Small Molecule Screen in ALS

Small Molecule activators of Glutamate Transporter EAAT2 for Treatment of Neurological disorders

7470 Neuromuscular Biology and Disease
March 5, 2015
C Glenn Lin
Neuroscience

Glutamate transporter EAAT2

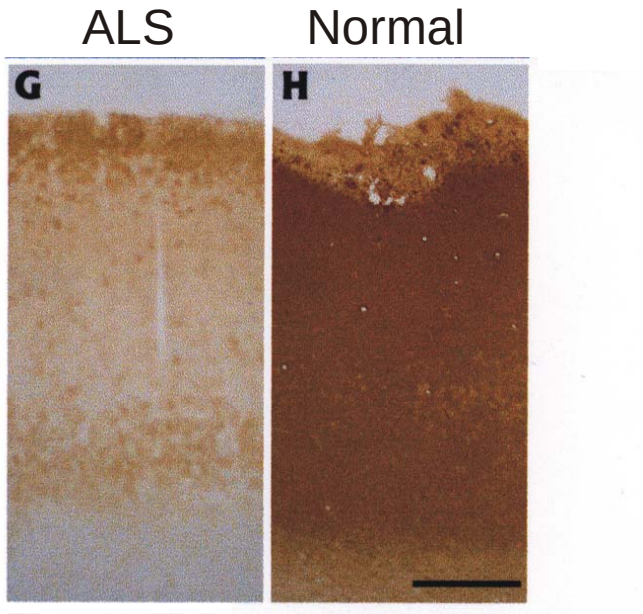
- on peri-synaptic processes of astrocytes closely associated with excitatory synaptic contacts
- for maintaining low extracellular glutamate concentrations and preventing excess glutamate-mediated toxicity
- plays an essential role in regulating synaptic plasticity and cognitive functions

- Mice lacking the EAAT2 gene exhibit elevated extracellular glutamate levels in the brain and lethal spontaneous seizures
- Mice lacking one allele for EAAT2 show worsened outcomes following SCI, TBI, or stroke
- In a mouse model of ALS, SOD1(G93A) mice lacking one allele for EAAT2 exhibit earlier onset and death
- In a mouse model of Alzheimer's disease, AβPPswe /PS1ΔE9 mice lacking one allele for EAAT2 exhibit accelerated cognitive deficits
- Blockade of EAAT2 function in the prefrontal cortex induces depression symptoms in rats
- Literature also indicates that EAAT2 contributes to the pathophysiology of alcohol and drug addiction

EAAT2 and ALS

- Approximately 60–70% of ALS patients have a 30–95% loss of the EAAT2 protein in the motor cortex and spinal cord
- Loss of EAAT2 protein is also observed in animal models of ALS, mutant SOD1, TDP-43
- When motor neurons are intact histologically and physiologically, there was a patchy loss of EAAT2 expression around motor neurons
- Loss of EAAT2 protein is due to disturbances at the post-transcriptional level because EAAT2 mRNA is not decreased

Loss of EAAT2 protein and function is commonly found in ALS patients

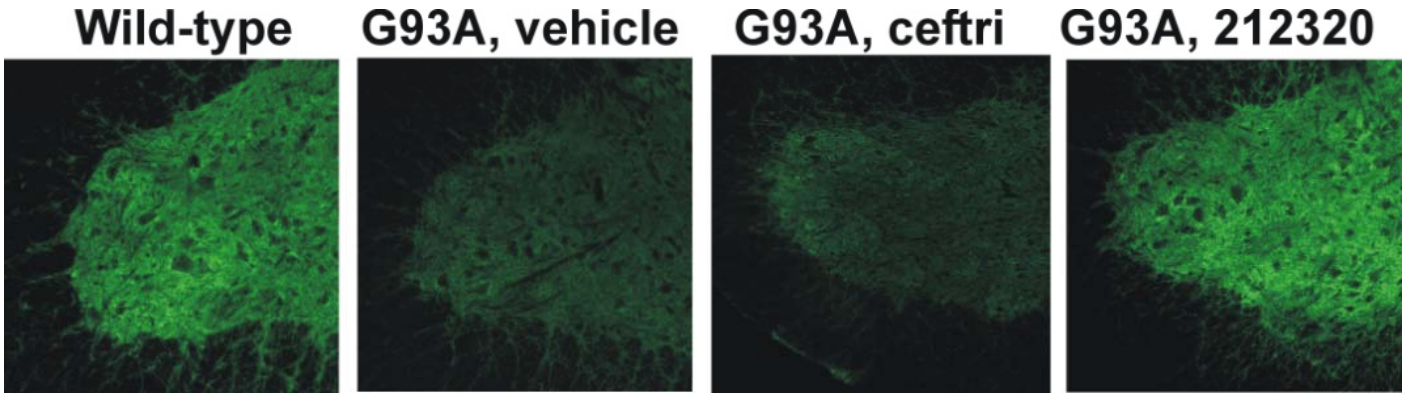


- Up-regulation of EAAT2 protein provides significant beneficial effects in many animal models of disease, including ALS, AD, Parkinson's disease, Huntington's disease, epilepsy, stroke, trauma, chronic pain disorders, addictive disorders, and depression

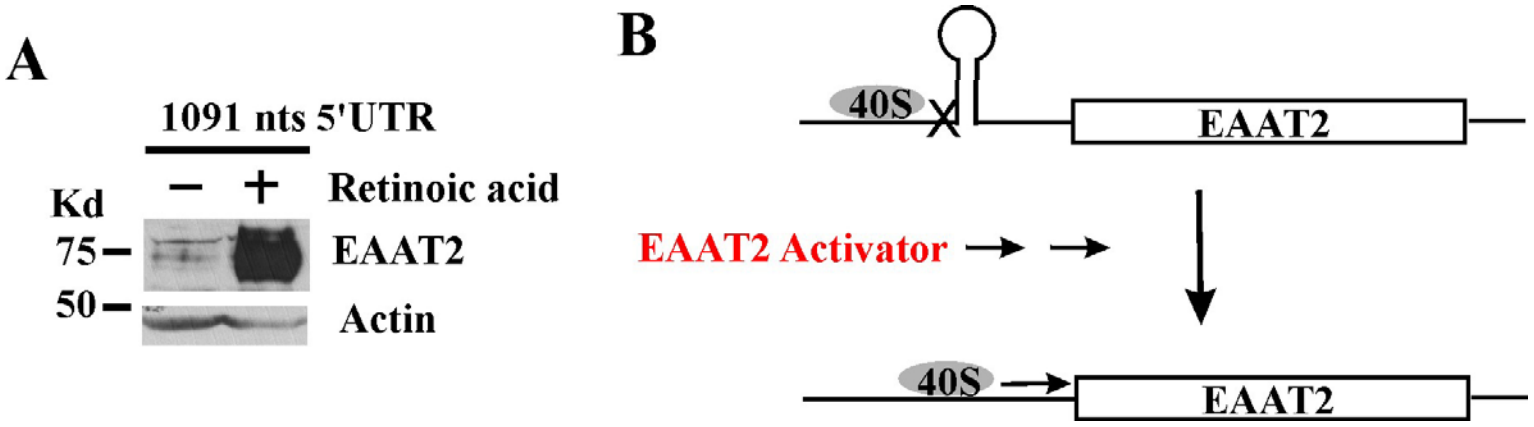
- Activation of EAAT2 expression is a potential therapeutic intervention

Ceftriaxone

- a β-lactam antibiotic that can increase EAAT2 expression via transcriptional activation
- Fail in ALS clinical trial
- Conflicting findings regarding ceftriaxone's ability to increase EAAT2 expression *in vivo*

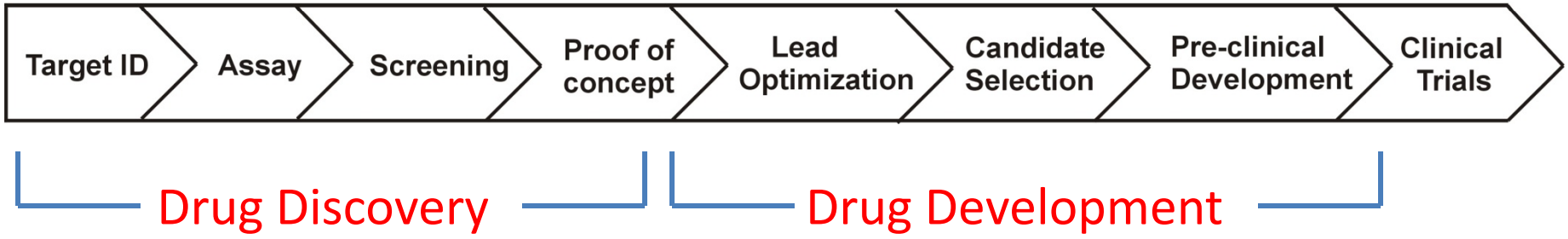


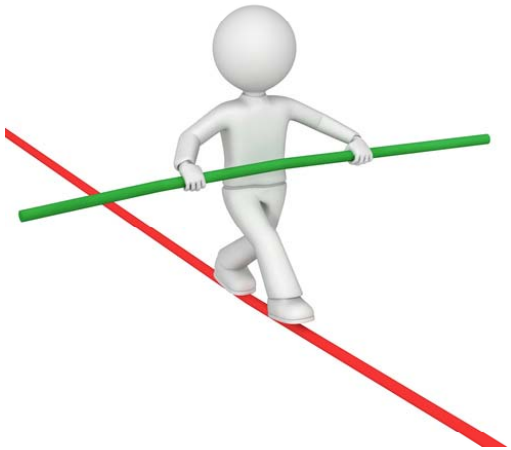
- It is unknown whether EAAT2 restored in ALS patients who received ceftriaxone in the trial

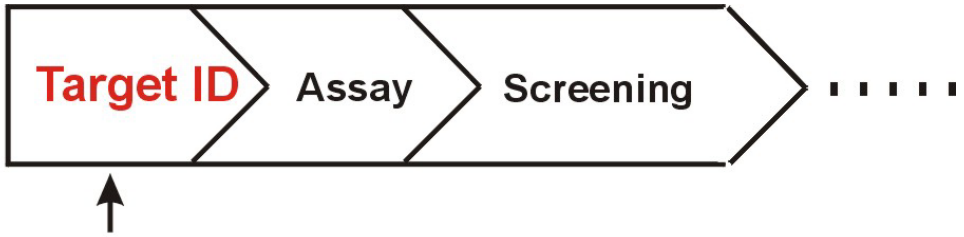


Tian G., Lai L., Guo H., Lin Y., Butchbach M.E.R., Chang Y., Lin C.-L. G. (2007) Translational regulation of glial glutamate transporter EAAT2 expression ***J. Biological Chemistry*** 282:1727-37

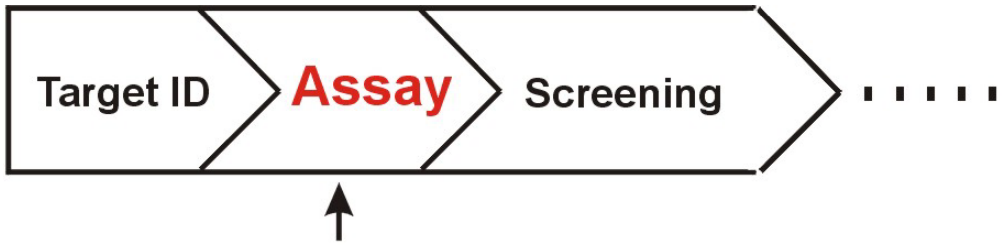
Drug discovery/development Pipeline





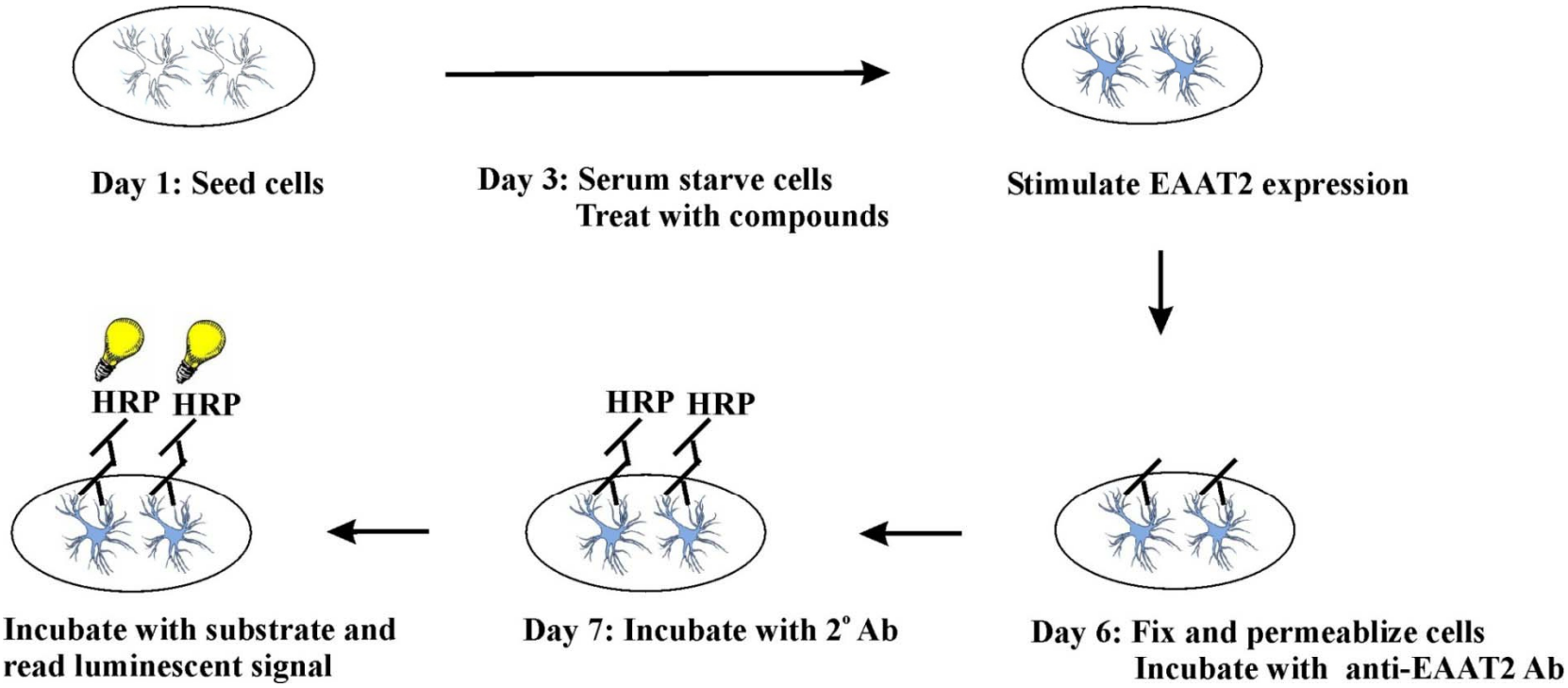


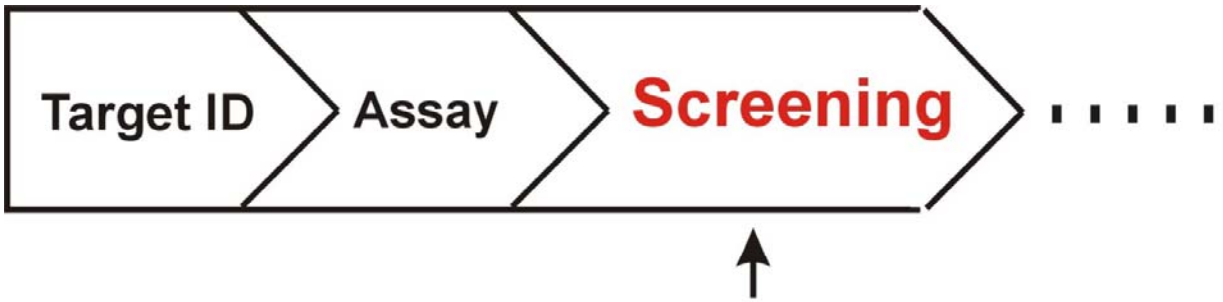
Search for compounds that increase EAAT2 translation



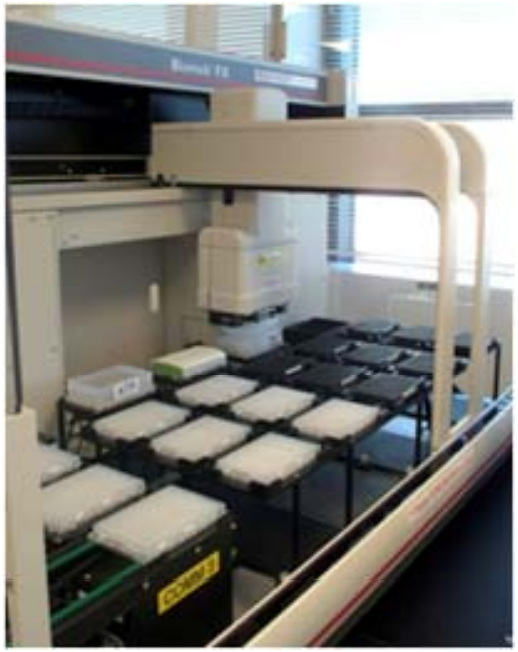
Established a cell-based ELISA

Laboratory for Drug Discovery in Neurodegeneration
Harvard Medical School

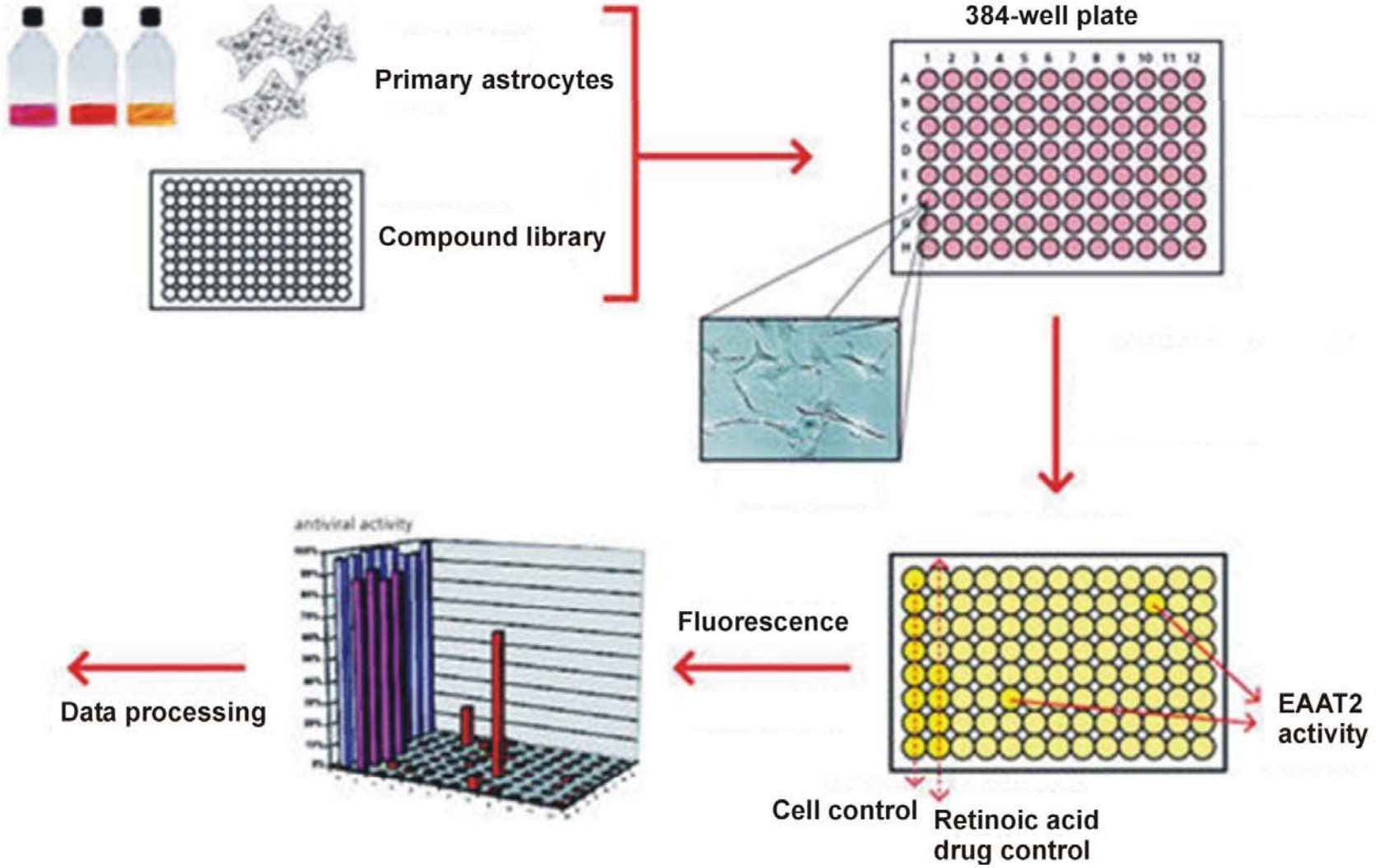

Day 1: Seed cells
Day 3: Serum starve cells
Treat with compounds
Stimulate EAAT2 expression
Day 6: Fix and permeablize cells
Incubate with anti-EAAT2 Ab
Day 7: Incubate with 2° Ab
HRP HRP
HRP HRP
Incubate with substrate and
read luminescent signal

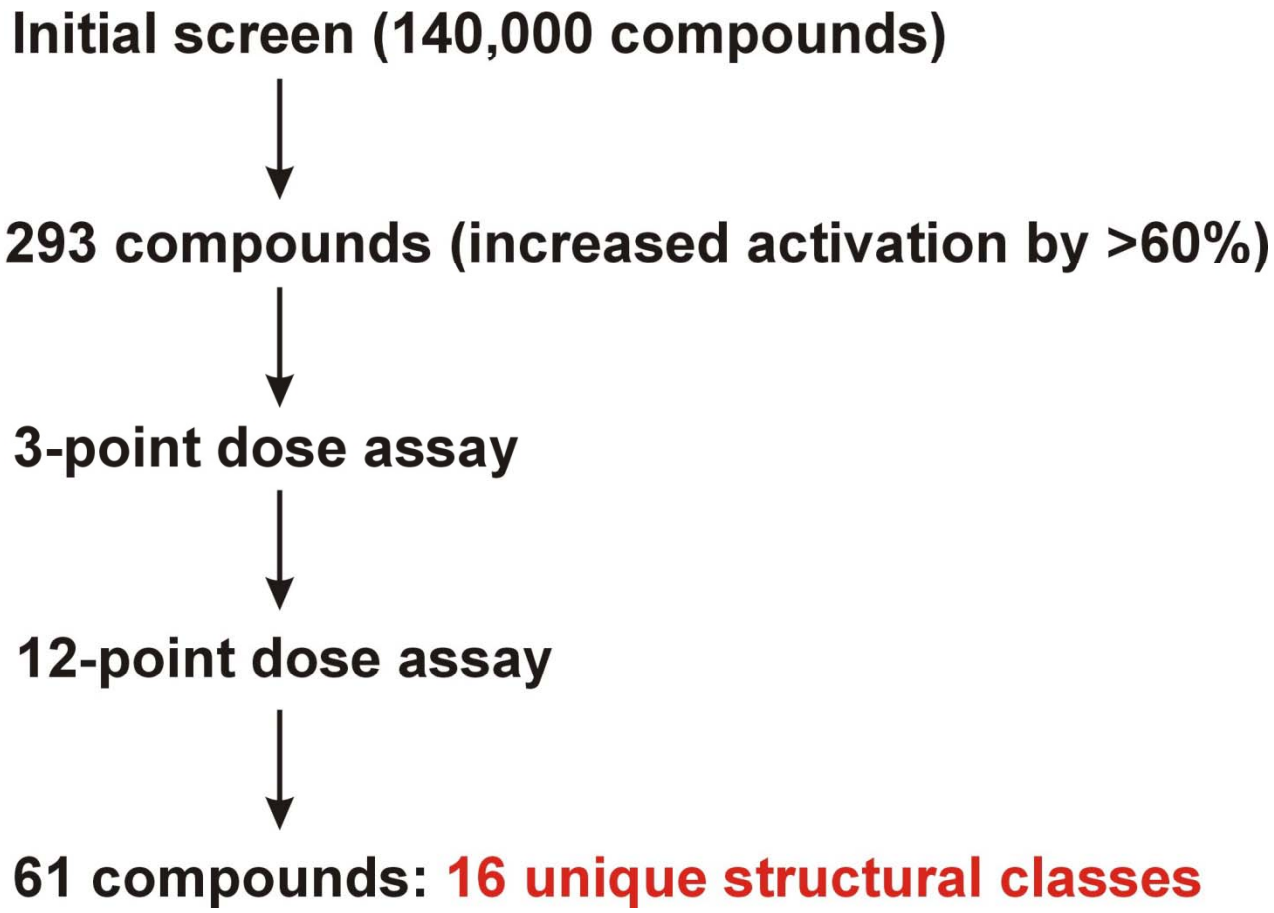


Performed a high-throughput screen



High Throughput Screen (HTS)

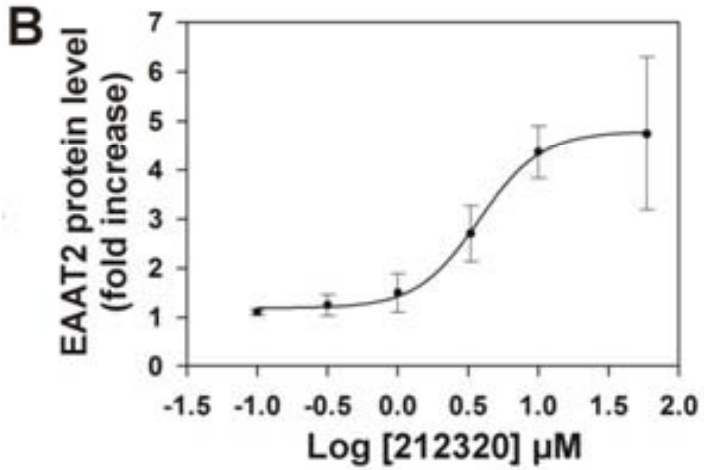


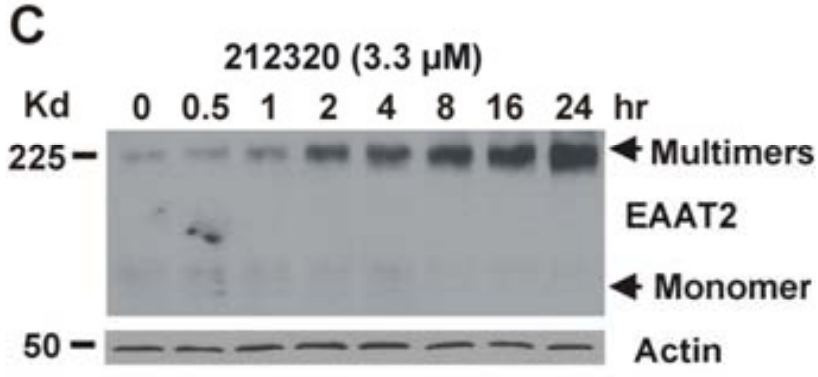


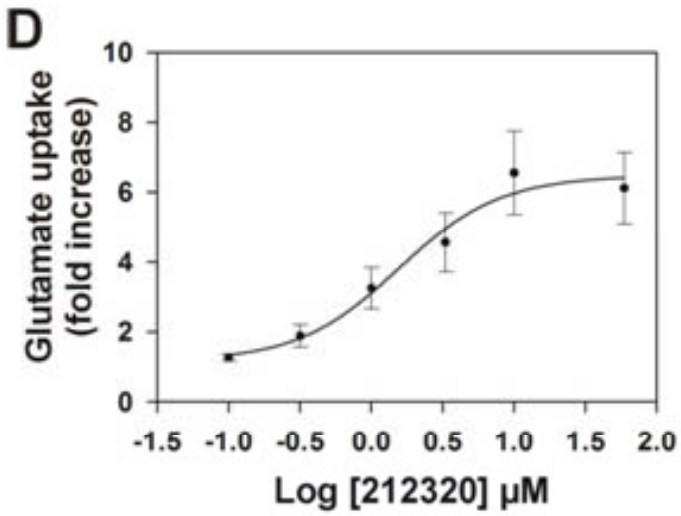
Colton C., Kong Q., Lai L., Zhu M.,1 Seyb K, Cuny G., Xian J, Glicksman M., Lin C.-L. G. (2010) ***Journal of Biomolecular Screening***, 15(6):653-662

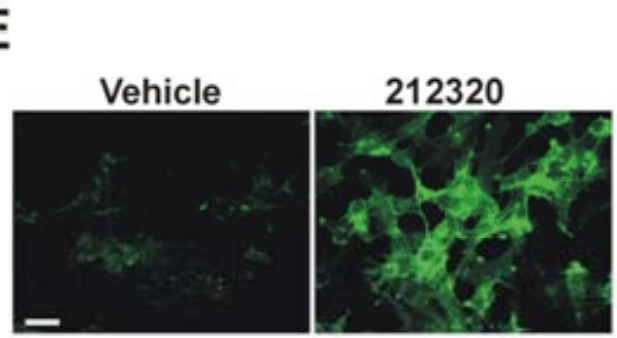
Class	Structural Analogues	Primary astrocytes	Primary neurons and astrocytes mixed cultures	Mice
1	33	dramatic, not dose-dependent, induction at 3.3 μM and higher doses	observed neuron degeneration	mice paralyzed and died
2	5	strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
3	4	low induction	low induction	
4	4			
5	2	strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
6	2			
7	2			
8	1	low induction	low induction	
9	1	Strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
10-16	1			

Primary astrocytes

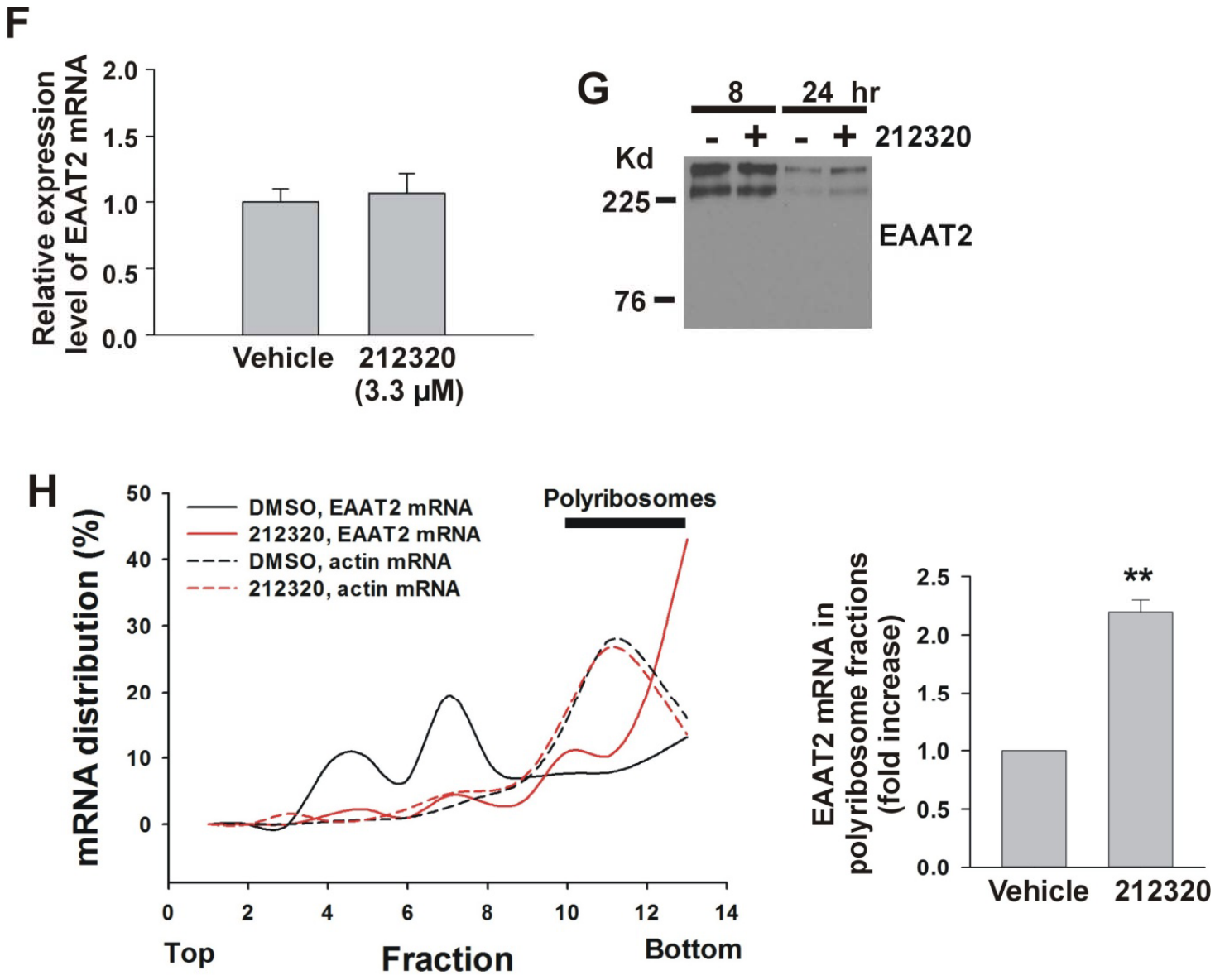




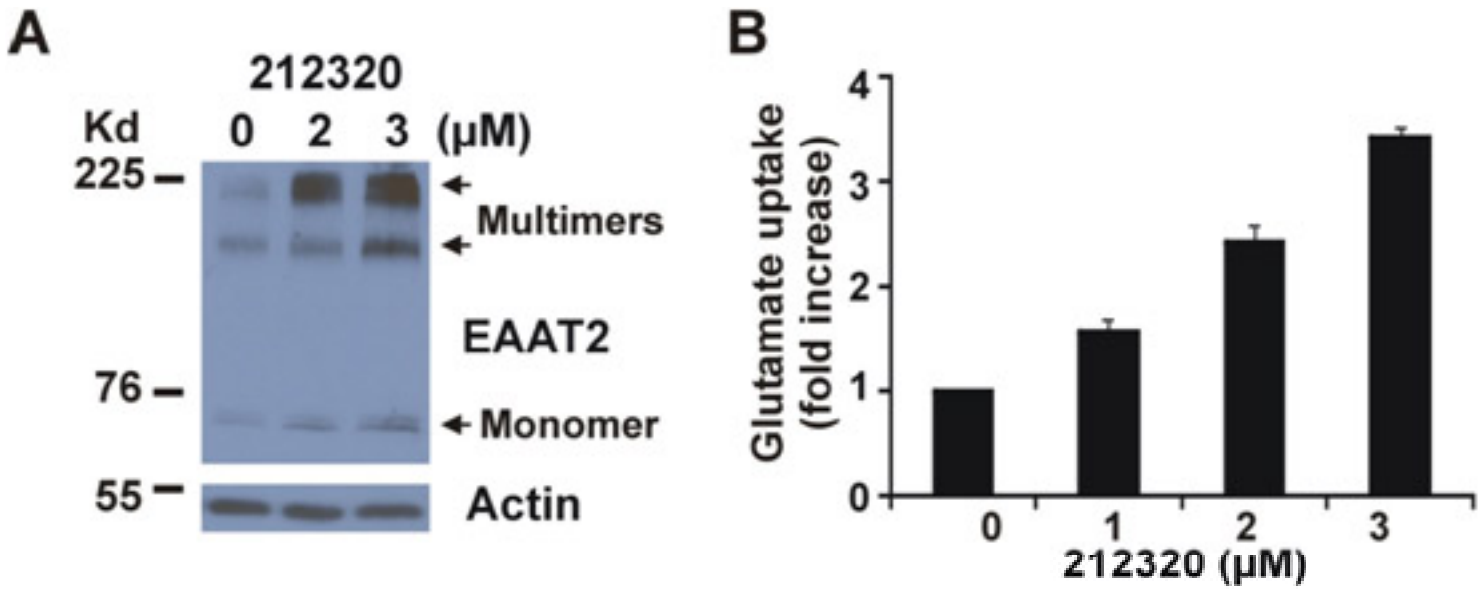




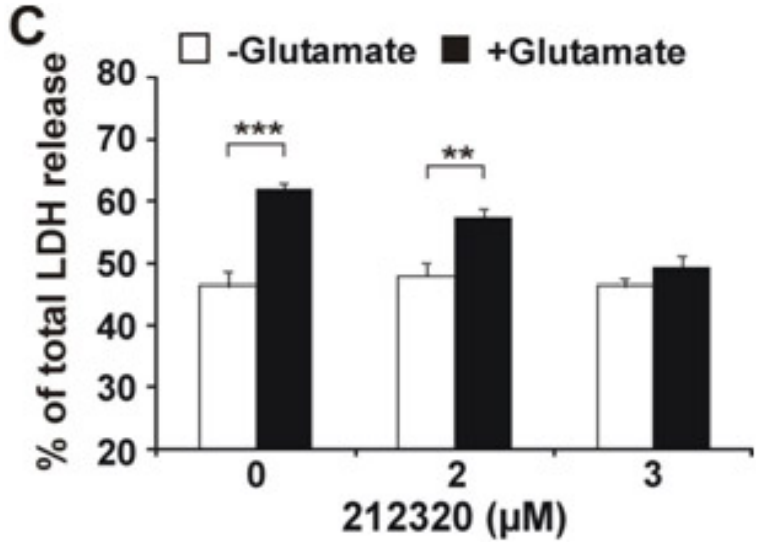
Primary astrocytes

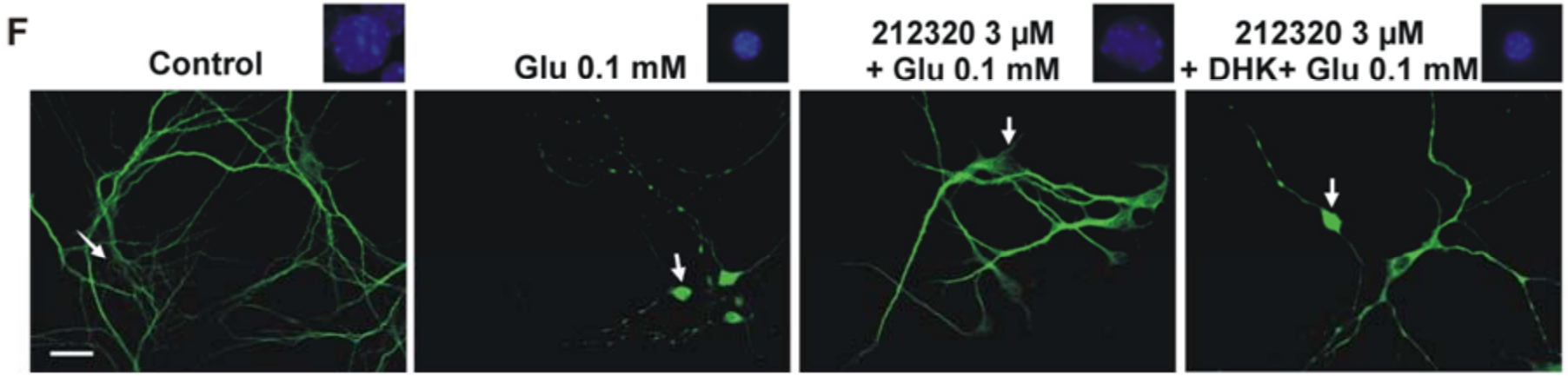


Primary neuron and astrocyte mixed cultures

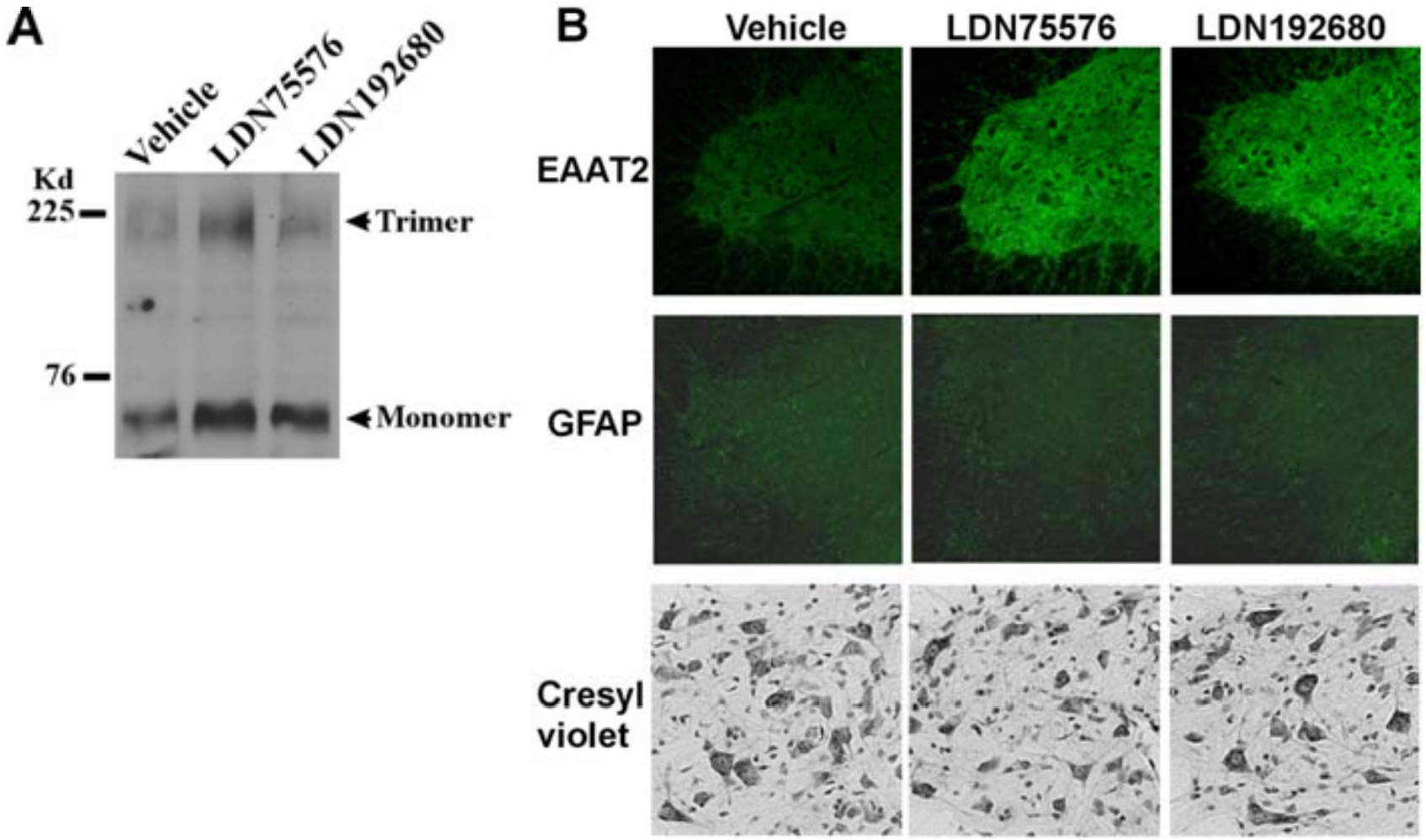


Protect neurons from glutamate-mediated excitotoxic injury/death

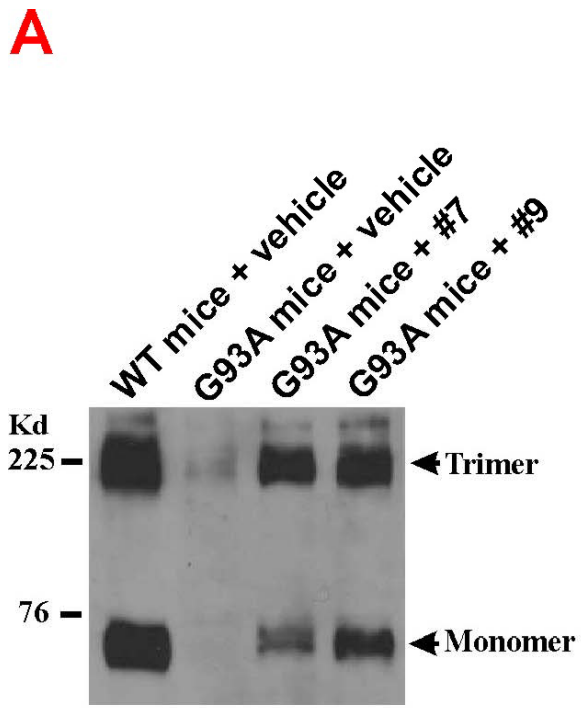


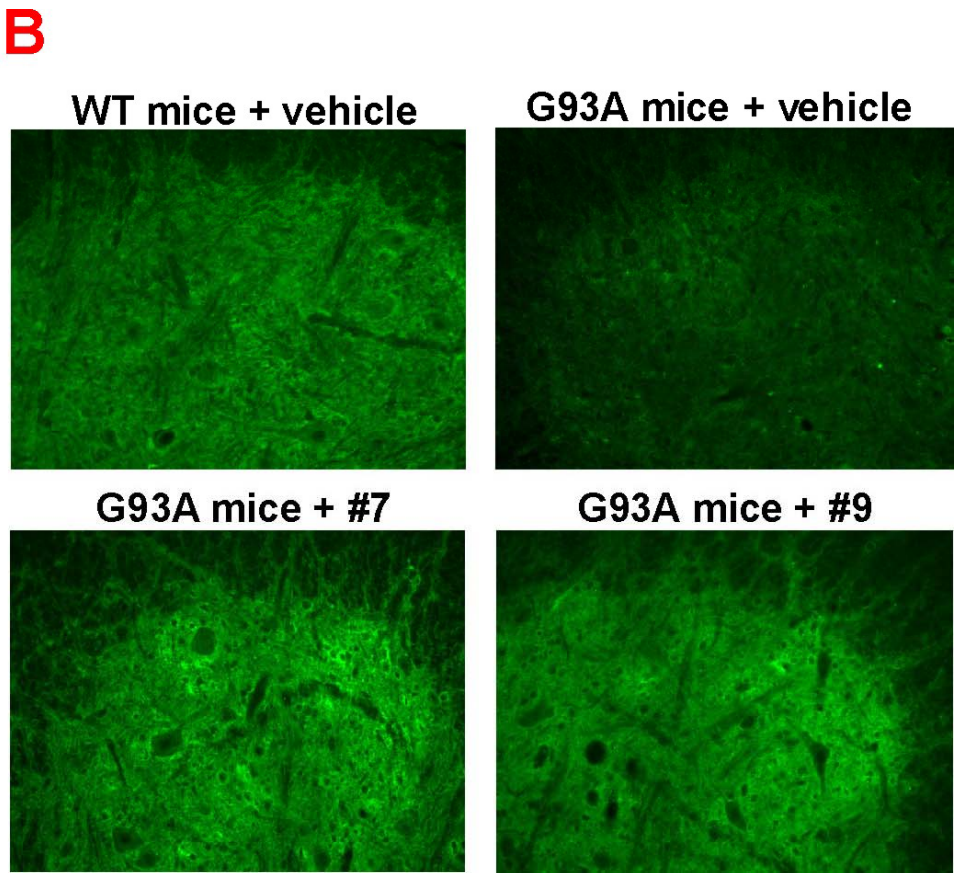


Evaluate compounds in wild-type mice by intrathecal injection

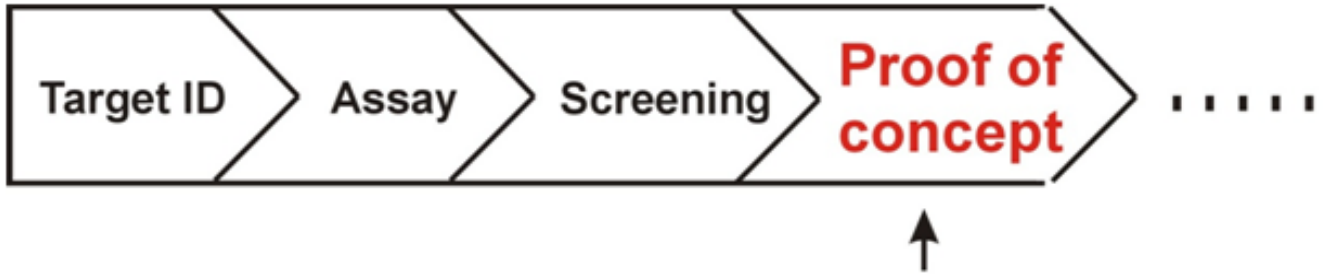


Evaluate compounds in SOD1(G93A) mice by intrathecal injection





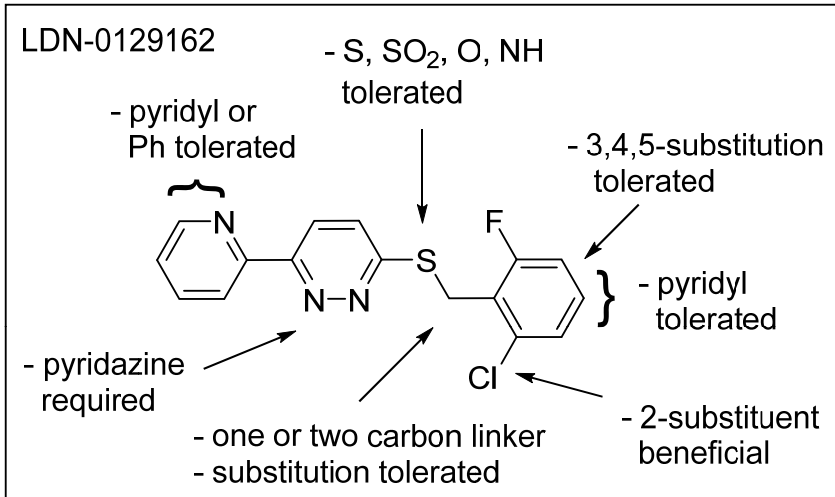
Class	Structural Analogues	Primary astrocytes	Primary neurons and astrocytes mixed cultures	Mice
1	33	dramatic, not dose-dependent, induction at 3.3 μM and higher doses	observed neuron degeneration	mice paralyzed and died
2	5	strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
3	4	low induction	low induction	
4	4			
5	2	strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
6	2			
7	2			
8	1	low induction	low induction	
9	1	Strong dose-dependent induction	strong dose-dependent induction; no toxicity was observed	strong induction; mice were normal and healthy
10-16	1			

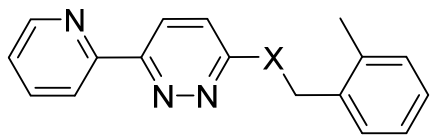


Performed
preliminary lead optimization,
***in vivo* pharmacokinetics,**
***in vivo* pharmacodynamics,**
***in vivo* toxicity,**
selectivity,
***in vitro* profiling**
efficacy studies
mechanism

Wexner Medical Center

Structure-activity relationship (SAR) study





LDN-	X	EAAT2*	cLogP	Sol. (μM)	μsome+
0129162	-	2.0	5.1	<1.5	1.5
0212320	S	3.5	4.1	12	2.0
0213826	SO_2	2.9	3.3	46	0.9
0212408	O	2.3	4.3	62	0.7
0213776	NH	2.4	3.9	26	NT
0213791	CONH	1.4	3.5	NT	NT

*fold increase, 24 h @10 μM, +% remaining after 30 min (mouse)
TBD = to be determined at LDDN, NT = not tested

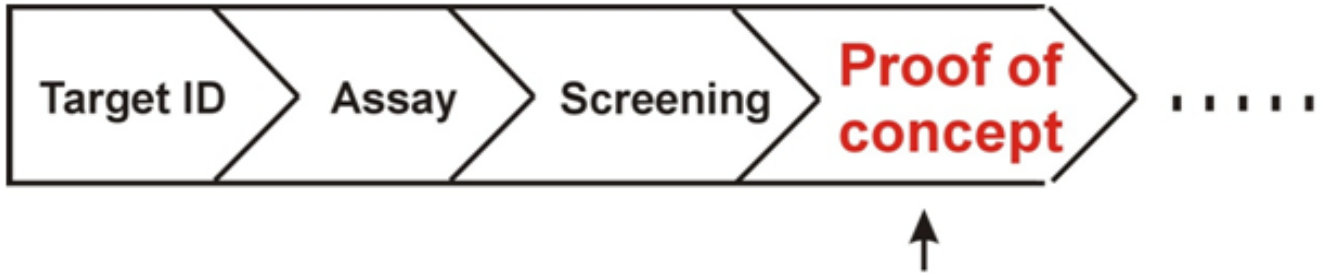
Blood brain barrier penetration (MW, cLogP, PSA)
Solubility
Stability
Potency
Toxicity

Patent

Pyridazine derivatives as glutamate transporter EAAT2 translational activators

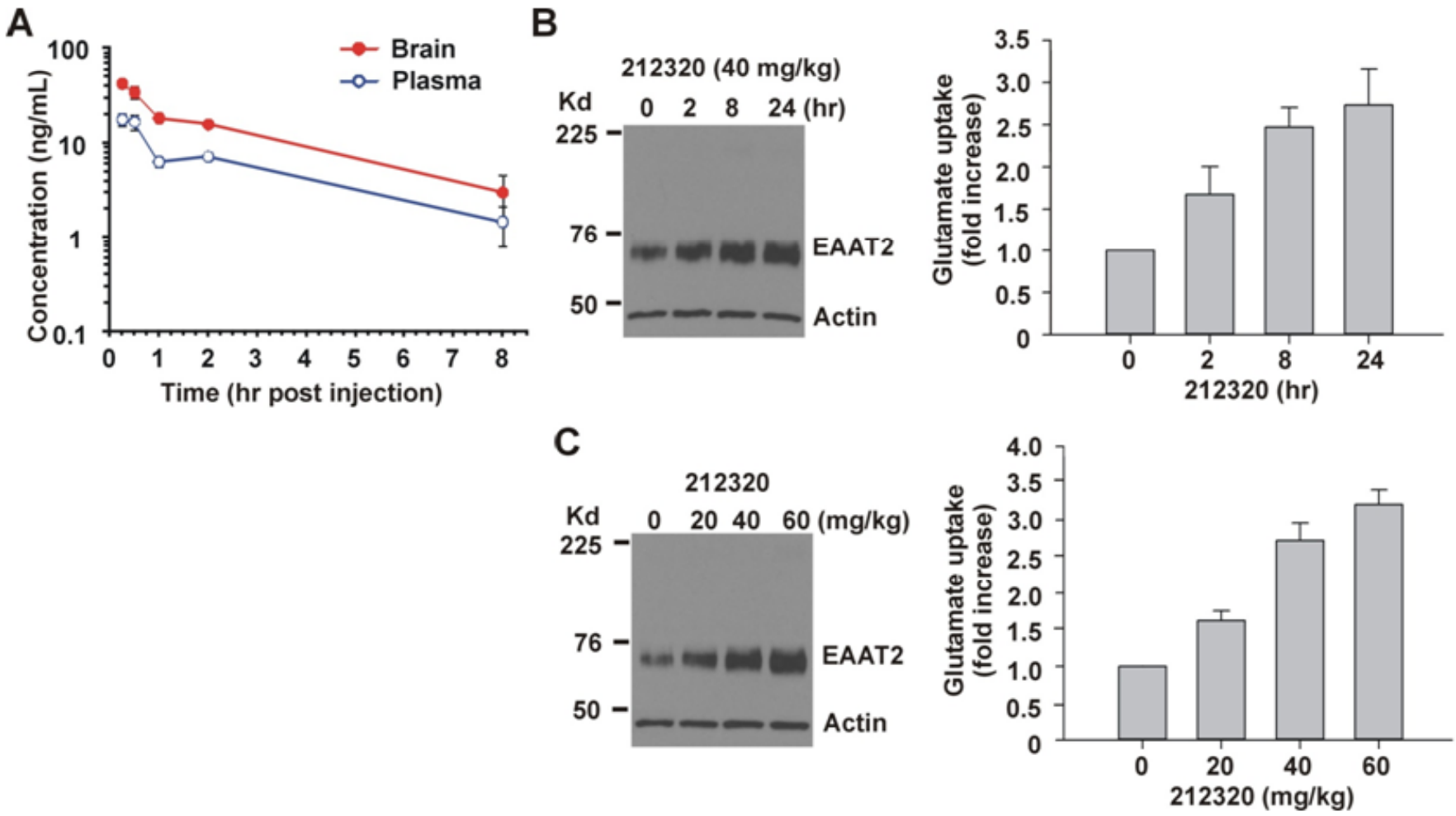
ischemic stroke, epilepsy, trauma, Parkinson's disease, Alzheimer's disease, multiple sclerosis, mesial temporal sclerosis, Huntington's disease, AIDS dementia complex, amyotrophic lateral sclerosis (ALS), migraine, temporomandibular disorders, neuropathic pain, visceral pain, complex regional pain syndrome, alcohol addiction, cocaine addiction, glioblastoma, depression

Xing X., Chang L., Kong Q., Colton C., Lai L., Glicksman M., Lin C.-L. G., Cuny G. (2011) ***Bioorganic & Medicinal Chemistry Letters***, 21(19):5774-7.



Performed
preliminary lead optimization,
***in vivo* pharmacokinetics,**
***in vivo* pharmacodynamics,**
***in vivo* toxicity,**
selectivity,
***in vitro* profiling**
efficacy studies
mechanism

In vivo pharmacokinetics & pharmacodynamics

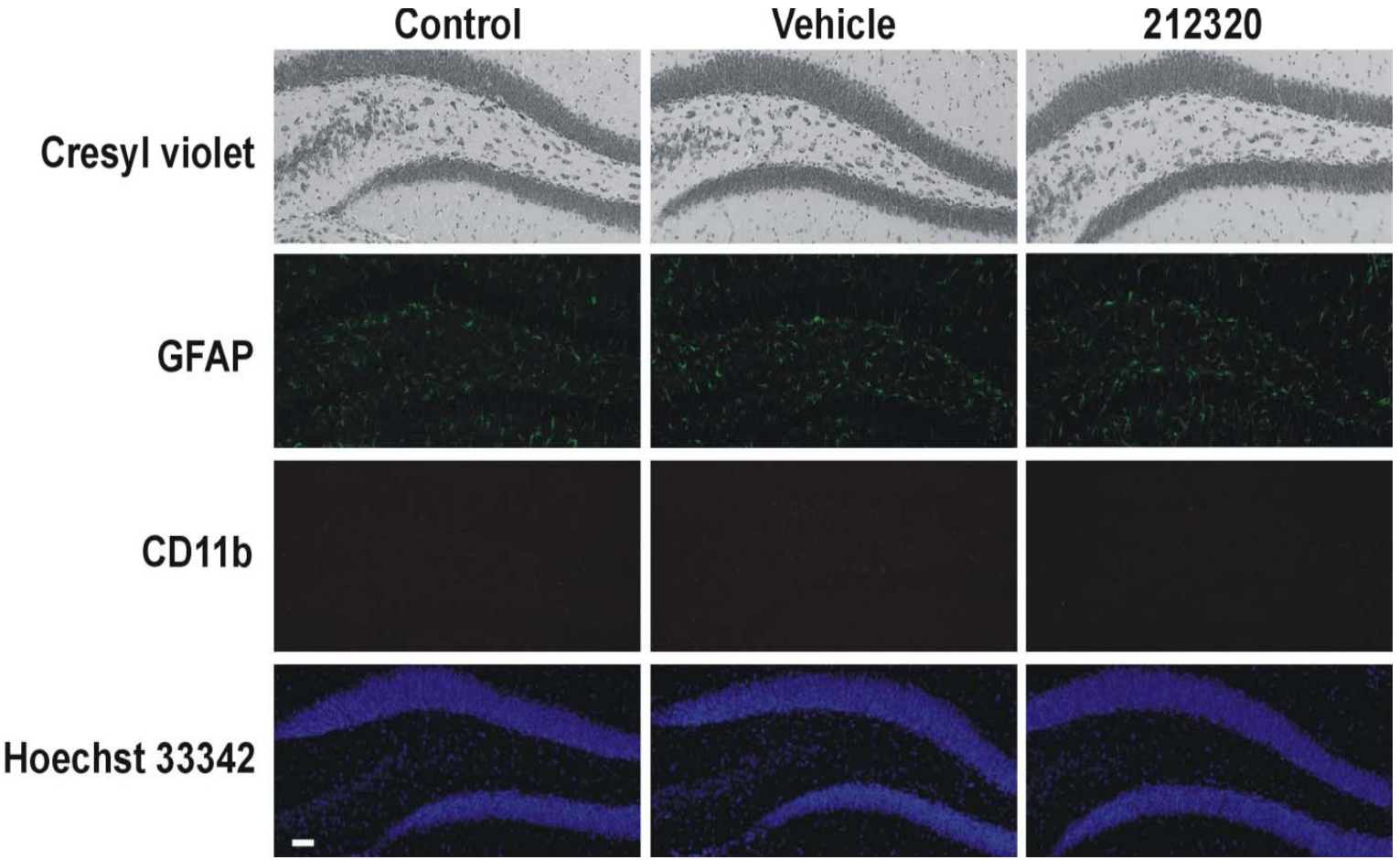


In vivo toxicity

- **5-day repeat dose tolerability study at 10, 25, 50, 100 mg/kg i.p. with no adverse effects**
  - No adverse clinical observations
  - No weight differences between vehicle and treated animals
  - No abnormal hematology, serum chemistry, coagulation
  - Organs examined, weighed, no abnormal gross pathology

In vivo toxicity

- daily treatment for 60 days at 40 mg/kg by ip: no adverse effects

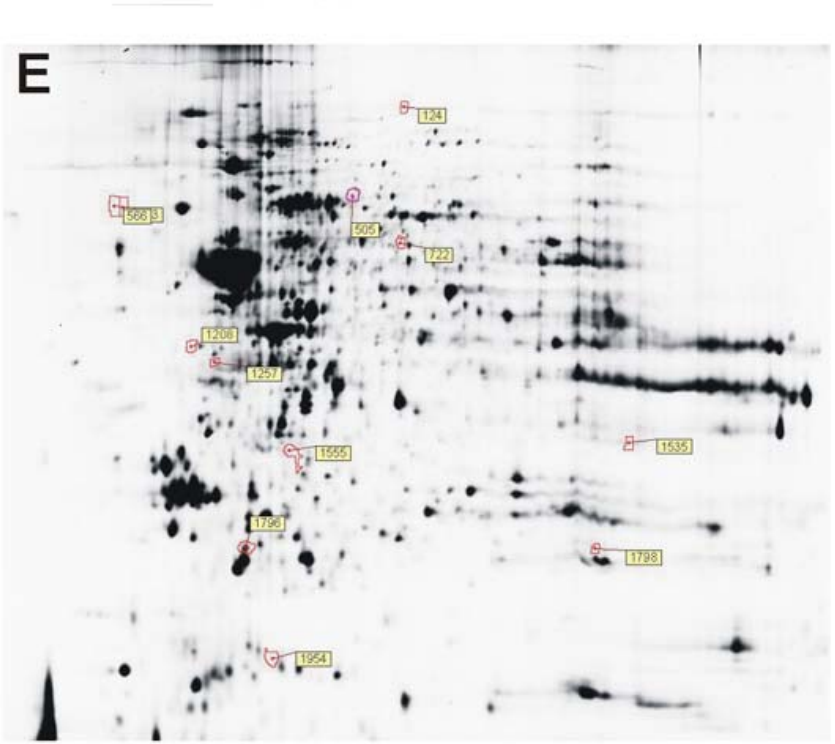


Selectivity studies

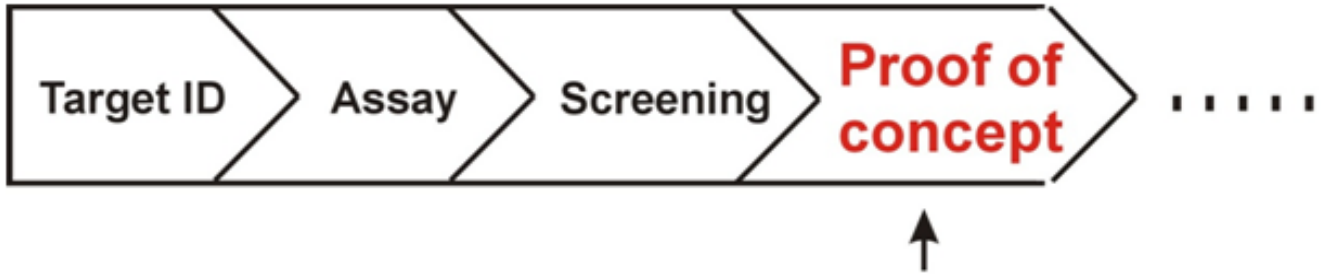
- ***In vitro* side effect profiling study**: LDN/OSU-0212320 had 69% inhibition on the cytochrome p450 CYP1A2 and 43% inhibition on calcium channel L-type at 10 µM. All other targets demonstrated <30% inhibition at 10 µM
- **Protein kinase profiling study:** EphA4 and EphA5 were inhibited by 44% at 10 µM. All other kinases tested showed insignificant activity

Specificity

- **Proteomic analysis:** LDN/OSU-0212320 did not induce global protein synthesis

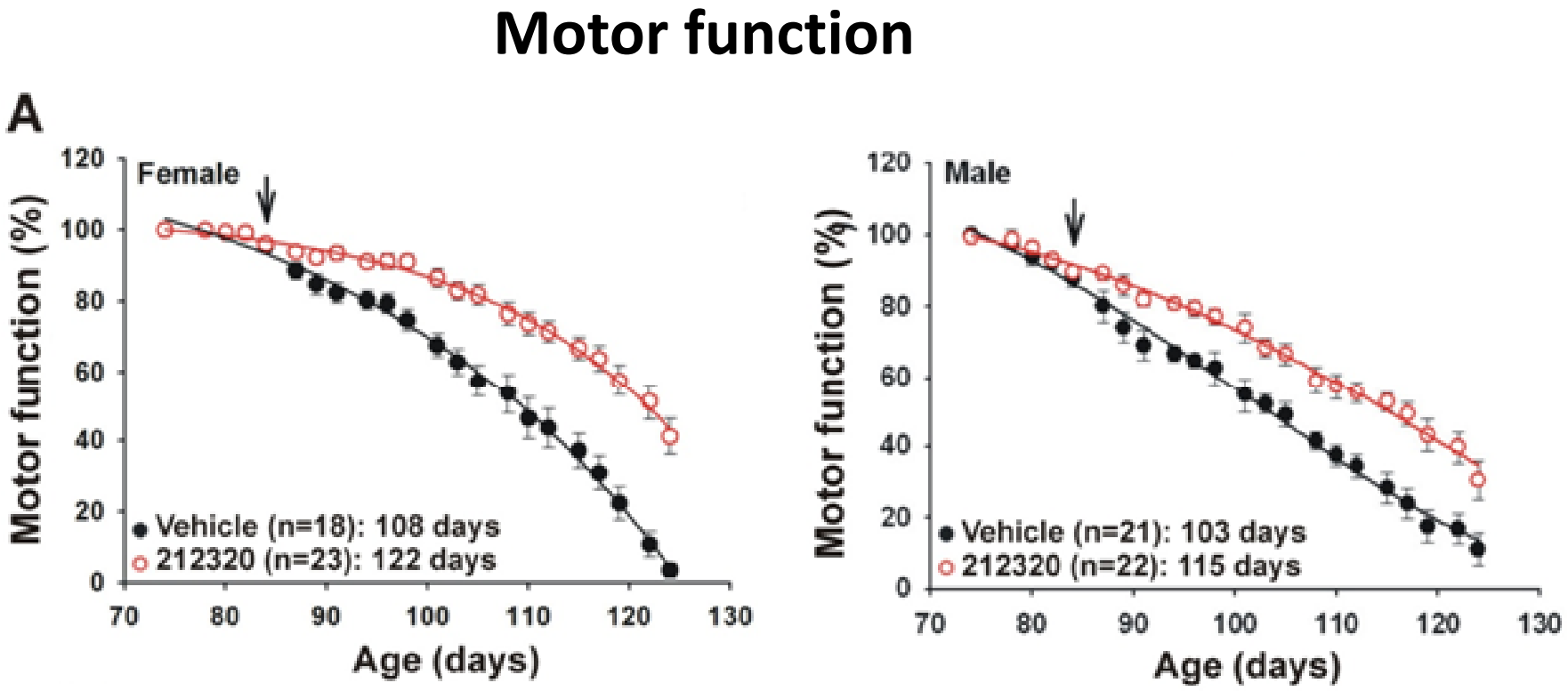


No.	T-test	Av. Ratio
505	0.016	1.3
511	0.0041	1.33
566	0.0064	1.39
722	0.0065	1.24
1208	0.01	1.2
1257	0.043	-1.21
1535	0.021	-1.26
1555	0.016	-1.18
1796	0.032	-1.18
1798	0.031	-1.22
1954	0.035	-1.18



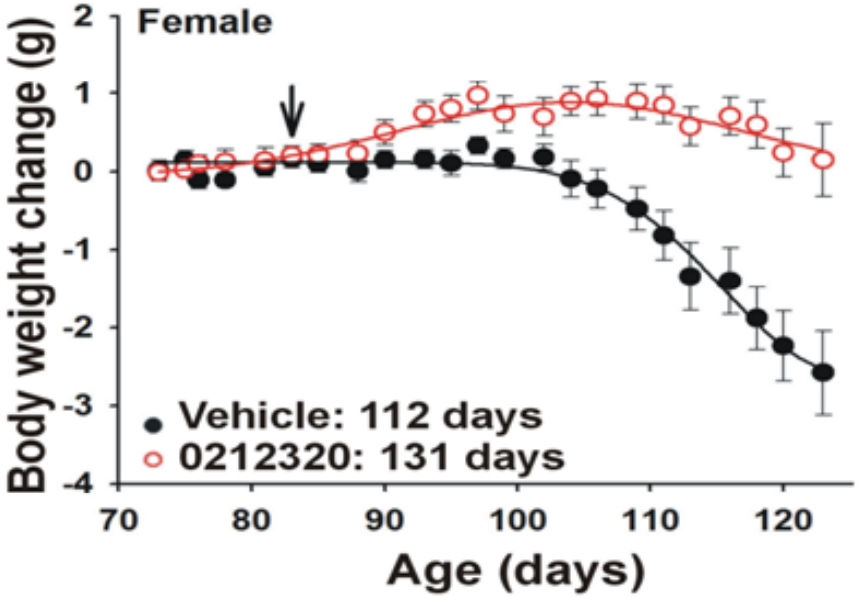
Performed
preliminary lead optimization,
***in vivo* pharmacokinetics,**
***in vivo* pharmacodynamics,**
***in vivo* toxicity,**
selectivity,
***in vitro* profiling**
efficacy studies
mechanism

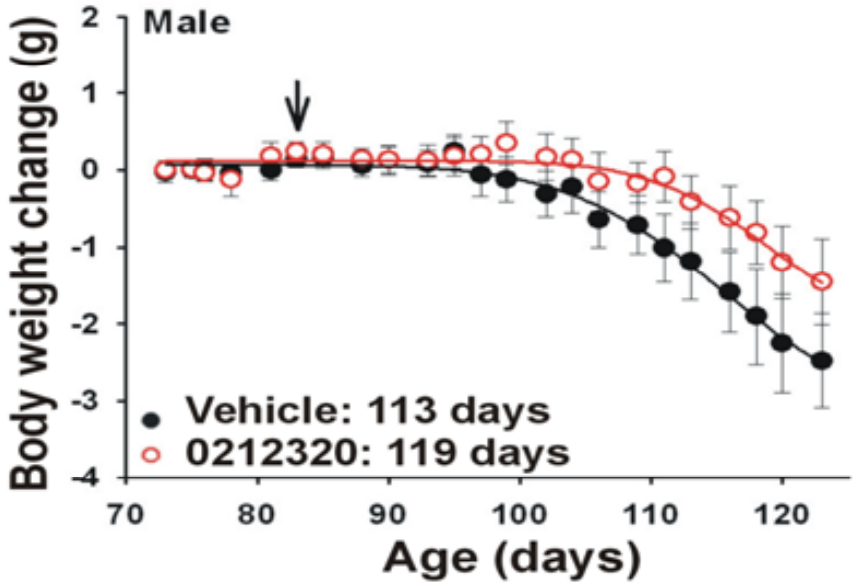
LDN/OSU-0212320 slows disease progression of SOD1(G93A) mice



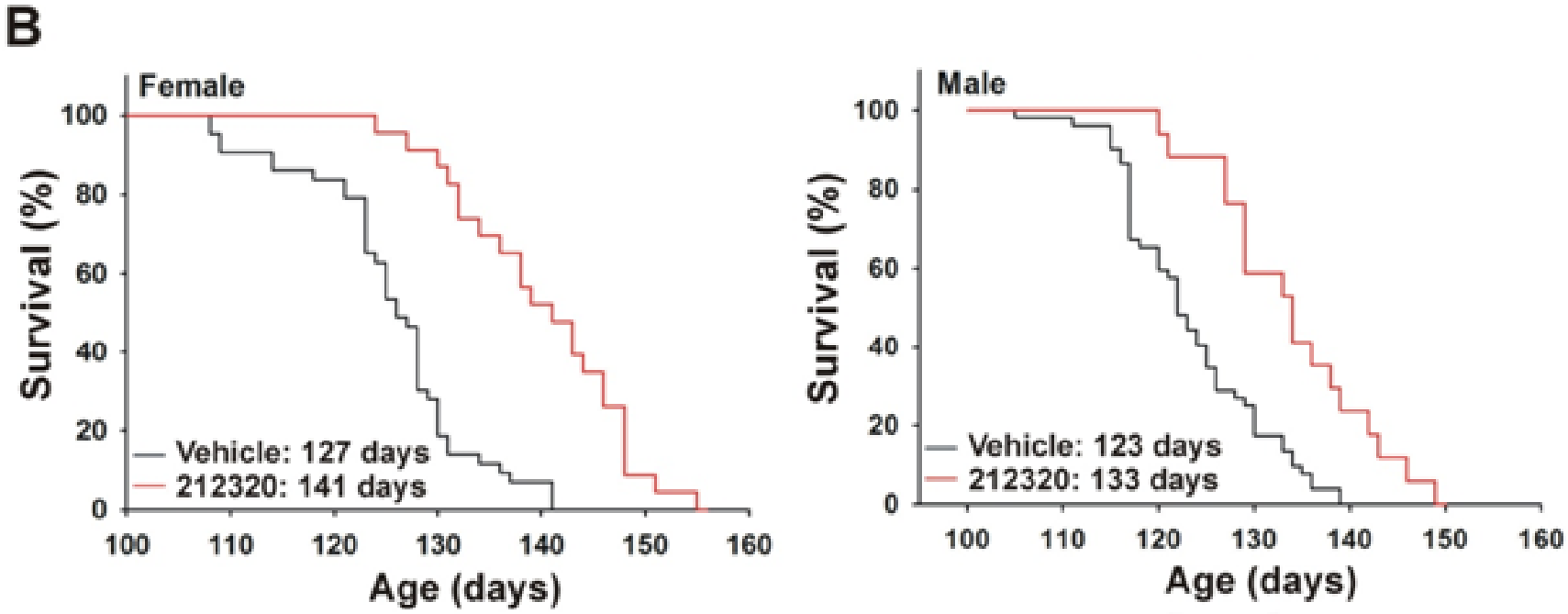
LDN/OSU-0212320 slows disease progression of SOD1(G93A) mice

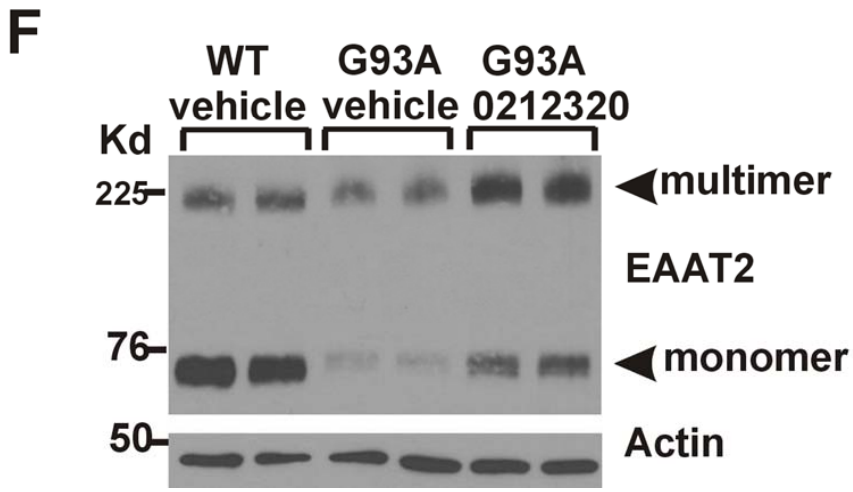
Body weight

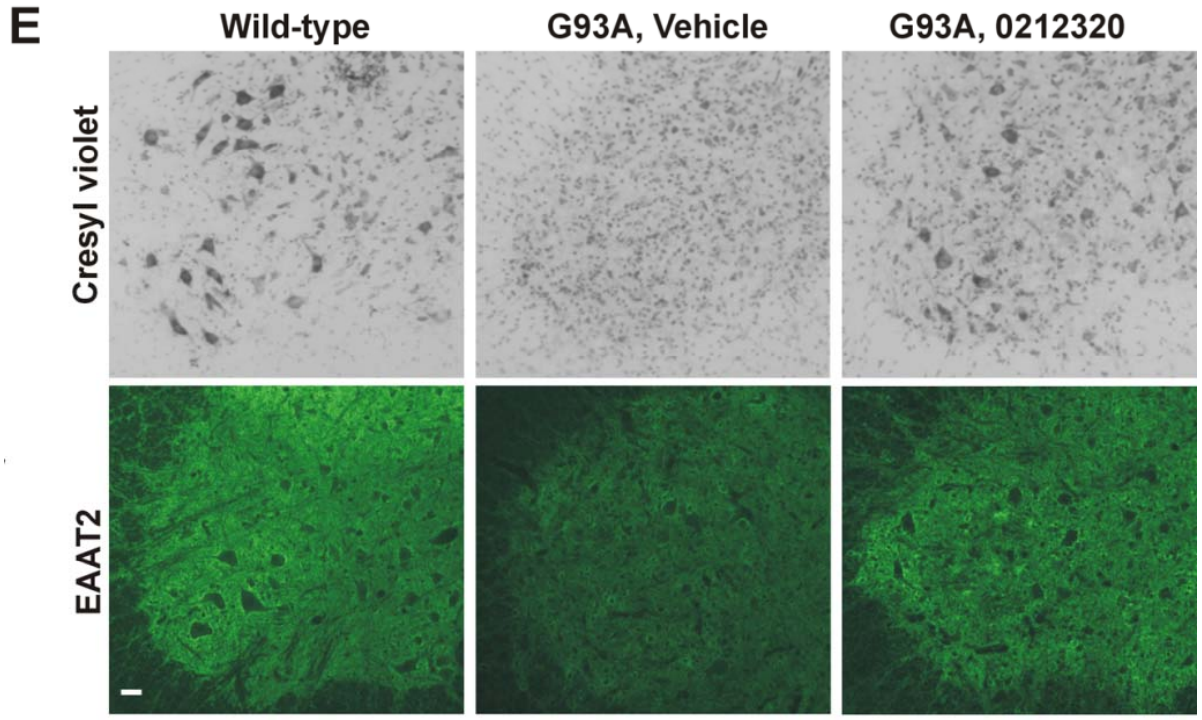


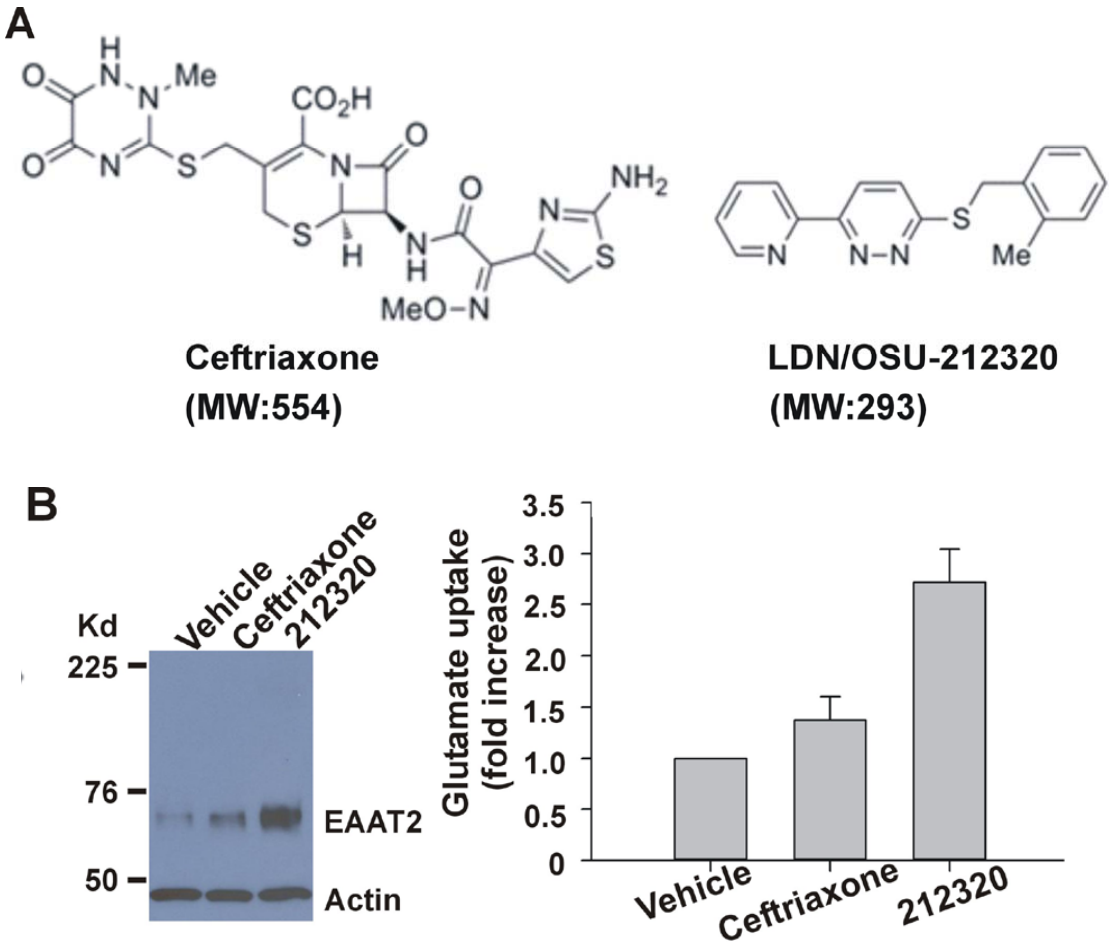


LDN/OSU-0212320 extends the life span of SOD1(G93A) mice



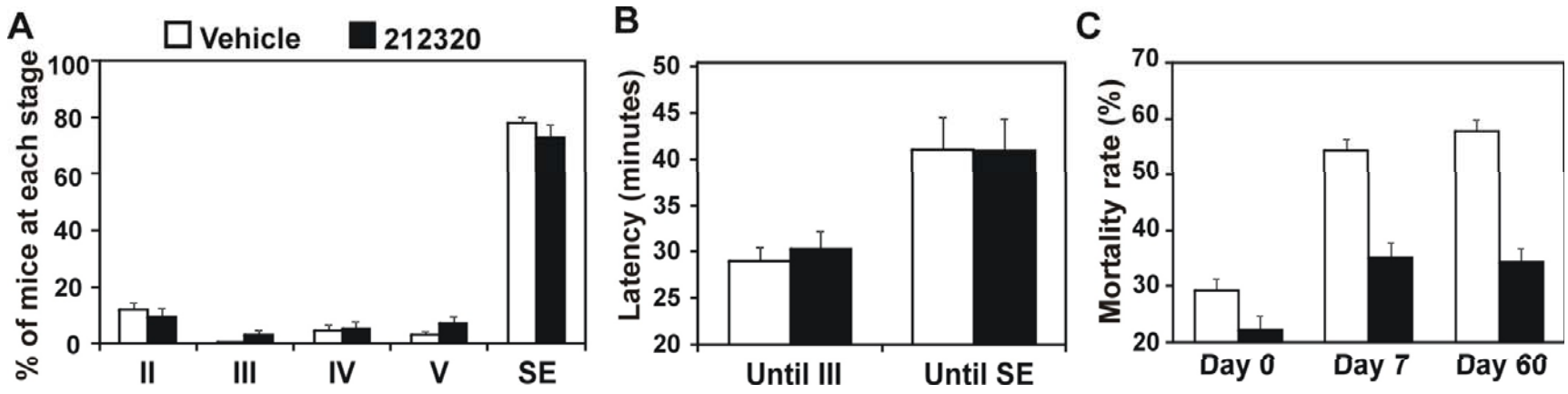

F
WT vehicle
G93A vehicle
G93A 0212320
Kd
225
76
50
multimer
EAAT2
monomer
Actin


E
Wild-type
G93A, Vehicle
G93A, 0212320
Cresyl violet
EAAT2

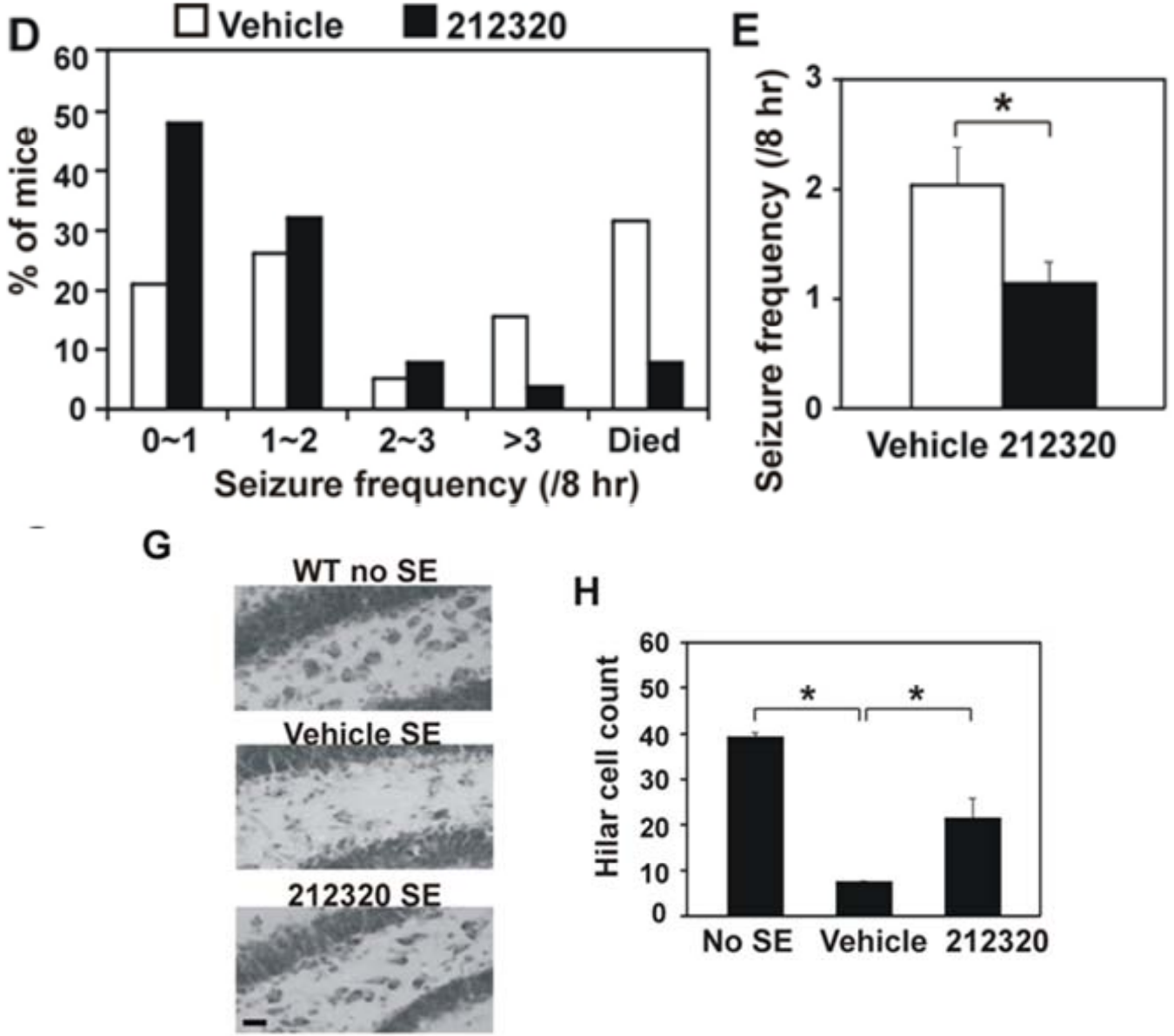

A
Ceftriaxone
(MW:554)
LDN/OSU-212320
(MW:293)
B
Kd
Vehicle
Ceftriaxone
212320
225
76
50
EAAT2
Actin
Glutamate uptake
(fold increase)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Vehicle
Ceftriaxone
212320
C
Wild-type
G93A, vehicle
G93A, ceftri
G93A, 212320

Pilocarpine-induced status epilepticus mouse model

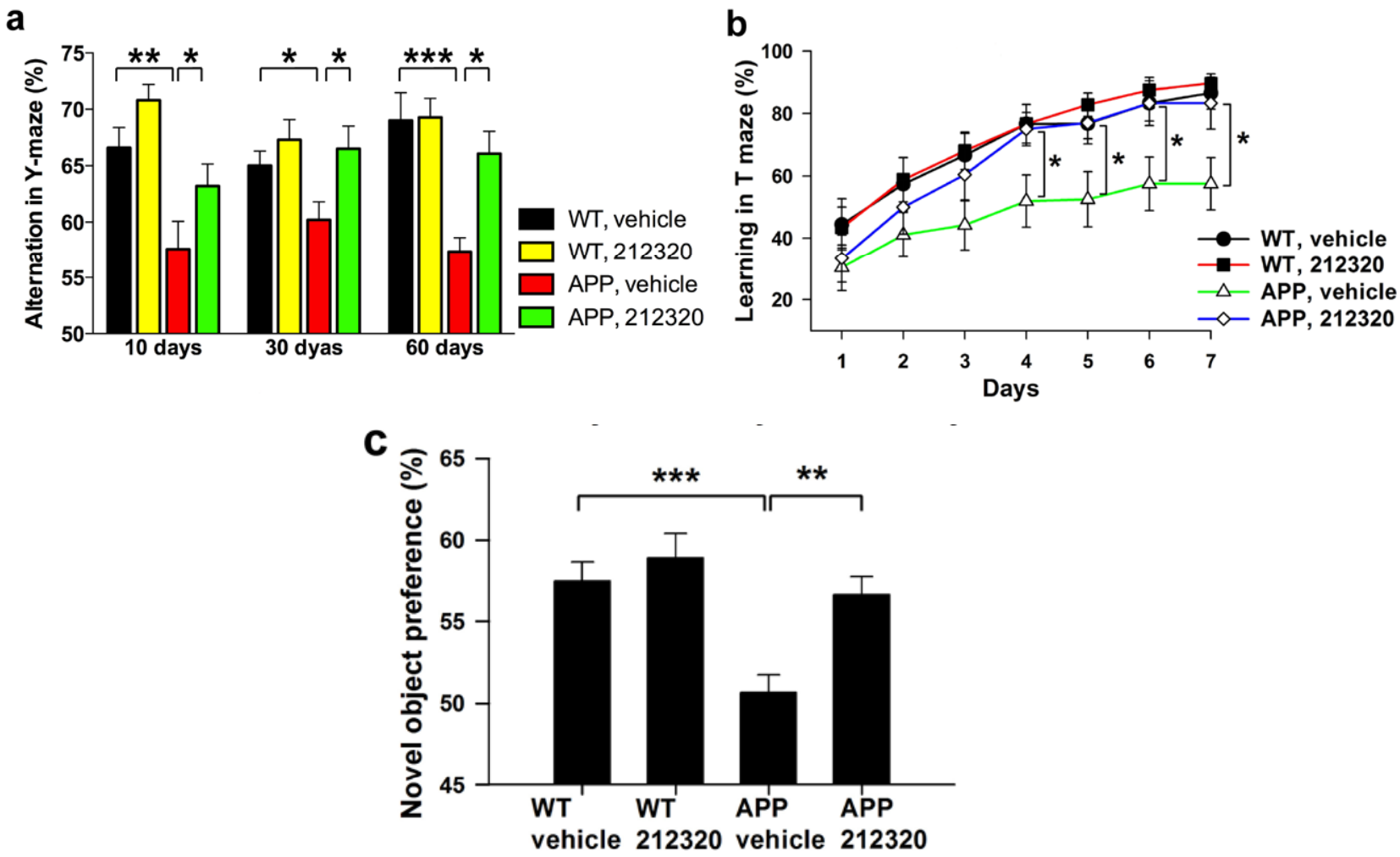
LDN/OSU-0212320 has no significant effects on early acute seizure activity but significantly reduces mortality rate

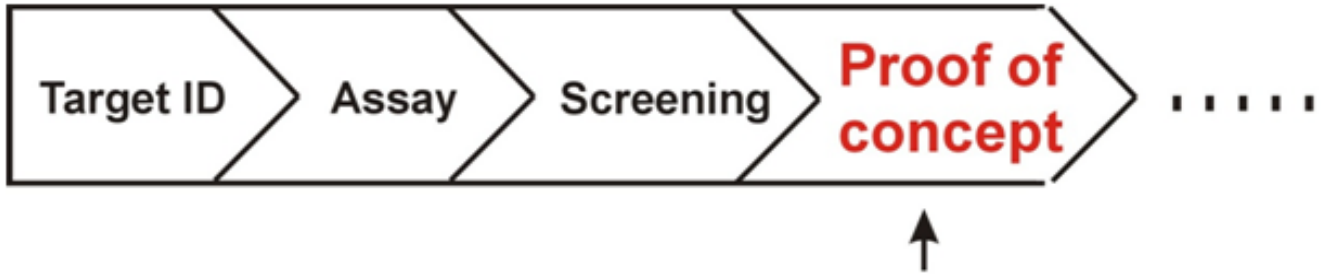


LDN/OSU-0212320 significantly reduces spontaneous recurrent seizures and neuronal death in an epilepsy model



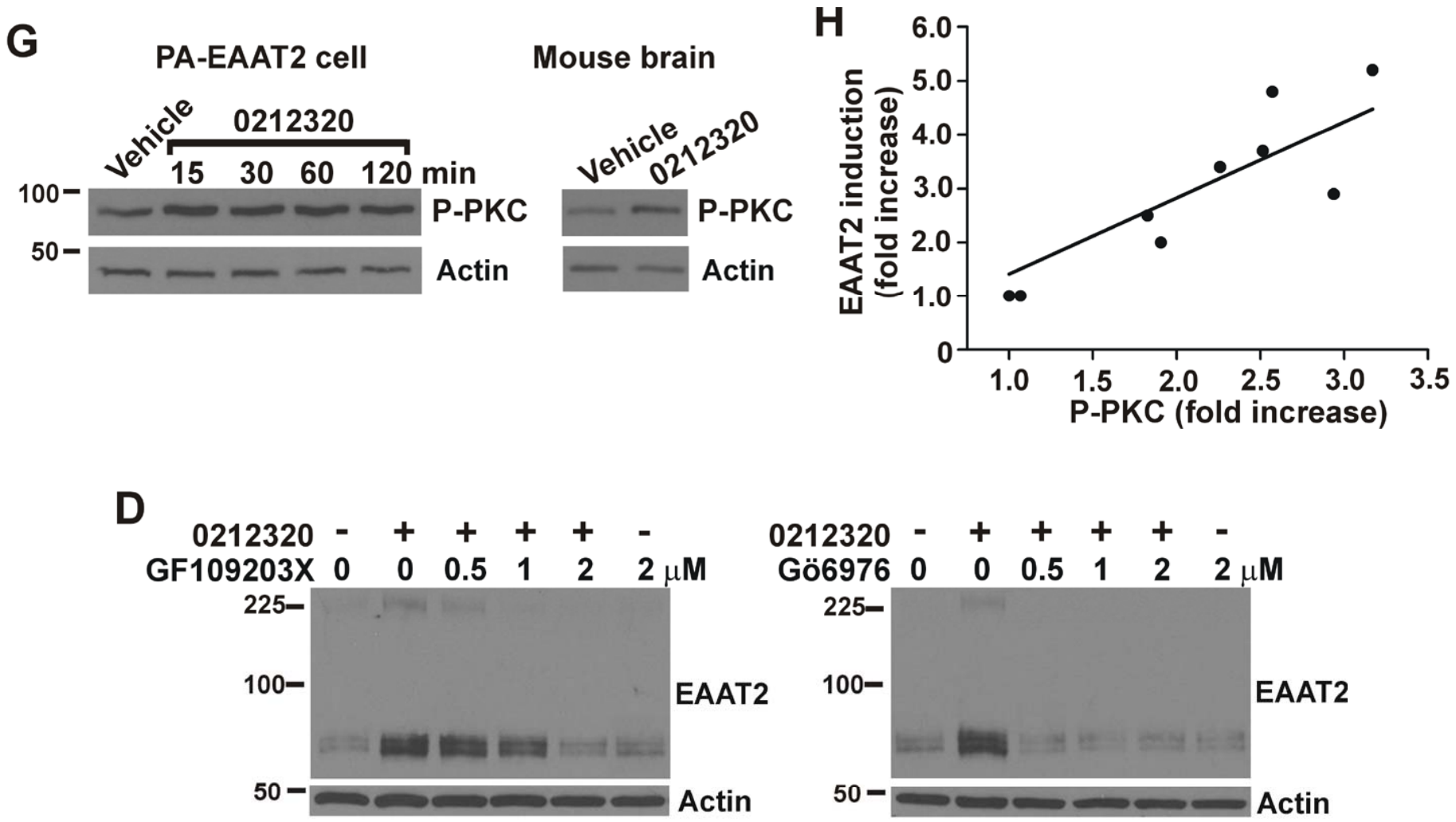
LDN/OSU-0212320 improves cognitive functions in a mouse model of Alzheimer's disease



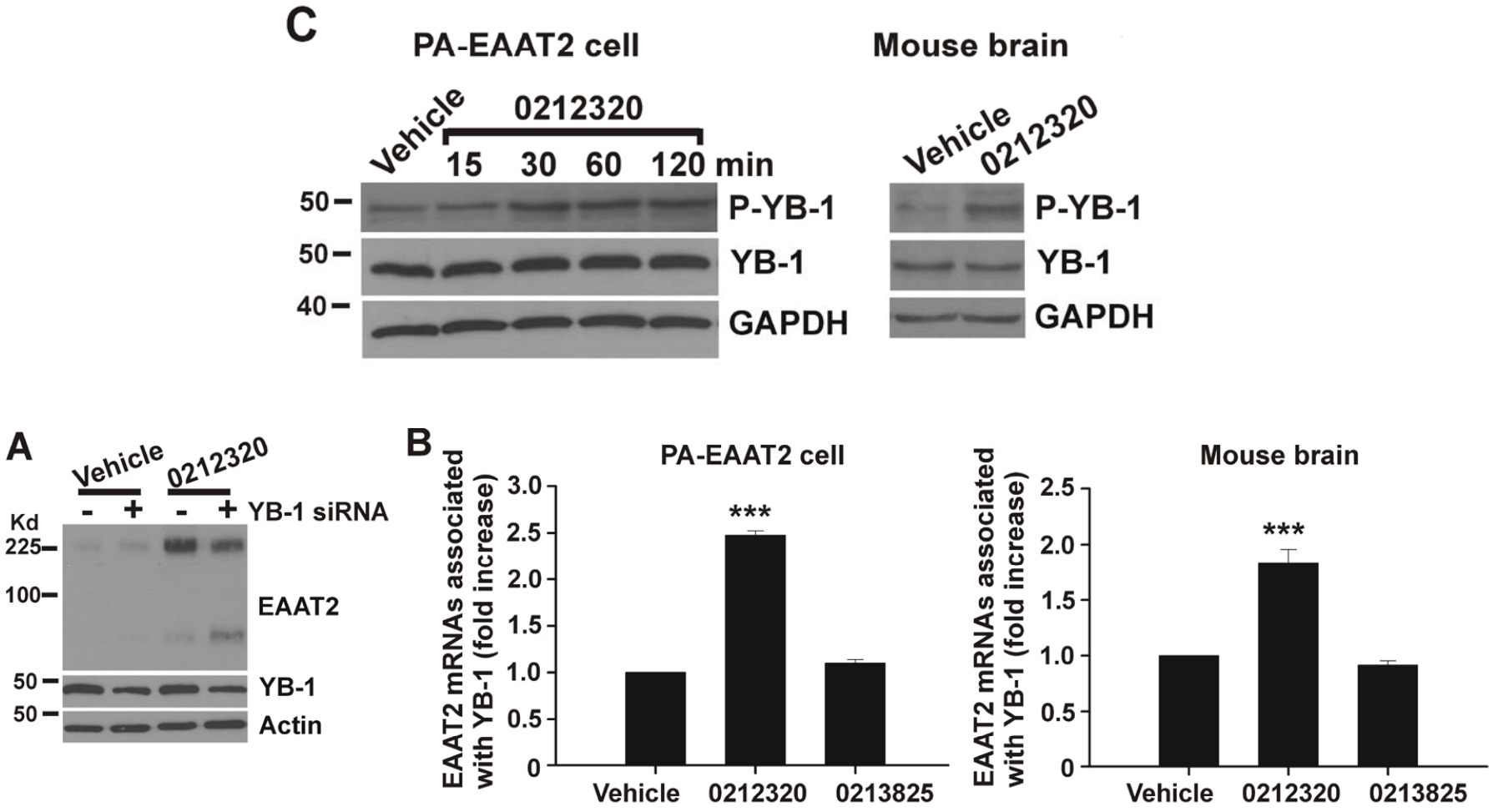


Performed
preliminary lead optimization,
***in vivo* pharmacokinetics,**
***in vivo* pharmacodynamics,**
***in vivo* toxicity,**
selectivity,
***in vitro* profiling**
efficacy studies
mechanism

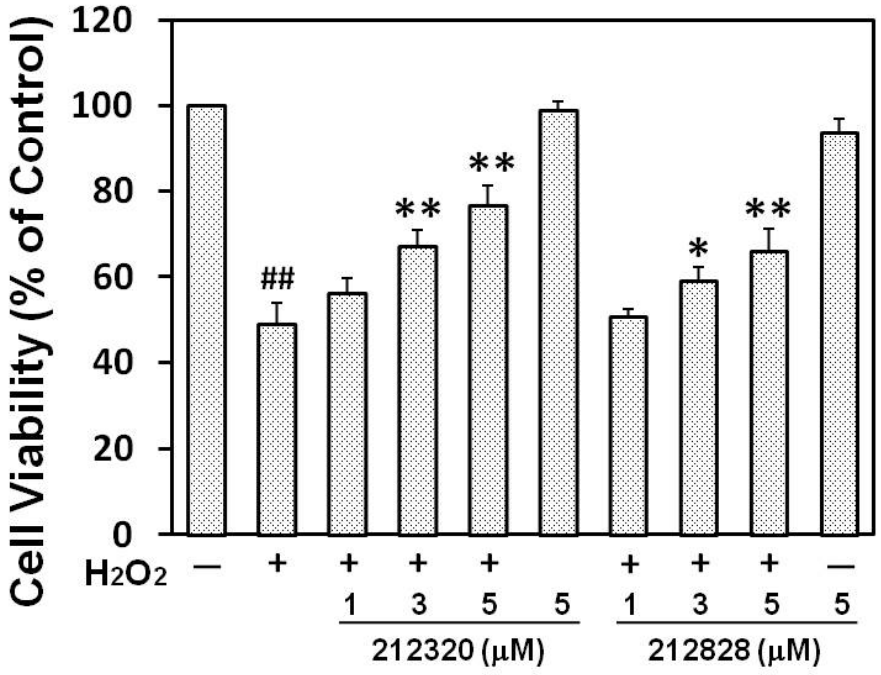
LDN/OSU-0212320 treatment results in activation of PKC

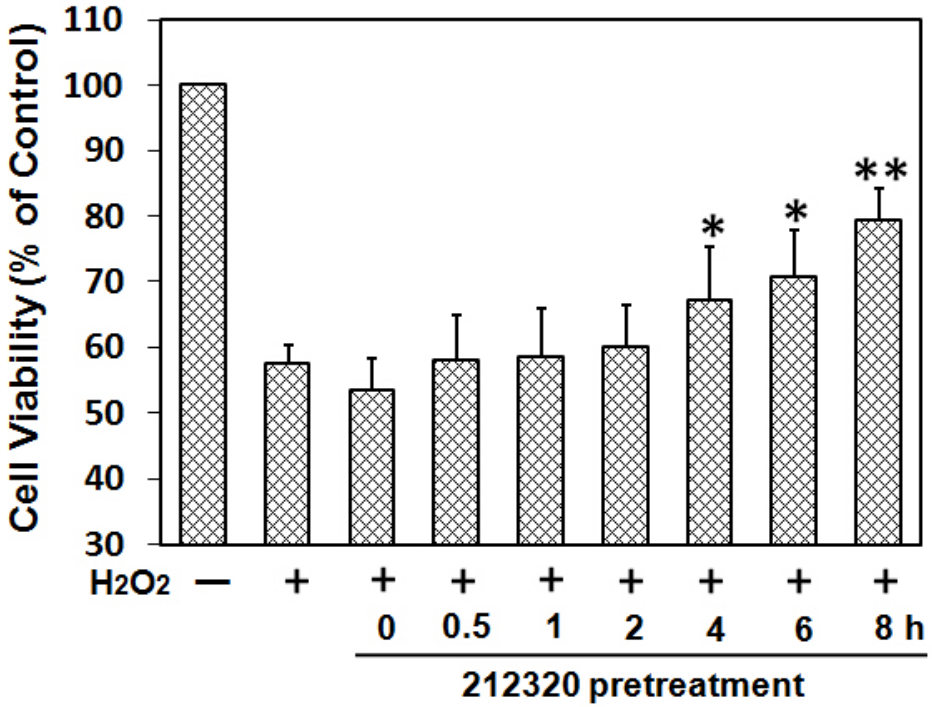


LDN/OSU-0212320 treatment results in activation of PKC, which subsequently activates YB-1

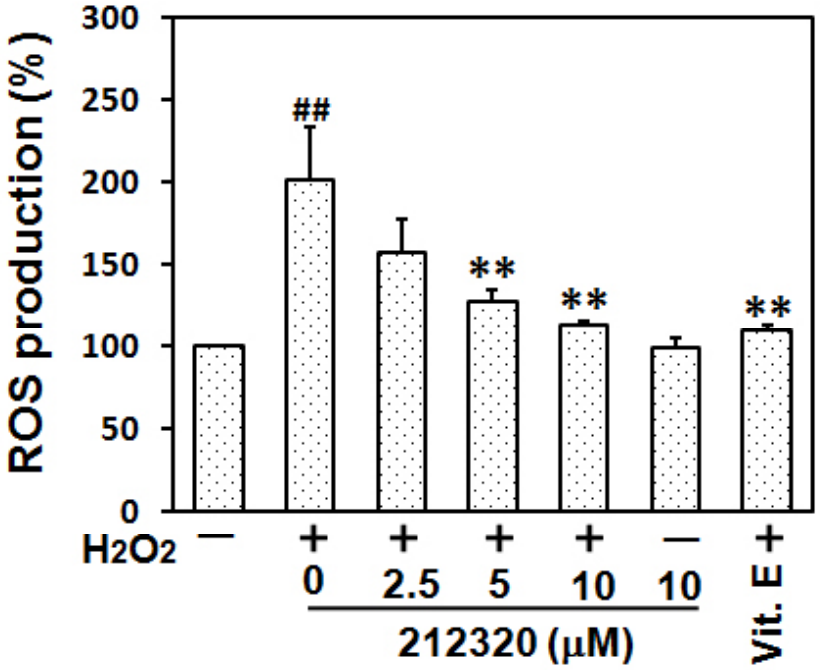


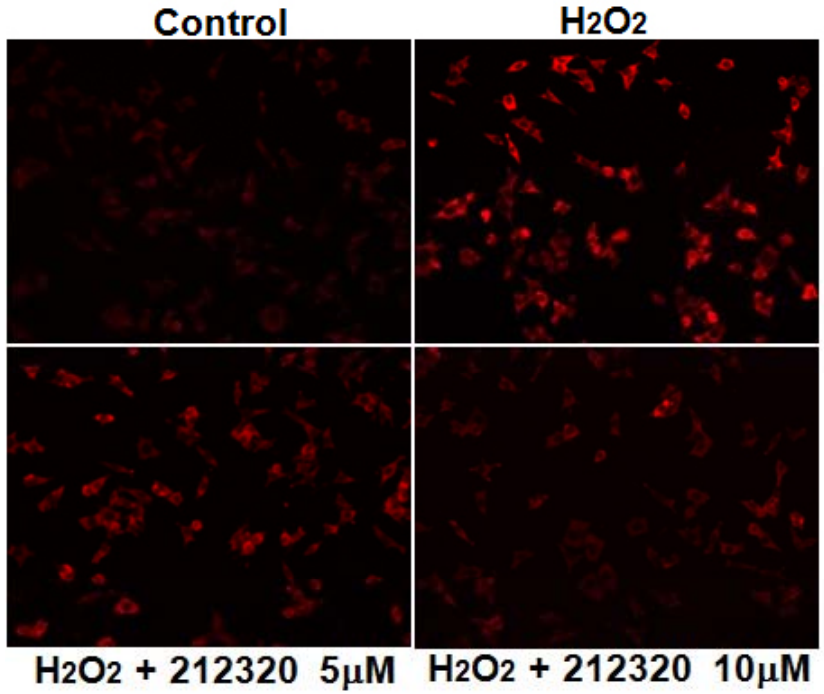
Our compounds have anti-oxidative stress function



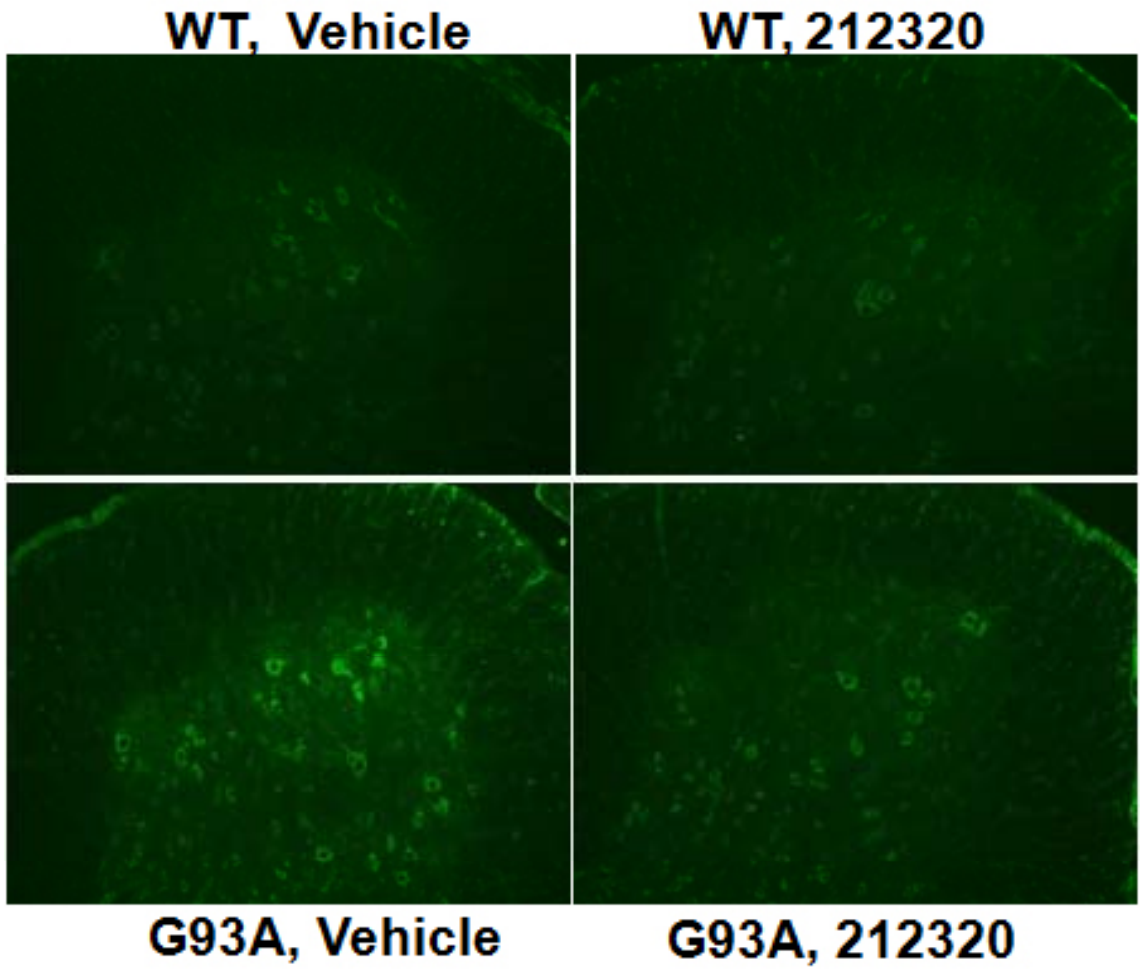


LDN/OSU-0212320 reduces H_2O_2-induced ROS and oxidative damage

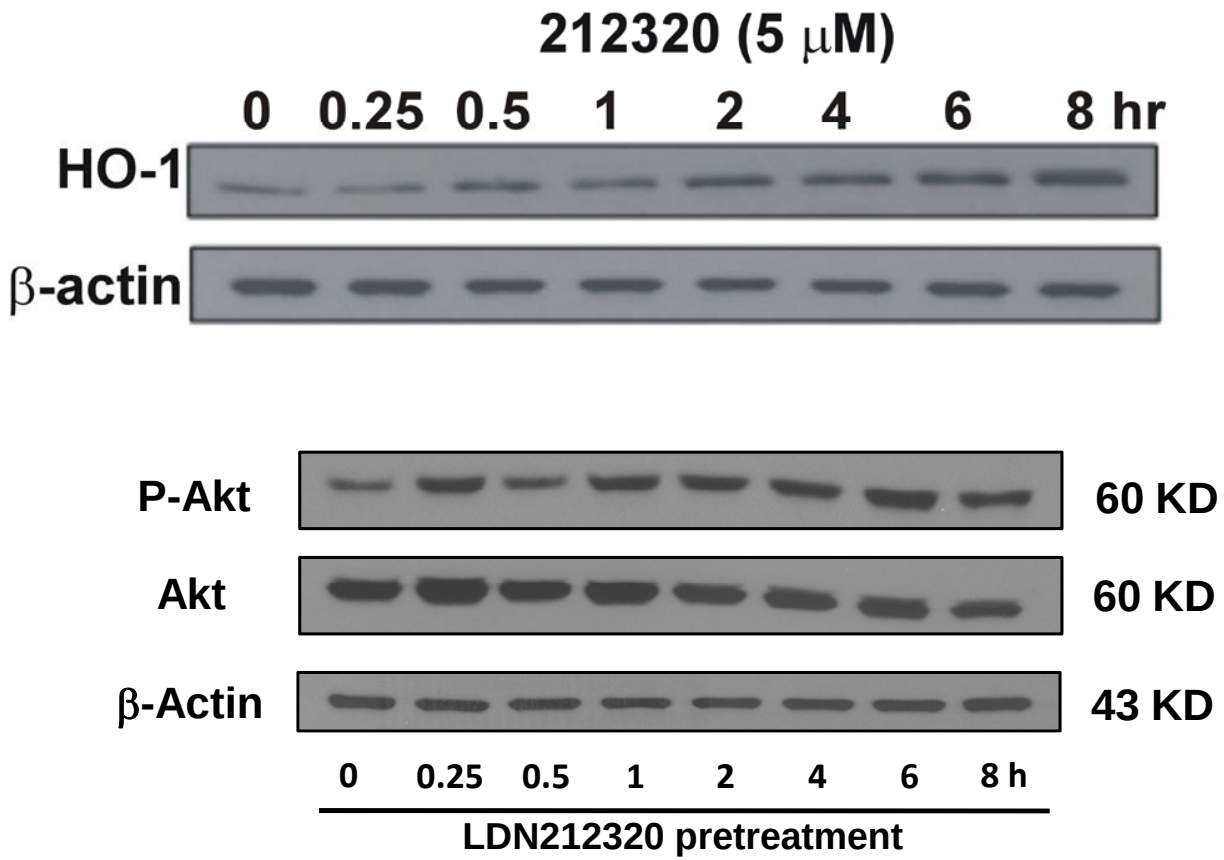


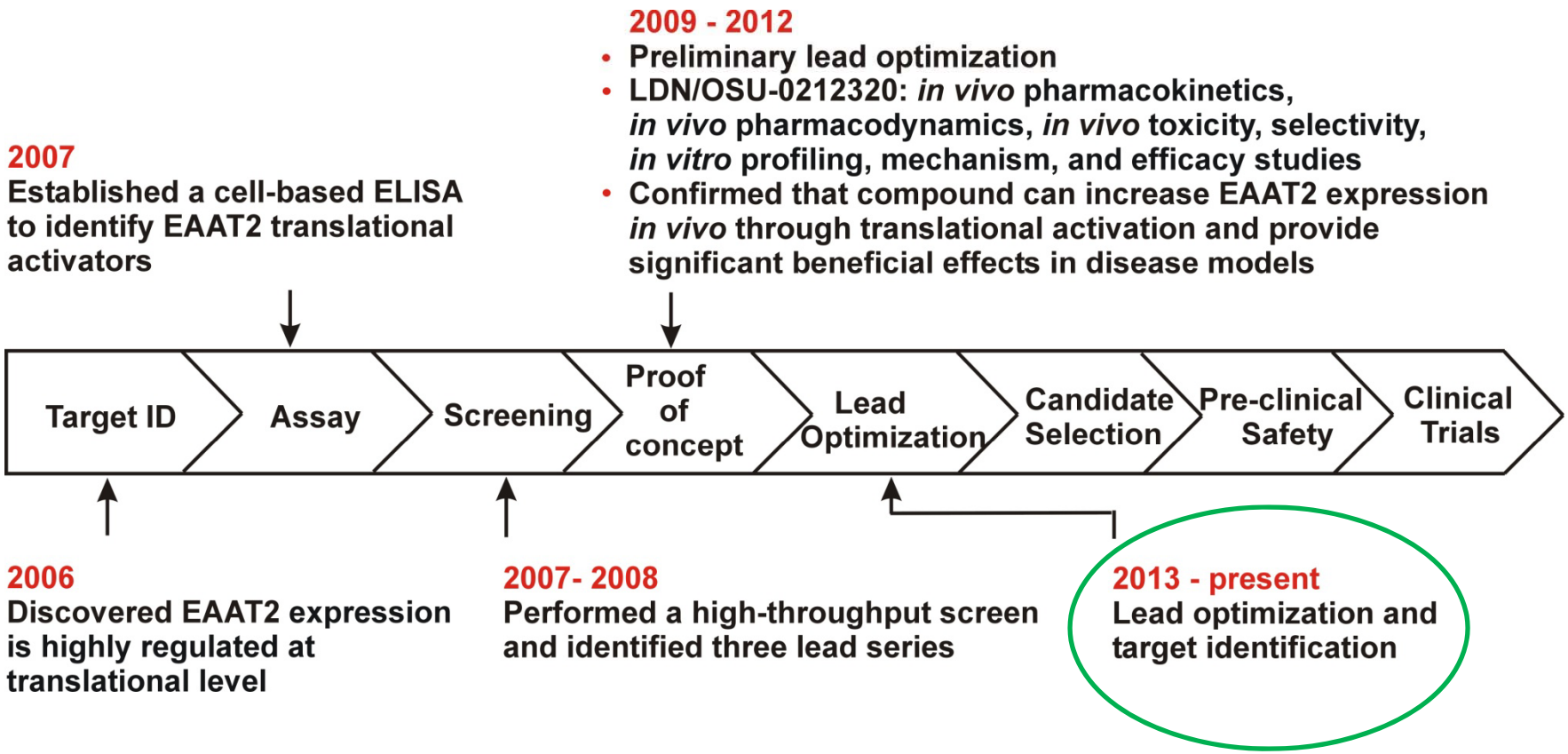


LDN/OSU-0212320 reduces RNA oxidation in the spinal cord of 60-day-old SOD1(G93A) mice



Heme oxygenase 1 (HO-1) was induced by the compound, via Nrf2/ARE signaling which is activated by AKT




2009 - 2012
• Preliminary lead optimization
• LDN/OSU-0212320: *in vivo* pharmacokinetics, *in vivo* pharmacodynamics, *in vivo* toxicity, selectivity, *in vitro* profiling, mechanism, and efficacy studies
• Confirmed that compound can increase EAAT2 expression *in vivo* through translational activation and provide significant beneficial effects in disease models
2007
Established a cell-based ELISA to identify EAAT2 translational activators
Target ID
Assay
Screening
Proof of concept
Lead Optimization
Candidate Selection
Pre-clinical Safety
Clinical Trials
2006
Discovered EAAT2 expression is highly regulated at translational level
2007- 2008
Performed a high-throughput screen and identified three lead series
2013 - present
Lead optimization and target identification

Lead optimization

LDN/OSU-0212320

MW = 293, cLogP = 4.9, PSA = 37

EAAT2: 3.1-fold @ 12.5 µM

EC_{50} = 1.8 µM

Sol = 12 µM @ pH 7.4

$T_{1/2}$ = 7 min

***In vivo* EAAT2 protein:**

10 mg/kg IP: no increase
20 mg/kg IP: ~1.5 fold
40 mg/kg IP: ~2.0 fold

10 mg/kg PO: no increase
20 mg/kg PO: no increase
40 mg/kg PO: ~1.5 fold

LDN/OSU- 0214733

MW = 294, cLogP = 3.2, PSA = 66

EAAT2: 2.4-fold @ 10 µM

EC_{50} = 39 nM

Sol = 7.5 µM @ pH 7.4

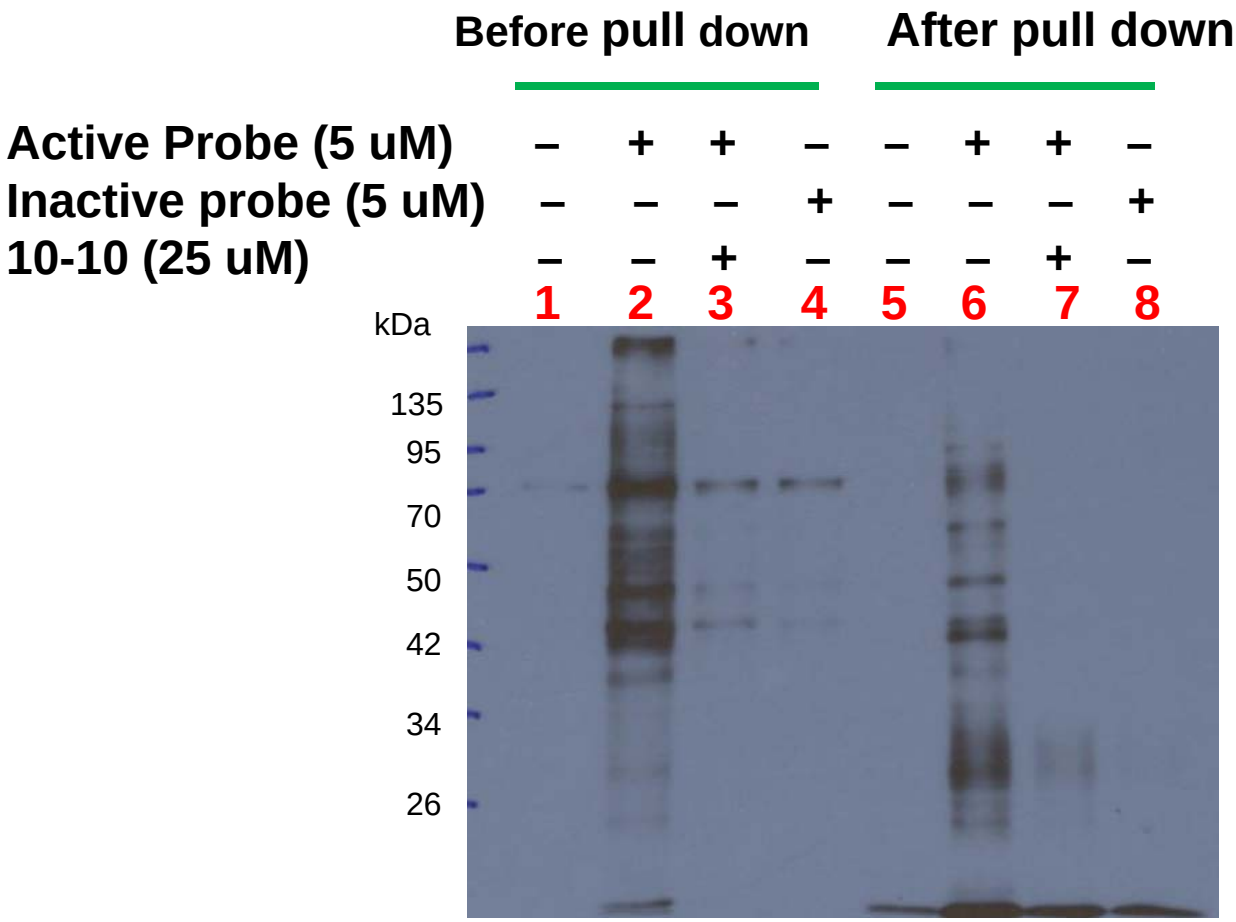
$T_{1/2}$ = 22 min

***In vivo* EAAT2 protein:**

10 mg/kg IP: ~3.8 fold
20 mg/kg IP: ~6.5 fold
40 mg/kg IP: ~5.2 fold

10 mg/kg PO: ~1.6 fold
20 mg/kg PO: ~1.9 fold
40 mg/kg PO: ~2.9 fold

Target identification



Drug discovery/development Pipeline