In vitro pharmacological profile of Compound A, a novel potent and selective LRRK2 inhibitor

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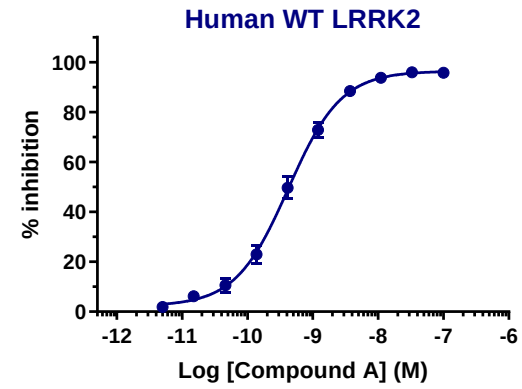
OBJECTIVES

Strong genetic evidence has validated Leucine-Rich Repeat Kinase 2 (LRRK2) as a target of interest for Parkinson's Disease, leading to the development of LRRK2 inhibitors as potential therapeutic approach. Among them, Compound A has recently been selected as a potent and selective LRRK2 inhibitor. This poster presents the *in vitro* pharmacological profile of Compound A.
Please see the accompanying poster for Compound A *in vivo* profile.

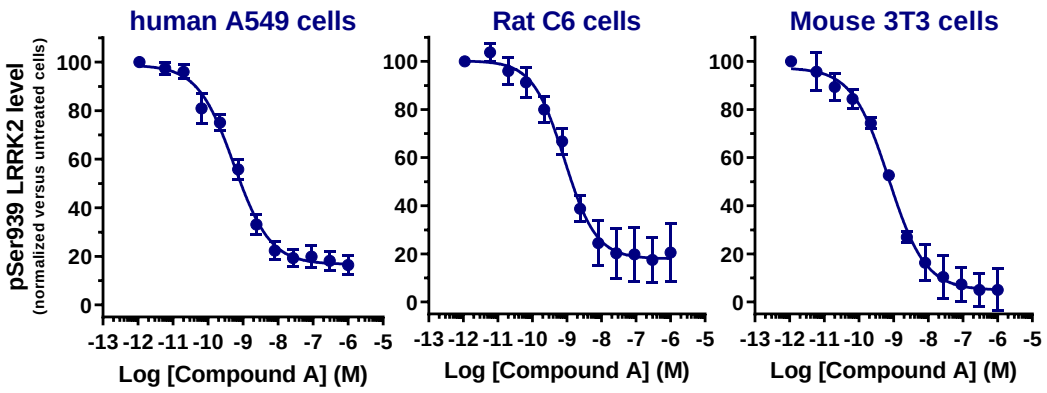
Strong affinity for LRRK2 in cell-free assays



	Human WT-LRRK2
IC_{50}	0.4 nM [0.3; 0.6] (n=22)

Biochemical ADP-GloTM assay used for cell-free assessments is a luminescent Adenosine DiPhosphate (ADP) detection assay that measures the kinase enzymatic activity of LRRK2 by quantifying the amount of ADP produced in a reaction (Promega).

High potency confirmed in cellular assays



	human A549	Rat C6	Mouse 3T3
IC_{50}	0.6 nM [0.4; 1.0] (n=14)	0.6 nM [0.1; 3.0] (n=4)	0.8 nM [0.2; 3.6] (n=3)

The In vitro activity of Compound A was measured in human A549, rat C6 and mouse 3T3 cells using phosphorylated Enzyme Linked Immunosorbent Assay (ELISA) (pSer935-LRRK2). pSer935-LRRK2 is phosphorylated form of LRRK2 which is reduced following LRRK2 kinase inhibition and is commonly used *in vitro* as well as *in vivo* as a marker of LRRK2 target engagement (Reynolds et al., 2014*; Rideout et al., 2020*). Following treatment with Compound A, pSer935-LRRK2 levels were reduced in human A549, rat C6 and mouse 3T3 cells.

* Reynolds A et al. (2014) LRRK2 kinase activity and biology are not uniformly predicted by its autophosphorylation and cellular phosphorylation site status. Front. Molec. Neurosci. 7:54
* Rideout HJ et al. (2020) The current state-of-the art of LRRK2-based biomarker assay development in Parkinson's disease. Front. Molec. Neurosci. 14:865

High selectivity for LRRK2

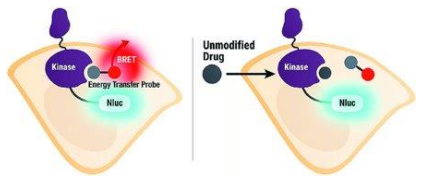
Selectivity towards other kinases was assessed using cell-free (full profiling on 413 kinases via binding assays, Eurofins) and cellular assays (full KiNativTM kinases profiling via an ATP probe binding and kinases identification and quantification by Liquid Chromatography-Tandem mass spectrometric detection (LC-MS/MS) on human A549 cells or PBMC, ActivX). The safety binding profile of Compound A was evaluated *in vitro* on 113 receptors, enzymes or channels at concentrations up to 10 µM by *In vitro* competitive binding assay on recombinant cell membranes.

	Eurofins Kinases profiling cell free	Kinativ Kinases profiling Human A549	Kinativ Kinases profiling Human PBMC	CEREP Safety binding profile Other off-targets
IC_{50}	LRRK2: 6 nM	LRRK2: 2.1 nM &	LRRK2: 2.9 nM &	-
Selectivity versus LRRK2	Safety margin ≥ 100 fold on 96.5% on the panel of 413 kinases	Safety margin ≥ 100 fold on 100% on the panel of kinases & 278 probe-labeled peptides corresponding to about 217 unique kinases	Safety margin ≥ 100 fold on 100% on the panel of kinases & 297 probe-labeled peptides, corresponding to about 220 unique kinases	Safety margin ≥ 4 000 fold on 100% on the panel of 113 receptors, enzymes and ion-channels

& Versus LRRK2 IC_{50} determined on the same cell

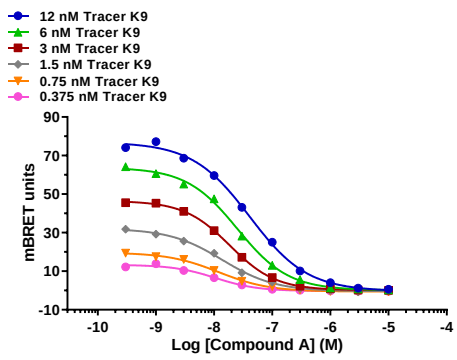
Comparable inhibition on different LRRK2 mutants

HEK293 cells were transfected with human WT LRRK2 or with G2019S and other LRRK2 mutants. An intracellular kinase assay based on an energy transfer technique, NanoBRET (Nano Bioluminescence Resonance Energy Transfer), was used (Promega). This assay measures the affinity of the compound in intact cells by competitive displacement of a NanoBRET tracer K9 or K10 (two ATP-probes), reversibly bound to a NanoLuc luciferase-LRRK2 fusion construct (Vasta et al., 2018*). Upon binding of Compound A to LRRK2, the BRET signal was attenuated.



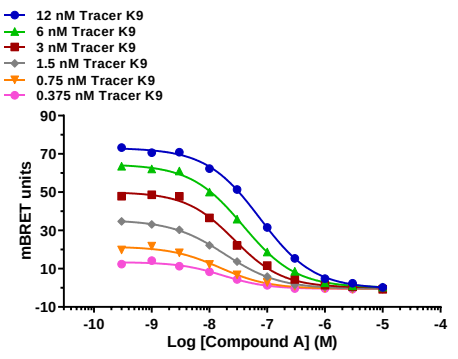
*Vasta JD et al. Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement. Cell Chem. Biol.2018;25:206–214..

Transfected HEK293, WT LRRK2



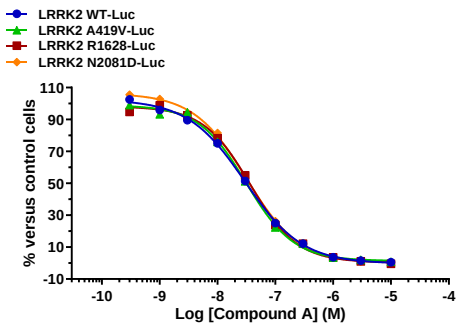
	WT-LRRK2	
	Tracer K9	Tracer K10
Kd	9 nM	6 nM

Transfected HEK293, G2019S LRRK2

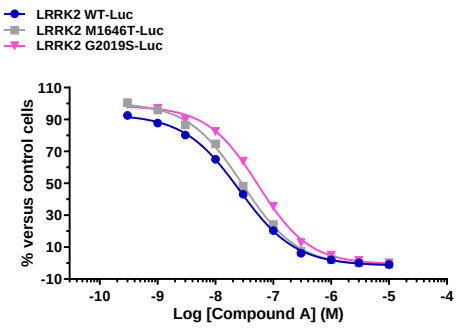


	G2019S-LRRK2	
	Tracer K9	Tracer K10
Kd	13 nM	8 nM

Transfected HEK293, WT and mutants LRRK2



	WT	A419V
IC_{50}	19 nM	32 nM
	R1628P	N2081D
IC_{50}	35 nM	37 nM



	WT	M1646T	G2019S
IC_{50}	32 nM	28 nM	51 nM

CONCLUSION

Compound A is a highly potent and selective LRRK2 inhibitor:

- **Nanomolar activity in various *in vitro* assays performed in cell-free or on rat, mice and human cells**
- **Equipotency on wild type, G2019S and other common LRRK2 mutants**
- **Selectivity ≥ 100 fold for LRRK2 (Kinome and CEREP safety binding)**

Further PK/PD evaluations confirmed the target engagement of Compound A *in vivo* with a similar nM activity in rat, mice and non-human primate brain (see accompanying poster).
Taken together, these data demonstrate that Compound A is an attractive drug candidate for the treatment of Parkinson's disease.