Identification and characterization of a novel LRRK2 kinase inhibitor: ODS2005294

763.12

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Introduction

Leucine-rich repeat kinase 2 (LRRK2) is a potential target for disease-modifying therapy in Parkinson's disease (PD) because mutations in its catalytic core are associated with both autosomal-dominant and late-onset sporadic PD. Most described LRRK2 mutations and notably the well characterized G2019S enhance kinase activity suggesting that small molecule LRRK2 kinase inhibitors may serve as potential therapeutic agents.
Our strategy is to design and develop LRRK2 kinase inhibitors crossing the blood-brain barrier (BBB) using the Nanocyclix® technology based on small macrocycles that readily cross the BBB.

Results

ODS2005294

▪ In vitro potency and selectivity profiles

Assay	Characteristic	Results
Biochemical activity (nM)	Wt IC_{50} Wt Ki % inh @ 100 nM G2019S, I2020T, R1441C	9 - 17 1 80-100%
Cellular activity (pLRRK2 IC_{50} nM)	h Wt SH-SY5Y cells h G2019S SH-SY5Y cells 3T3 mouse fibroblasts	79 52 62
Kinases selectivity @ 100 nM	S50 in % ; 96K S50 in % ; 386 K (included mutants)	6% 7%

ODS2005294 shows low-nanomolar activity for LRRK2 in an in vitro purified LRRK2 kinase assay and a cellular assay monitoring dephosphorylation of LRRK2 pS935 and a minimal inhibition of other kinases

A radiometric protein kinase assay (PanQinase® Activity Assay) was used for measuring the kinase activity of the selected protein kinases from ProQinase.
Cellular LRRK2 kinase activity was measured using LanthaScreen technology from Invitrogen. SH-SY5Y neuroblastoma cells are transfected with h G2019S or hWt LRRK2.
LRRK2 pS935/total LRRK2 ratios were measured in mouse fibroblast 3T3 cell line to evaluate LRRK2 kinase inhibition.

▪ Ex vivo inhibition of pLRRK2 in human peripheral blood cells:

see poster 763. 04: Ex-vivo inhibition of LRRK2 phosphorylation by a new kinase inhibitor in peripheral blood cells of subjects with and without G2019S LRRK2 mutation. P. Plas et al

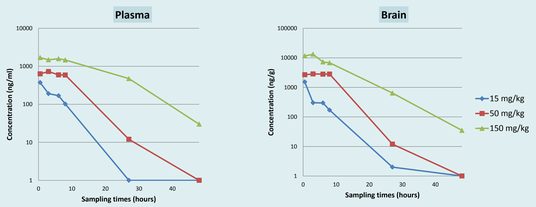
▪ ADME parameters

Assay	Test concentration	Species	Results	
CYPs Inhibition	1µM	Human	• CP 1A2 67% => IC50 0.77 µM • CYP3A4 BFC 47%	
Metabolic stability	10µM	Human Mouse	• Human T1/2 : 17 min => Clint = 81 µL/min/mg • Mouse T1/2 : 45 min => Clint = 36 µL/min/mg	
Plasma Stability	1µM	Human Mouse Rat	• ODS2005294 is stable up to 6 hours in rat/mouse/human plasma	
Plasma & Brain Protein Binding	5µM		Plasma	Brain
		Human	99,4	
		Mouse	99,4	98,2
		Rat	98,5	98,0
MDR1-MDCK Permeability	2µM	Human	• Moderate permeability (Papp=3,36 10-6 cms^{-1}) and no efflux due to MDR1	
Caco-2 Permeability	2µM	Human	• High permeability (Papp= 7.75 10-6 cms^{-1}) and no efflux due to transporters in Caco-2 cells	

ODS2005294 was dissolved in the appropriate matrix with 1% DMSO.
The seven main cytochrome P450 isoforms (CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were investigated in the Cytochrome P450 Inhibition assay. In vitro metabolism, permeability and protein binding were evaluated at Cyprotex, UK.

▪ PK parameters

Dose-related PK profile in mouse



Species	Dose mg/kg	route	Results
Mouse CD1	15; 50 & 150	PO	• Rapid and high distribution in all tissues especially in kidney , liver and brain leading to high tissue exposure. • High level in striatum, hippocampus, midbrain & cortex • Supralinearity between 15 and 150 mg/kg. • High oral bioavailability (estimated at 60%).
Mouse CD1	50 x 4d	PO	• No significant accumulation between day1 and day4 in plasma, brain, heart, kidney, liver and muscle.
Rat SD	15; 50 & 150	PO	• High distribution in brain Kp = 3,6 ; **Kpu,u = 4,7** (15mg/kg) • Rapid and high distribution in all tissues especially in kidney and liver (all doses) leading to high tissue exposure. • Supralinearity between 15 and 150 mg/kg.

ODS2005294 displays a good PK profile in rodents and a good in vivo brain penetration

ODS2005294 was dissolved in 1% tween 80 and 1% HPMC in water and administered by oral route. Rodents were sacrified at different times after administration and blood and tissues were collected. ODS2005294 was quantified using LC/MS-MS method.

▪ In vivo inhibition of pLRRK2

Species	Dose mg/kg	% inh Brain	Brain	Kidney	% inh Kidney	% inh PBMC
CD1	50	64%	Vehicle 5294 LRRK2-p(S935) LRRK2	Vehicle 5294 LRRK2-p(S935) LRRK2	87%	99%
hWT	15 50 100	12% 52% 27%	Vehicle 15 mg/kg 50 mg/kg 100 mg/kg 100 nM 267nM 254nM	Vehicle 15 mg/kg 50 mg/kg 100 mg/kg	73% 91% 94%	91% 100% 100%
		[5294] free				
hG2019S	15 50 100	35% 67% 69%	Vehicle 15 mg/kg 50 mg/kg 100 mg/kg 96 nM 337 nM 316 nM	Vehicle 15 mg/kg 50 mg/kg 100 mg/kg	89% 86% 87%	99% 100% 100%
		[5294] free				

In CD1 or in transgenic mice expressing human LRRK2 protein (Wt or G2019S mutation) robust concentration-dependent knockdown of pLRRK2 in brain and peripheral tissues (kidney and PBMC) was observed (from 15 to 100 mg/kg), using pSer935 levels and determination of compound concentrations.

*ODS2005294 was dissolved in 1% tween 80, 1% HPMC in water and administered by oral route into CD1 (Charles Rivers France) or Transgenic FVB/N-Tg(LRRK2*G2019S)1Cjli/J and Wt mice (Jackson Laboratories; USA). Ninety minutes after administration, mice were sacrified and blood, brain and kidney were rapidly dissected and snap-frozen in liquid nitrogen.*
For immunoblot procedures, antibodies against pS935 and total LRRK2 were used. Western blot detection and quantification were used and LRRK2 pS935/total LRRK2 ratios were calculated to compare LRRK2 kinase inhibitor-dosed groups with vehicle control group.

▪ In vitro Toxicological profile

Assay	Test concentration	Results
SafetyScreen 44 selected targets	1,7µM	70 % inh 5HT-1A and 5HT-2B
Drug abuse Potential profile	1,7µM	No alert
Cardiac toxicity		hERG IC_{50} : 0,7 µM Nav1.5 IC_{50} : >3 10^{-5}M
Genotoxicity	30µM	Ames : negative In vitro micronucleus: negative
Action potential in rabbit Purkinje fibres	1 – 10µM	Low risk of QT interval prolongation

ODS2005294 displays a good *in vitro* safety profile

All these studies were conducted at Cerep; France

References:
Maron, D.M. and Ames, B.N. (1983), Mutation Res., 113 : 173-215. Mathes, C. (2006), Expert Opin. Ther. Targets, 10 (2) : 230-241. Diaz, D. et al. (2007), Mutat Res. 630 (1-2):1-13. Krafte, D.S. et al. (1995), J. Mol. Cell Cardiol. 27 : 823-830

▪ In vivo Toxicological profile

Assay	Treatment –related changes
Body weight	No effect
Hematology	WBC: +10%
	Platelets: -1%
	Reticulocytes: -19%
	RBC: -10%
Serum biochemistry	No alterations
Histopathological findings	Kidney, spleen, liver, lung, intestines: no alterations
	Slight decrease in bone marrow cellularity

Oral administration of ODS2005294 at 100 mg/kg, daily for 4 days was well tolerated

Male CD1 mice were orally administered at 100 mg/kg once daily for 4 days. On completion of the treatment period, blood was removed for hematology and biochemistry, then all animals were euthanized and submitted to a full microscopic post mortem examination.

Conclusions

- This small molecule inhibitor is characterized by nanomolar biochemical and cellular activities, moderate broad kinome selectivity, good in vivo oral exposure and unbound brain concentrations, dose-related inhibition of in vivo pLRRK2 with no obvious adverse effect at pharmacological dose.
- This compound provides a starting point for developing a candidate that could ultimately be used to address the therapeutic benefit of inhibiting LRRK2 in Parkinson's patients harboring the LRRK2 G2019S mutation.

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