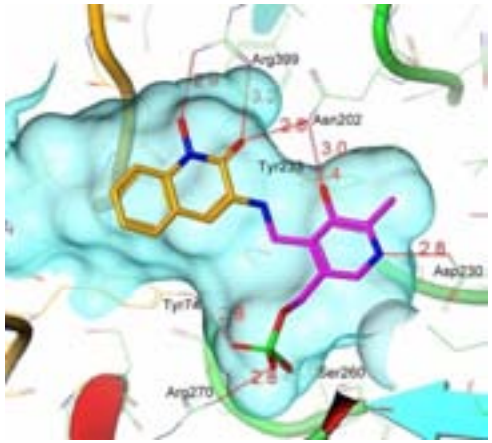
Wadenburg, M.-L. *Neuroscience and Biobehavioral Reviews* 23 (1999) 851–862

Summary

- Discovered a series of novel, brain penetrant KATII inhibitors
- Inhibitors are irreversible, forming a covalent complex with enzyme co-factor PLP
- X-ray structures helped to guide medicinal chemistry efforts towards more potent compounds
- PF-04859989 demonstrated *in vivo* activity both at lowering levels of KYNA in the brain and in disease relevant *in vivo* models.



PF-04859989



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Dounay, A.B.; *ACS Med. Chem. Lett.* 2011
MacAllister, L. A.; *et.al. J. Org. Chem*. 2011, 76, 3484–3497
Tuttle, J. B.; *et al. Tetrahedron Lett.* (2011), doi:10.1016/j.tetlet.2011.07.083