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

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

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*

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

HTS identifies potent KATII inhibitor

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

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

- 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

- 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

Is PF-04859989 an irreversible inhibitor of KATII?
Defining irreversibility

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 µM each)

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

Confirming structural requirements for irreversible inhibition

- Small series of analogues confirmed structural requirements for KATII inhibition

![Structural formula and IC50 values for hKAT II and rKAT II](image)
What does irreversibility mean for the project

• Irreversible inhibitors often avoided
  - Covalent modification of enzyme can lead to immune response
  - Idiosyncratic 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

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

\[
\text{Ph-}N\text{N-}O\text{tBu + R-}N\text{O}_2\text{Br} \rightarrow \text{H}_2\text{N-}O\text{H} + \text{NO}_2\text{O}R
\]

1. Catalyst (10 mol\%)  
   \[\text{CsOH, DCM, } -30 \, ^\circ\text{C}\]
2. TFA/DCM

50-92\% yield, 75 - 99\% ee

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

\[
\text{H}_2\text{N-}O\text{H} \rightarrow \text{SnCl}_2, \text{NaOAc} \\
\text{THF-MeOH, r.t}
\]

35-86\%

Concise synthesis of KATII inhibitors

• SnCl₂ reduction ineffective when nitro group is ortho-substituted.

\[
\text{SnCl}_2, \text{NaOAc}\quad \text{THF-MeOH, r.t}
\]

• Examined activated esters to enhance rate of cyclisation

\[
\begin{align*}
\text{H}_2, 5\% \text{Pt/C} \\
R = H - 1:2.3 \\
= \text{Me} - 2:1 \\
= \text{CH}_2\text{CF}_3 - 1:0
\end{align*}
\]

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

\[
\begin{align*}
\text{I-} \quad \text{NHBOc} & \quad \xrightarrow{\text{Zn}^+} \quad \text{I-Zn} \quad \text{NHBOc} \\
\text{CO}_2\text{Me} & \quad \text{I}_2 \text{ or TMSCl} \quad \text{DMF} \\
\end{align*}
\]

\[
\begin{align*}
\text{Pd(OAc)}_2 \text{ (}1 \text{ mol}\%\text{)} & \quad \text{XPhos} \text{ (}2 \text{ mol}\%\text{)} \\
\text{DMF, } 40 ^\circ\text{C} & \quad \text{1M DMF, 40 }^\circ\text{C}
\end{align*}
\]

\[
\begin{align*}
\text{F}_3\text{CO} & \quad \text{NO}_2 \\
\text{Br} & \quad \text{40 g}
\end{align*}
\]

\[
\begin{align*}
\text{I-Zn} \quad \text{NHBOc} & \quad \xrightarrow{\text{Pd(OAc)}_2 \text{ (}5 \text{ mol}\%\text{)} \quad \text{XPhos} \text{ (}10 \text{ mol}\%\text{)} \quad \text{DMF, r.t.}} \\
\text{CO}_2\text{Me} & \quad \text{5% Pt/C,} \quad 150 \text{ psi } \text{H}_2, \text{pyridine} \quad \text{HCl, IPA}
\end{align*}
\]

Initial SAR – is there room to maneuver?

<table>
<thead>
<tr>
<th></th>
<th>hKATII</th>
<th>rKATII</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>40</td>
<td>631</td>
</tr>
<tr>
<td>OMe</td>
<td>572</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Cl</td>
<td>252</td>
<td>&gt;10000</td>
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<tr>
<td>Me</td>
<td>1050</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>CF₃</td>
<td>174</td>
<td>&gt;6810</td>
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</tbody>
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<tr>
<td>OMe</td>
<td>22</td>
<td>137</td>
</tr>
<tr>
<td>Cl</td>
<td>29</td>
<td>118</td>
</tr>
<tr>
<td>Me</td>
<td>37</td>
<td>368</td>
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PF-04859989
hKAT II IC50 = 23 nM
rKAT II IC50 = 263 nM
SAR observations consistent with X-ray structure

Human KAT II structure

Rat KAT II model

• 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

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<tbody>
<tr>
<td>hKATII (nM)</td>
<td>23</td>
<td>23</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>hkinact/Ki (M⁻¹s⁻¹)</td>
<td>18,500</td>
<td>26,800</td>
<td>31,700</td>
<td>129,000</td>
</tr>
</tbody>
</table>

**Ki**: Initial binding affinity (rapidly reversible)
**kinact**: Reactivity (covalent bond formation)

Overall potency—inhibition rate constant: **kinact/Ki (M⁻¹s⁻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

Enhanced potency is derived from a novel flexible domain \( \alpha \)-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.

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<td></td>
<td>37</td>
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<td></td>
<td>129,000</td>
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</table>
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  
$hKi/kinact = 26800$ M$^{-1}$s$^{-1}$  
$rKAT II  IC_{50} = 263$ nM  
$MW = 178; \ logD = -0.7$  
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
HTS hit proves to be a useful *in vivo* tool

<table>
<thead>
<tr>
<th>Brain fraction unbound</th>
<th>Plasma fraction unbound</th>
<th>Free plasma conc (nM)</th>
<th>Free brain conc (nM)</th>
<th>CSF conc (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>39.3</td>
<td>89.3</td>
<td>10200 ± 403</td>
<td>3760 ± 776</td>
<td>4060 ± 969</td>
</tr>
</tbody>
</table>

**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 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

Rat Radial Arm Maze (Ketamine Deficit)

- 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

Strick et al. 2010, SFN
Cognitive effects in rats are confirmed in a primate model

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

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

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.
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