AU-403
Novel selective LXRβ-PPAR selective agonist for Alzheimer's Disease

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Innovation

AU403 helps mitigate ADRD, including APOe4 patients, develop neurodegeneration due to dysregulation of neuro-metabolism and neuro- cholesterol that induces aggregation of proteins, neuroinflammation and pathological hallmarks that lead to neurodegeneration.

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Introduction

- AU 403 was designed *in silico* to selectively interact with Liver-X-Receptor-β plus PPAR-α and PPARδ. Liver X receptors (LXRs) are nuclear receptors that serve as lipid-responsive transcription factors and thus targeted for patients with **ApoE** mutations.
- However, current LXR agonist have ***failed*** at the clinical level due to association with hepatic steatosis and elevated cholesterol levels.
- Our in silico design avoids LXRα activation in the liver and increases OXPHOS in the liver and muscle.
- *Our in silico design and recent findings of AU403 help increase brain cholesterol levels, while preventing hepatic steatosis and elevated cholesterol levels.*

Objective

- Show proof of mechanism in multiple relevant in vivo models demonstrating good safety pharmacology prevent unwanted side effects of current LXRα agonists:
  - Hepatic steatosis
  - Elevated circulating cholesterol
  - Neutropenia
- Robust biomarker clearance:
  - Amyloid β and P-Tau clearance.
- Improved AD-mediated memory impairment and neuronal health.

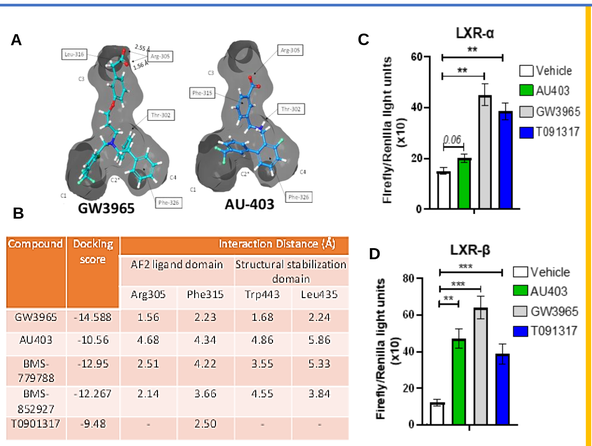
Results

- Ongoing research supports our *in silico* design that AU403 selectively activates LXRβ and PPARα/δ.
- Further that AU403 avoids LXRα and thus avoids development of hepatic steatosis and elevated circulating cholesterol levels.
- AU403 passes the BBB and positively effects biomarkers clearance,
- Improves synaptic plasticity and behavioral deficits.
- Most importantly AU403 shows no signs of toxicity observed with other LXR or PPAR agonist.

Conclusions

- AU403 is the first in class to exhibit both LXRβ and PPARα/δ activity.
- AU403 exhibits all of the desired characteristics of a drug at this stage of development.
- It is safe.
- Has good bioavailability.
- Is effective at low concentrations.
- Acts on target *in vivo.*
- AU403 will move to phase 1 and 2 SBIR and then Pre-IND
- Potential therapeutic for patients with APOe4 or APOe3/4 by improving the forms of cholesterol patterns in the brain.

Selected Data: Design



Compound	Docking score	Interaction Distance (Å)			
		AF2 ligand domain		Structural stabilization domain	
		Arg305	Phe315	Trp443	Leu435
GW3965	-14.588	1.56	2.23	1.68	2.24
AU403	-10.56	4.68	4.34	4.86	5.86
BMS-779788	-12.95	2.51	4.22	3.55	5.33
BMS-852927	-12.267	2.14	3.66	4.55	3.84
T0901317	-9.48	-	2.50	-	-

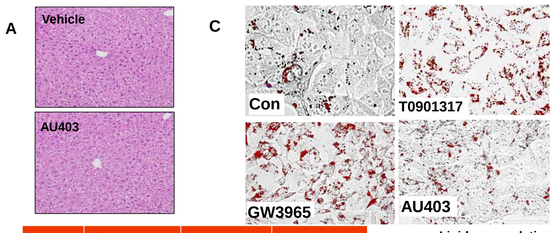
Figure 1 Design of AU403. A) In Silico predicted images if AU403 interacting with select aminao acids in he ligand binding domain of LXRα. **B)** Table displaying the docking scores based upon computational analysis using Schrodinger software suit of various LXR compounds interacting with amino acids in the ligand binding domain of **LXRα. LXR activity**. **(C & D)** LXR Promoter activity (luciferase assay) using human LXRα or LXRβ plus ABCA1-LXR recognition element values standardized to renilla; N>3 independent experiments in triplicate. Values average per group and analyzed using student t-test. *;p<0.05, **;p<0.001, ***;p<0.0001.

Drug	MW	logP Neutral H2O	logP Citrate buffer (pH 4)	logP PBS pH 7.4	Solubility in DMSO (mg/ml)	Solubility in etoh (mg/ml)
AU403	403	.783	1.23	1.660	35	28
AU403 ME	403	0.615	1.900	3.394	38	32
Fenofibrate	360.83	0.3788	3.7316	5	36	36
GW0742	471.49	-1.405	.37889	5	24.23	48.5

Drug	PAMPA GI Permeability Rate (cm/s) (Calculated)			
		Control High permeability	Control Medium permeability	Control Low permeability
AU403ME	$8.69x10^{-06}$			
AU403	$1.43x10^{-05}$	$5.15x10^{-06}$	$2.21x10^{-06}$	$6.17x10^{-07}$

Figure 2 *Calculated values* for AU403. A) LogP of AU403 and AU403ME (methyl ester). **B)** Parallel Artificial Permeability Assays (PAMPA) for gastrointestinal (GI) permeability of AU403 compounds. All compounds were evaluated at same dose (500μM/dmso) based upon manufactures instructions (BioAssay Systems) Values are based upon cm/sec. Control unknowns were offered in kit for comparisons.

Safety



Drug	Plasma Triglyceride (mg/dL)	Plasma total cholesterol (mg/dL)	Liver triglycerides (mg/g protein)
Vehicle	58.3± 12	75.3± 14	152± 11
AU403 (5mg/kg)	62.7± 8	77.5± 9	157± 10

Figure 3 Hepatic steatosis. A) Liver H&E staining from vehicle vs AU403 (5mg/kg) daily, i.p. for 1 month. Micron bar represents 200X. **B**) No significant change in plasma triglycerides, total cholesterol and hepatic triglyceride levels between AU403 and control mice. **B)** Blood values are from mice B A, n=8 mice per group. Student t-test were used to compare averages between groups. **C & D)** Lipid accumulation (OilRedO stain) in HEPG2 cells treated with 10μM of LXR agonists (GW3965 and T0901317) or AU403 for 72 hours. Endpoint reading of OilRedO stain and is standardized to total protein content. Data are tabulated from mean ± SEM and shown as percentage change from control using Prism software and determined significant by student T-test; N=4 repeated studies, *p<0.05.

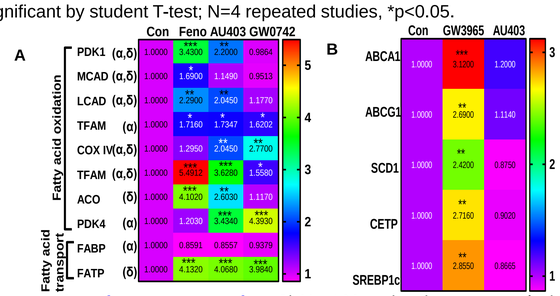


Figure 4 Hepatic gene expression. A) Heat Map showing gene analysis profile of **PPAR α and δ** targets in primary rat hepatocytes treated with AU403 **B) LXRα** targets by gene expression (qRT-pCR) from primary rat hepatocytes. Hepatocytes **(A)** were treated with either Feno (Fenofibrate), GW0742, AU403 or GW3965 (1μM) for 4 hours. Where fenofibrate; PPARα agonist, GW0742; PPARδ agonist, **B)** GW3965; LXR agonist and AU403. Averages from n=4 independent studies in triplicate and compares to control (saline) treated cells using student t-test. Values were based ΔΔct values standardized to β-actin and displayed as fold change from control, from 4 independent experiments in triplicate. *; p<0.05; **; p<0.00, ***; p<0.0001.

AD Pathology,Behavior and Neuronal activity

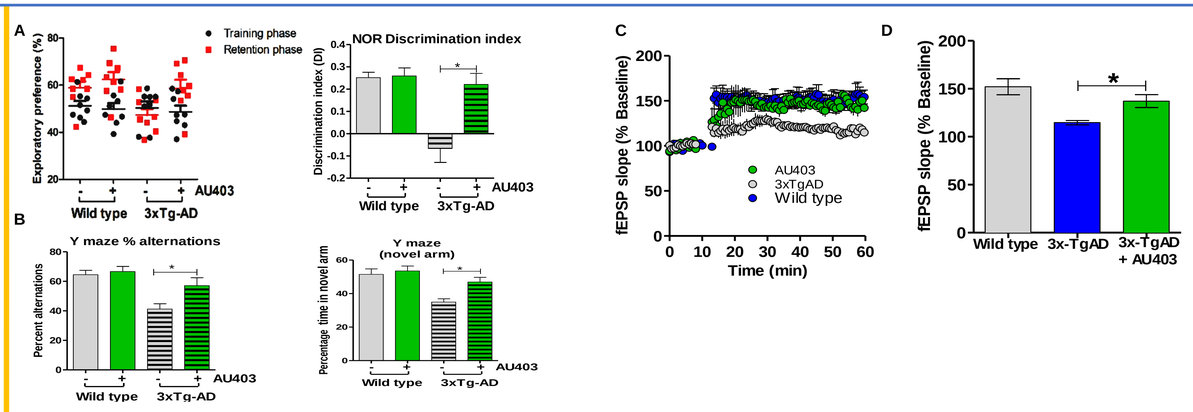


Figure 5 AU403 improves behavior and synaptic plasticity.AU403 A) AU403(5mg/kg) 1 month (i.p.) improves working declarative memory in 10 month old 3xTg-AD mice, as determined by novel object recognition (NOR) (**B**) and Y-maze. Values were based upon averages and S.E.M. and graphed % from control, n=8 mice per group; *p>0.05.
C) LTP is improved in 3xTgAD mice (10 months in age) following administration of AU403 (1 month daily i.p., 5 mg/kg). Time courses of normalized mean D) fEPSP slopes showing reduced levels of LTP in slices from 3xTgAD mice treated with saline. In comparison, restored LTP levels in slices from 3xTg-AD mice treated with AU403. Indicates fEPSP in relation to stimulus intensity. Bar graphs show LTP levels measured 50–60 min after induction. Data are shown as mean ± SEM. Data analyses were determined significant by one way-ANOVA; N=8 per group, *p<0.05.

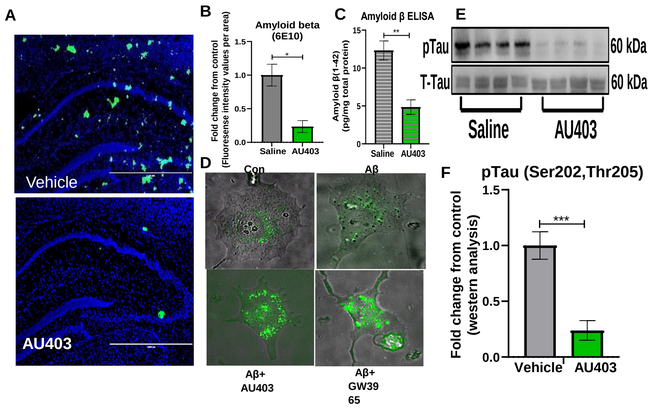


Figure 6 AU403 reduces Aβ and pTau in 10 month old 3xTgAD mice. A) Immunostaining for Aβ (Green), from vehicle treated mice and AU403 treated 3XTgAD mice, 5mg/kg/daily for 1 month, orally. Images were captured at 10X. **B**) values were obtained by measuring fluorescent units per area from 8 mice with 4 sections per mouse. **C**) Elisa for 1-42 soluble Aβ form was obtained the same as in panel A. Concentrations of Aβ were based upon a standard curve from the ELISA kit. Values were based upon averages where n=8 mice per group; **p>0.001. **D)** *Microglial uptake of Aβ*: Primary rat astrocytes were treated with either AU403 (1μM) or GW3965 (1μM) for 48 hours. Media was collected and condensed using Centricon-10 filters. Primary rat microglia cells (imaged here) were then treated with condensed astrocyte media (AM) ± drugs for 24 hours. Primary rat microglial cells were treated with fluorescent (HiLyte Fluor 488)-labeled ß-Amyloid (1-42), cat# A-60479-01 at 500nM concentration for 2. Images were captured using a Nikon Ti inverted fluorescence microscope. **E & F**) Western analysis pTau (Ser202/Thr205) from 10 month old female 3xTgAD mice treated similar to mice in A.