A Novel, Brain Penetrant KAT-II Inhibitor, KYN-5356, Reduces Brain Kynurenic Acid Levels and Enhances Glutamatergic and Cholinergic Transmission

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Background

- Kynurenic Acid **(KYNA**) is elevated in schizophrenia, AD, and other neurocognitive disorders, and impairs cognition
- Kynurenine aminotransferase II **(KAT-II)** is the primary regulator of KYNA synthesis in the brain
- KYNA inhibits α7nACh and NMDA receptors, **disrupting key cognitive pathways**
- KAT-II **inhibition lowers KYNA** and improves neurotransmission
- KYN-5356 is a potent KAT-II inhibitor currently being tested for efficacy in Phase 2 for CIAS

Methods

Brain KYNA Quantitation
Common marmosets (*Callithrix jacchus*) (12-13 mo) were orally dosed with KYN-5356 (1, 3, 10 *or* 30 mg/kg.) After 90 min, L-kynurenine (10 mg/kg, IV) was administered to elevate KYNA levels. Animals were euthanized 60 min later, and hippocampus and prefrontal cortex tissues were collected. L-kynurenine and KYNA concentrations were quantified using high-performance liquid chromatography (HPLC).

Hippocampal Acetylcholine Measurement
In vivo microdialysis was performed in cynomolgus monkeys using probes implanted in the hippocampus.

Electroencephalography (EEG): Eight common marmosets were implanted intraperitoneally with HD-S02 telemeters (DSI) with a single channel EEG lead over the prefrontal cortex. Signals were recorded via telemetric receivers, acquired using Ponemah, and analyzed with NeuroScore (DSI).

Scopolamine challenge: Animals received scopolamine (40 µg/kg) to establish baseline effects, followed by KYN-5356 (30 mg/kg, BID, 7 days) and rechallenge with scopolamine. Both drugs were co-administered, and EEG was recorded for 6 h post-dose. FFT analysis was performed in 5-s epochs to assess spectral power across frequency bands.

Results

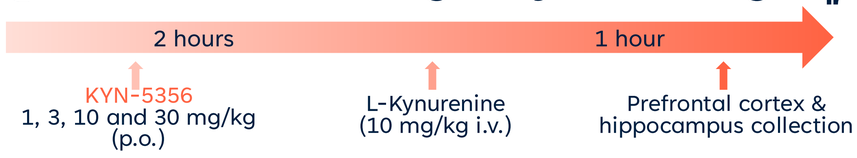
KYN-5356 is a highly selective and potent inhibitor of KAT-II

	IC_{50}	Ki
KAT-II	**16.05 nM (9.17-28.07nM)**	**10.48 nM (5.99-18.33 nM)**
KAT-I	>100 µM	nd
KAT-III	>100 µM	nd
KAT-IV	>100 µM	nd

Table 1. In vitro KYN-5356 activity against human KAT isoforms

- KYN-5356 potently and selectively inhibited recombinant human KAT-II (E. coli–expressed)
- Reducing KYNA production in an HPLC enzyme assay, with no activity at other human KAT isoforms (≤100 µM)

KYN-5356 inhibits KYNA production in the brain following L-Kyn challenge



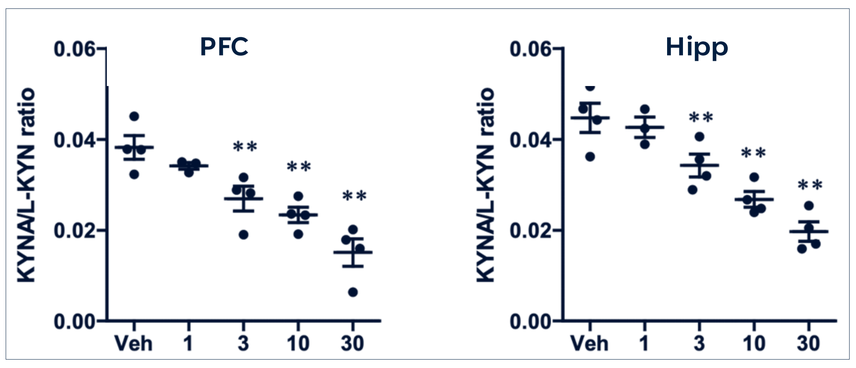


Figure 1. Effect of KYN-5356 on KYNA/L-Kyn ratio in the prefrontal cortex and hippocampus of marmosets

- L-Kyn was administered IV to elevate KYNA levels
- KYN-5356 pretreatment produced robust, dose-dependent reduction in KYNA/L-Kyn ratio (up to 50%) in the tissue homogenate, confirming inhibition of KYNA production despite supraphysiologic L-Kyn exposure
- A 10 mg/kg dose reduced the ratio by 30%, establishing the threshold for predicted human efficacious dose

KYN-5356 robustly increases acetylcholine levels in hippocampus

N=5 , Means: ±SEM; *P<0.05, **P<0.01 vs vehicle (2-way ANOVA, Bonferroni)

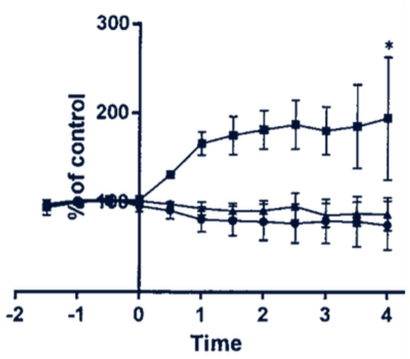


Figure 2. Intrahippocampal infusion of KYN-5356 increased acetylcholine levels in cynomolgus monkeys

- KYN-5356 was infused into the hippocampus via reverse dialysis (100 µM to ensure full KAT-II inhibition due to low probe recovery).
- A baseline was recorded 2 h pre-infusion; post-dose changes were normalized to four pre-drug samples.
- KYN-5356 induced a steady, robust increase in hippocampal acetylcholine levels.

KYN-5356 attenuates scopolamine-induced muscarinic blockade

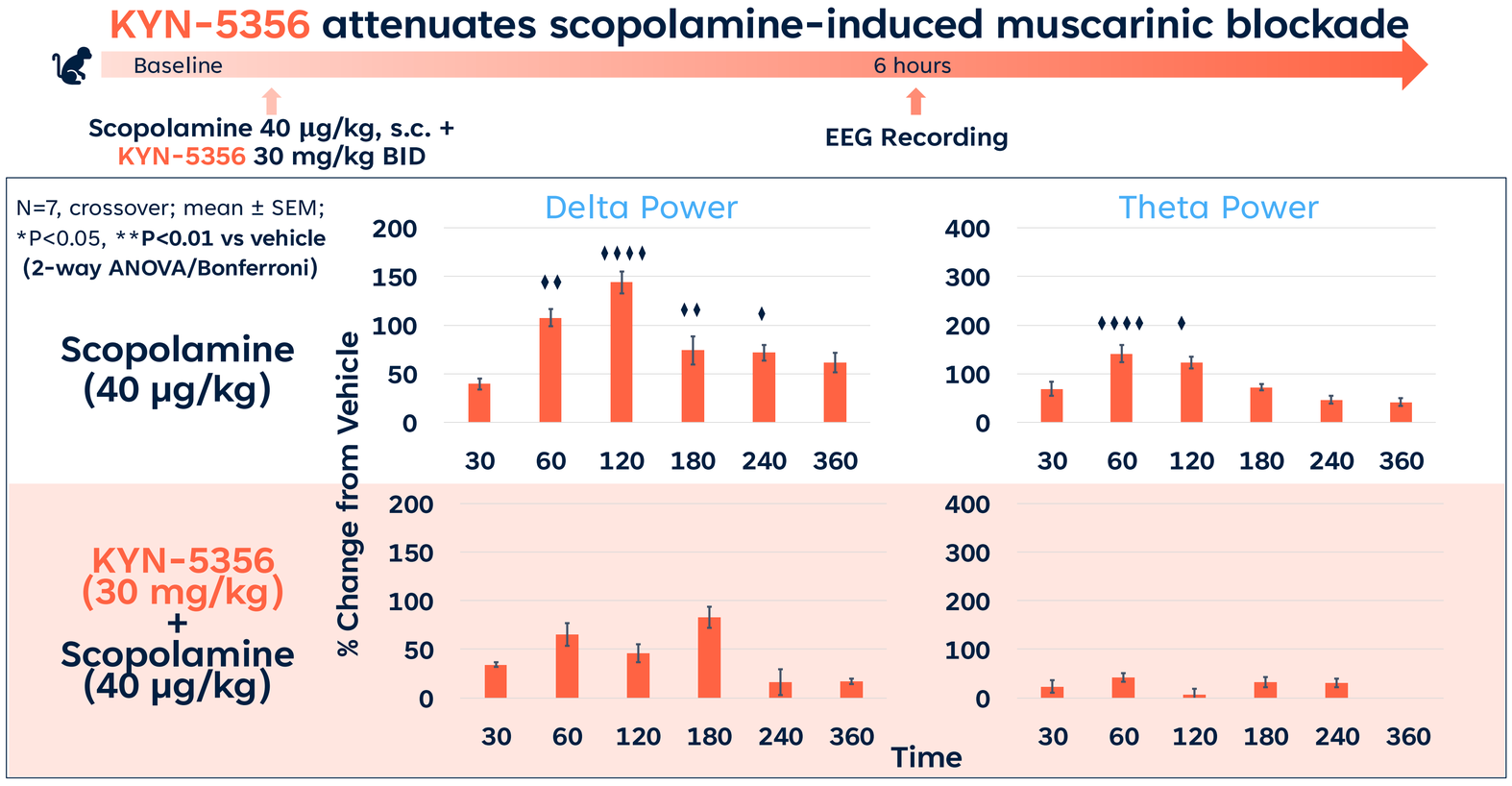


Figure 3. Effect of KYN-5356 on EEG network activity following scopolamine in cynomolgus monkeys

- Scopolamine significantly increased delta and theta power consistent with muscarinic blockade
- KYN-5356 robustly attenuated the effect of scopolamine on delta power and nearly completely inhibited the increase in theta power
- Data show that KYN-5356 robustly enhances cholinergic activation that can reverse acute blockade by scopolamine
- KYN-5356 also increases gamma power, suggesting enhanced glutamatergic signaling

KYNA inhibits α7nACh receptor function

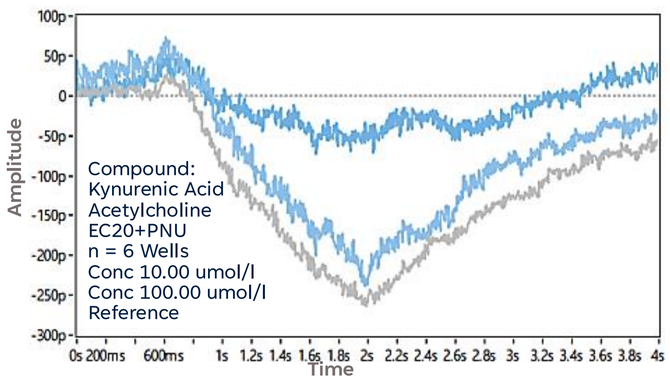
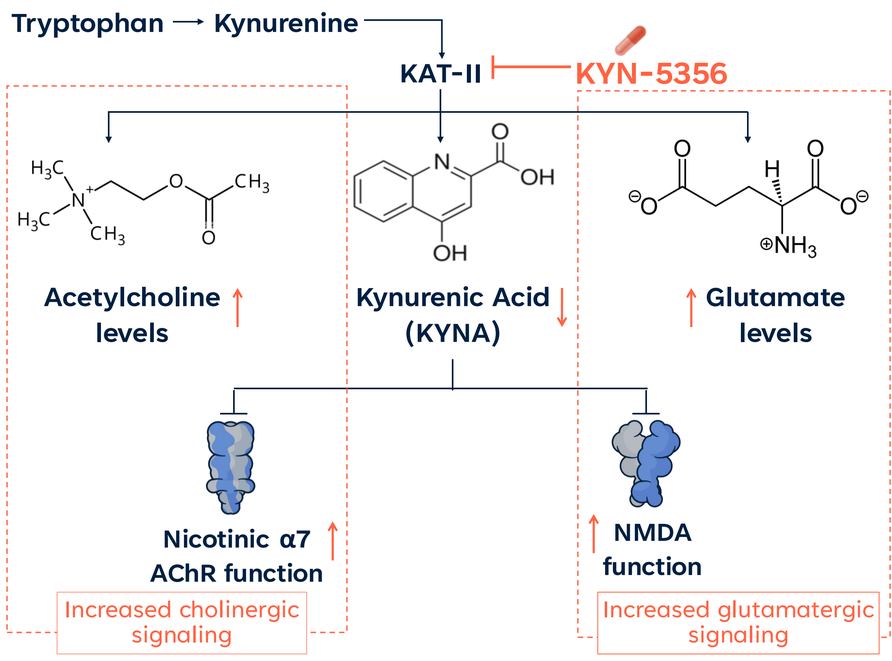


Figure 4. Assessment of KYNA inhibition of α7nAChR current in inhibition mode in SyncroPatch

N = 29 cells; averaged receptor current traces (DMSO vs. two KYNA concentrations)

- KYNA inhibits α7 nicotinic receptor currents; reducing KYNA expected to disinhibit α7 nicotinic receptor
- Increasing concentration of KYNA inhibit acetylcholine-induced activation of α7nACh receptor.
- At higher concentrations, KYNA nearly completely inhibits receptor activation, confirming the role of KYNA in α7nACh modulation

Multimodal mechanism of action of KYN-5356



- KAT-II inhibition with KYN-5356 *reduces* KYNA, and *increases* glutamate and acetylcholine levels
- Reducing KYNA with KYN-5356 increases NMDA and nicotinic α7 receptor function

Conclusions

- KYNA is elevated in schizophrenia, AD, and other neurocognitive disorders, and impairs cognition
- KYN-5356 robustly reduces brain KYNA/L-Kyn ratio
- KYN-5356 increases acetylcholine levels and attenuates the effect of muscarinic blockade
- Multimodal mechanism KYN-5356 enhances glutamatergic and cholinergic transmission
- KYN-5356 is a potent KAT-II inhibitor currently being tested for efficacy in Phase 2 for CIAS

Heramb Chadchankar[1] , Nick DeMartinis[1], and Jens Wendland[1] are employees of Kynexis B.V. and hold equity in the company. Robert Schwarcz [1, 2] and Carol Tamminga[1,2] hold equity shares in Kynexis[1] as scientific co-founders and are affiliated with University of Maryland and UT Southwestern, respectively. [2]