Discovery and Preclinical Development of SM15685, a Novel Highly Selective Oral DYRK1A/B Inhibitor as a Potential Therapeutic for Alzheimer's Disease

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Background

- Abnormal accumulation of hyperphosphorylated Tau protein is a pathological hallmark of many neurodegenerative diseases such as Alzheimer's disease (AD)[1]
- DYRK1A promotes Tau self-aggregation and fibrillization via abnormal hyperphosphorylation of Tau[2,3] and contributes to neurodegenerative diseases like AD
- Targeting DYRK1A has emerged as a novel approach for neurodegenerative diseases[4,5] but developing DYRK1A selective inhibitors has been a difficult challenge
- There has been a paucity of potent, selective, and orally bioavailable DYRK1A/B inhibitors that did not inhibit the closely related family of kinases including cdc2-like kinases (CLKs) and glycogen synthase kinase 3β (GSK3β)[6]
- We identified SM15685, a novel highly potent and selective oral DYRK1A/B inhibitor by utilizing an HTS campaign, structure-based drug design and lead optimization strategies
- In this study, we characterized SM15685 and assessed its potential to reduce Tau hyperphosphorylation both in vitro and in vivo

Conclusions

- We have identified SM15685, a novel, potent and ultra-selective DYRK1A/B inhibitor
  - Metabolically stable in human liver microsomes with no significant hERG and CYP inhibition
  - Negative in CYP induction, AMES and Micronucleus assays
  - Pharmacokinetic profiles with high oral bioavailability and CNS penetration across the species
  - Low efflux ratio in hMDR1-MDCK assay
- SM15685 significantly reduced Tau phosphorylation, Tau aggregates and neurofibrillary tangles in hTau P301S transgenic mouse model
- SM15685 is a promising candidate for the treatment of tauopathies that warrants further development

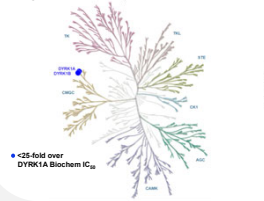
Results

Fig. 1: SM15685 is a highly potent and ultra selective DYRK1A/1B inhibitor that reduced Tau phosphorylation in vitro

A Biochemical and Target Engagement profiles

Compound	Biochemical IC_{50} (nM)		Target Engagement (TE) IC_{50} (nM)							pTau (Thr212) IC_{50} (nM)	No. of off-target kinases with ≥80% inhibition at 1 μM
	DYRK1A	GSK3β	DYRK1A	DYRK1B	DYRK2	CLK1	CLK2	CLK3	CLK4		
SM15685	1	9950	18	55	1789	5452	7932	9980	4150	14	6/400

B SM15685 full kinome profile



C Eurofins SafetyScreen87 Panel

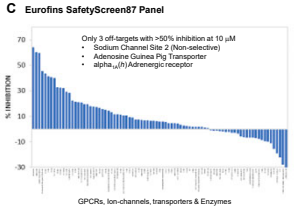
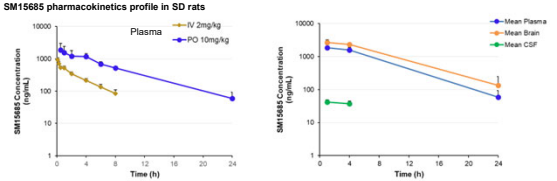


Fig. 2: SM15685 is orally bioavailable and highly brain-penetrant with favorable in vitro ADME and toxicity profiles

A SM15685 pharmacokinetics profile in SD rats



B SM15685 plasma exposures in CD1 mice, SD rats and cynomolgus monkeys after oral administration

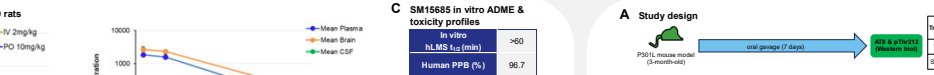
Species	Dose (mg/kg)	$AUC_{0\text{-last}}$ (h·ng/mL)	C_{max} (ng/mL)	t_{max} (h)	IV $t_{1/2}$ (h)	CL (mL/min/kg)	Vss (L/kg)	%F	$K_{puu,brain}$	$K_{puu,CSF}$
Mouse	10	9500	883	4.00	3.9	10.9	3.8	72	0.60	0.9
Rat	10	11426	1968	1.67	3.1	13.2	3.3	105	0.84	1.4
Monkey	5	4347	659	3.30	3.4	24.1	6.2	129	NA	1.0

C SM15685 in vitro ADME & toxicity profiles

In vitro hLMS $t_{1/2}$ (min)	>60
Human PPB (%)	96.7
Caco-2: A-B P_{app} (10^{-6} cm/s)	12.8
Caco-2 efflux ratio	0.5
hMDR1-MDCK: A-B P_{app} (10^{-6} cm/s)	20.5
hMDR1-MDCK efflux ratio	2.7
Cytotoxicity (HepG2) IC_{50} (μM)	>100
hERG IC_{50}/$C_{u,p\ eff}$	170-fold
CYP 1A2/2D6/3A4 IC_{50} (μM)	all >20
CYP induction	Negative (<1-fold)
AMES	Negative
Micronucleus	Negative

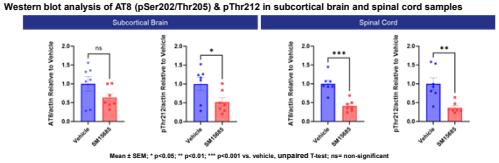
Fig. 3: SM15685 reduced Tau hyperphosphorylation in a PD study in JNPL3 hTau transgenic (P301L) mouse model

A Study design



Treatment	Dose (mg/kg)	Frequency	n/sex	Tissue collection	Time post-dosing at euthanize
Vehicle	NA	QD	7/F	Brain	2 h
SM15685	45	QD	7/F	Plasma & brain	2 h

B Western blot analysis of AT8 (pSer202/Thr205) & pThr212 in subcortical brain and spinal cord samples



Mean ± SEM; * p<0.05; ** p<0.01; *** p<0.001 vs. vehicle, unpaired T-test; ns= non-significant

C Unbound exposures of SM15685 at 2 h on day 7

Dose (mg/kg)	pTau T212 IC_{50} (nM)	Exposures at 2 h			
		$C_{u,p}$ (nM)	$C_{u,b}$ (nM)	K_{puu}	$C_{u,b}$/pTau IC_{50}
45	14	171.8	172.3	1	12.3

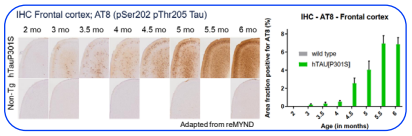
$C_{u,p}$ (unbound plasma concentration)
$C_{u,b}$ (unbound brain concentration)
K_{puu} (ratio of unbound brain concentration/unbound plasma concentration)

Fig. 4: Long term efficacy study in hTau transgenic (P301S) mouse model

A Study design

Treatment	Dose (mg/kg)	Frequency	n/sex	Tissue collection	Time post-dosing at take down
Vehicle	NA	QD	15/F	Plasma, brain & spinal cord	2 h
SM15685	6.25	QD	15/F	Plasma, brain & spinal cord	2 h
SM15685	12.5	QD	15/F	Plasma, brain & spinal cord	2 h
SM15685	25	QD	15/F	Plasma, brain & spinal cord	2 h

B reMYND P301S model characteristics: age dependent increase of Tau pathology



C SM15685 was well tolerated at all doses without significant BW changes compared to Vehicle

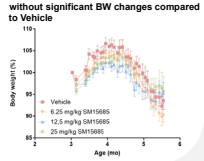


Fig. 5: SM15685 reduced Tau hyperphosphorylation, Tau aggregates, neurofibrillary tangles in hTau P301S transgenic mice

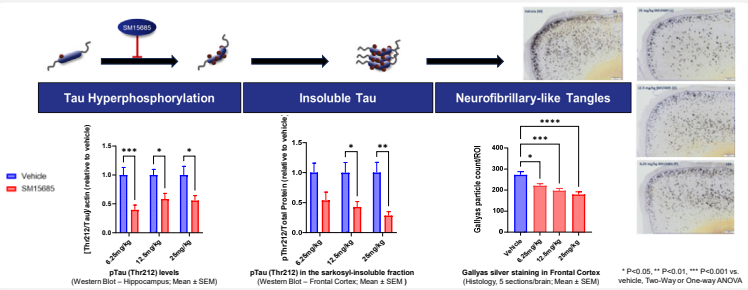
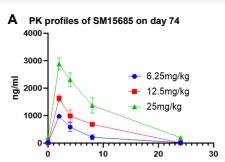


Fig. 6: Exposures of SM15685 in hTau P301S mice and PK/PD relationship

A PK profiles of SM15685 on day 74



Dose (mg/kg)	$AUC_{0\text{-last}}$ (h·ng/mL)	$C_{u,p}$ at 2 h (nM)
6.25	5999	37
12.5	13133	61
25	28185	108

B Unbound exposures of SM15685 at 2 h on day 77 (end of efficacy study)

Dose (mg/kg)	$C_{u,p}$ (nM)	$C_{u,b}$ (nM)	pTau IC_{50} (nM)	$C_{u,b}$/pTau IC_{50}
6.25	36	26		1.8
12.5	73	63	14	4.5
25	131	109		7.8

Figure Legends and Methodology

- **Fig. 1**: (**A**) Table listing biochemical DYRK1A and GSK3β kinase inhibition IC_{50}, the target engagement of CLK/DYRK family members and pTau Thr212 inhibition IC_{50} by SM15685. Biochemical kinase assay IC_{50} values were determined using the Thermo Fisher Scientific Z-LYTE™ platform. Target engagement assay IC_{50} values were determined using the Promega NanoBRET TE Intracellular Kinase Assay platform in transiently transfected HEK293T cells. Full kinome data is from Thermo Fisher Scientific SelectScreen ™ Profiling Service using compounds at 1μM. Inhibition of tau phosphorylation (pTau) was measured in human tau/DYRK1A-transfected HEK-293T cells using Tau Thr212 AlphaLISA assay. (**B**) Dendrogram of the human kinome. Kinases inhibited at 90% or more by SM15685 and showing an IC_{50} less than 25-fold over DYRK1A IC_{50} are highlighted with a blue circle and labeled accordingly. (**C**) %inhibition in SafetyScreen87 Panel at 10 μM.
- **Fig. 2**: (**A**) Pharmacokinetics profile in plasma, brain and cerebral spinal fluid (CSF) was analyzed from SD rats following a single oral (10mg/kg, PO) or intravenous (2mg/kg, IV) administration of SM15685. (**B**) PK parameters of SM15685 after oral administration in mouse (10 mg/kg, PO), rat (10 mg/kg, PO) and monkey (5 mg/kg, PO). (**C**) In vitro ADME and toxicity profile of SM15685.
- **Fig. 3**: (**A**) Schematic of the PD study design in 3-months old female JNPL3 transgenic (P301L) mice. (**B**) Levels of pTau in subcortical brain and spinal cord fractions were analyzed by western blot (WB) using AT8 or pTau Thr212 antibodies. Data were normalized to β-actin and expressed relative to vehicle. Each bar represents mean ± SEM. (**C**) Plasma and brain unbound exposure analysis of SM15685 at 2h after daily oral administration for 7 days (45 mg/kg).
- **Fig. 4**: (**A**) Schematic of the efficacy study design in hTau transgenic (P301S) mice. (**B**) reMYND hTau (P301S) model characteristics: age and Tau pathology relationship. (**C**) Body weight change throughout the study for all dosing groups.
- **Fig. 5**: Effect of SM15685 on the formation of pTau, insoluble Tau and neurofibrillary tangles (NFT) after daily oral administration for 11 weeks (6.25, 12.5 or 25 mg/kg, PO). Tau hyperphosphorylation was assessed by WB using total lysates from hippocampus samples and using pTau Thr212 antibody. Data were normalized to total Tau and β-actin and expressed relative to vehicle. Insoluble Tau was assessed by WB using sarkoyl-insoluble fractions from frontal cortex samples and using pTau Thr212 antibody. Data were normalized to total protein and expressed relative to vehicle. NFTs were assessed by histology using Gallyas silver staining of frontal cortex sections. Quantification was done across 5 sections per brain. Each bar represents mean ± SEM.
- **Fig. 6**: (**A**) Plasma PK profiles of SM15685 after daily oral administration for 74 days (6.25, 12.5 or 25 mg/kg, PO). (**B**) Exposure analysis of SM15685 at 2h after daily administration for 77 days (6.25, 12.5 or 25 mg/kg, PO). (**C**) Plasma and brain unbound exposure analysis of SM15685 at 2h after daily oral administration for 11 weeks.

References

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