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Discovery of a Pyrrolopyrimidine (JH-II-127), a Highly Potent, Selective, and Brain Penetrant LRRK2 Inhibitor

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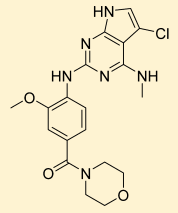
Supporting Information

ABSTRACT: Activating mutations in leucine-rich repeat kinase 2 (LRRK2) are present in a subset of Parkinson's disease (PD) patients and may represent an attractive therapeutic target. Here we report a 2-anilino-4-methylamino-5-chloropyrrolopyrimidine, JH-II-127 (**18**), as a potent and selective inhibitor of both wild-type and G2019S mutant LRRK2. Compound **18** substantially inhibits Ser910 and Ser935 phosphorylation of both wild-type LRRK2 and G2019S mutant at a concentration of 0.1−0.3 μM in a variety of cell types and is capable of inhibiting Ser935 phosphorylation in mouse brain following oral delivery of doses as low as 30 mg/kg.



JH-II-127 (**18**)

LRRK2-wild-type	:	6.6 nM
LRRK2-G2019S	:	2.2 nM
LRRK2-A2016T	:	47.7 nM

KEYWORDS: *LRRK2, leucine-rich repeat kinase 2, Parkinson's disease, pharmacokinetics*

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world. It affects over one million Americans, and more than 60,000 patients are newly diagnosed annually.[1,2] Current standards of care have serious limitations and largely address only symptoms.[3] Recent genetic studies have revealed an underlying genetic cause in at least 10% of all PD cases,[4] which provides new opportunities for the discovery of molecularly targeted therapeutics that may ameliorate neurodegeneration. Among the genes associated with PD, leucine-rich repeat kinase 2 (LRRK2) is unique because a missense mutation, G2019S, is the most frequent pathogenic substitution found in both familial and sporadic Parkinson's disease cases.[5,6] The G2019S mutation leads to a two- to three-fold increase in kinase activity, which may result in activation of neuronal death signal pathway, suggesting that small molecule LRRK2 kinase inhibitors may be able to serve as a new class of therapeutics for the treatment of Parkinson's disease.[7,8] Human genetic studies conclusively implicate the G2019S mutation as functionally relevant for Parkinson's disease.[9]

LRRK2 kinase inhibitors are being actively pursued both as "tools" to pharmacologically interrogate normal and pathological LRRK2 biology and as experimental therapeutic agents (Table 1).

Several 2,4-diaminopyrimidine based inhibitors of LRRK2 have been reported including LRRK2-IN-1 (**1**),[10] CZC-25146 (**2**),[11] and TAE684 (**3**).[12] Additionally, GSK2578215A (**4**),[13] an unusual benzamide ATP-site directed scaffold, was found to be a potent and selective inhibitor of LRRK2.

However, none of these compounds are capable of effectively inhibiting phosphorylation of Ser910 and Ser935 of LRRK2 in mouse brain following intraperitoneal doses of up to 100 mg/kg, which limits their use as tools in murine PD models.[10,11] More recently, we and others developed additional 2,4-diaminopyrimidines including HG-10-102-01 (**5**)[14] and GNE7915 (**6**)[15] that are potent and selective LRRK2 inhibitors that can traverse the blood−brain barrier. These diaminopyrimidines were shown to significantly reduce LRRK2 activity in the brain of G2019S LRRK2 transgenic mice after oral dosing, judged by their ability to reduce LRRK2 phosphorylation at Ser935, a phosphorylation that is dependent on LRRK2 catalytic activity.[14,15] The pyrrolopyrimidine PF-06447475 (**7**)[16] was recently reported to also be a potent and selective LRRK2 inhibitor capable of reducing LRRK2 activity in the brain of G2019S LRRK2 transgenic mice after oral dosing. Additional ATP-competitive LRRK2 inhibitors that have been reported include cinnolines,[17] triazolopyridazines,[18] and 3-cyanoquinazolines,[19] as well as several others that have appeared in the patent literature.[20−24] Based on the chemical structure of GNE7915 (**6**), we thought

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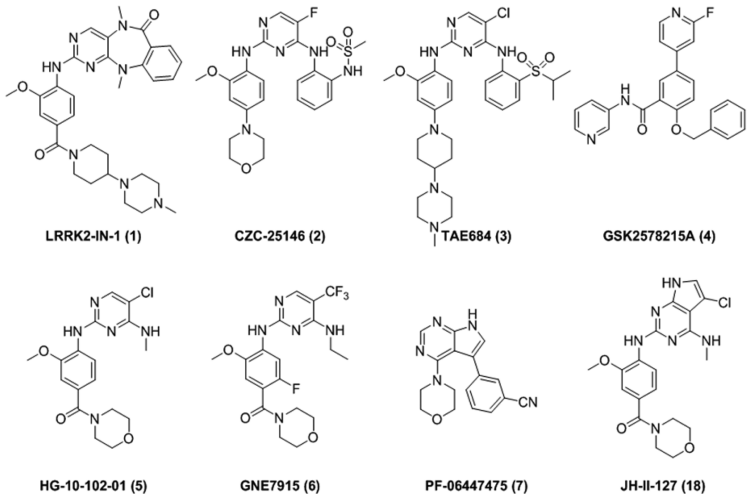
Table 1. Comparison of Known LRRK2 Inhibitors with JH-II-127



compd ID	IC_{50} (nM)[a] wild-type LRRK2	LRRK2-G2019S	LRRK2-A2016T	LRRK2-G2019S + A2016T
LRRK2 IN 1 (**1**)	13 ± 1.8	6 ± 0.9	2450 ± 16	3080 ± 10
TAE684 (**3**)	8 ± 0.5	6 ± 0.8	93 ± 2.2	22 ± 2.9
GSK2578215A (**4**)	11 ± 1.9	9 ± 1.4	81 ± 5.4	61 ± 5.5
HG-10-102-01 (**5**)	20 ± 3.2	3 ± 0.8	154 ± 8.7	96 ± 2.4
GNE7915 (**6**)	1 ± 0.5	0.3 ± 0.2	466 ± 6.8	382 ± 14
PF-06447475 (7)[b]	3[16]	11[16]	N/A	N/A
JH II 127 (**18**)	6 ± 0.5	2 ± 0.3	48 ± 3.5	20 ± 0.9

[a]GST-LRRK2(1326−2517), GST-LRRK2[G2019S](1326−2527), GST-LRRKT[A2016T](1326−2517) and GST-LRRK2[G2019S + A2016T] (1326−2517) were assayed using 20 μM Nictide in the presence of 100 μM ATP. Results are average of triplicate experiments. [b]Published results.

intramolecular hydrogen bonding was likely to exist between the C-5 trifluoromethyl group and the NH hydrogen to give the pseudobicycle shown in Figure 1.[15] We decided to construct the

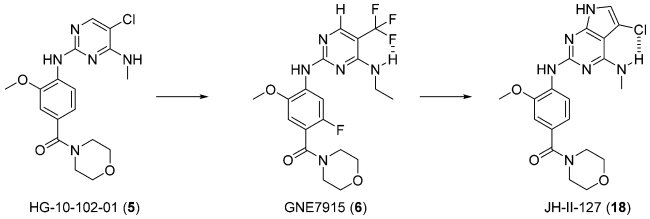


Figure 1. Rationale behind the design of JH-II-127.

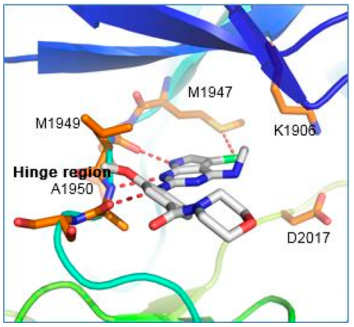


Figure 2. Molecular model of JH-II-127 with LRRK2.

ring-closed version of GNE7915 (**6**) by designing a series of 6,5-fused ring analogues. We reasoned that a fused bicyclic analogue would increase the binding affinity due to the additional hydrogen bond donor at the 7-position, which is predicted to hydrogen bond with M1949 as shown in Figure 2. In addition, a fused bicyclic compound should be able to better fill the hydrophobic area around the hinge region, thus leading to an increase in binding affinity. We have shown that a chlorine in the 5-position of a pyrimidine interacts with the gatekeeper Met790 of EGFR to increase the binding affinity;[25] therefore, we wanted to use a pyrrolopyrimidine, which would also allow the installation of a chlorine at the C-5 position. We hypothesized that a chlorine at the C-5 position would result in a more favorable interaction with the Met1947 residue. Herein, we report our efforts to identify bicyclic compounds with potent LRRK2 inhibition and favorable pharmacokinetic properties.

We began with the synthesis of pyrrolopyrimidines **8**−**20** with a variety of substituents at the C-4 position (R_2). As shown in Table 2, compounds with a range of amine substituents exhibited excellent potency in both the wild-type and LRRK2[G2019S] enzymatic assays, while compounds with a methoxy group (**9**) or no substituent at the C-4 position (**10**, **17**) showed reduced activity. Compounds **18**−**20** with a chlorine at the C-5 (R_3) position and amine substituents at the C-4 position also showed excellent potency in wild-type and LRRK2[G2019S] kinase assays.

The biochemical potency of **18** for inhibition of wild-type LRRK2 and LRRK2[G2019S] is similar to that observed for (**1**); however, **18** maintains inhibition of the A2016T mutation, which induces dramatic resistance to LRRK2-IN-1 (**1**) (Figure 1).

Being mindful of other 6,5-fused bicyclic analogues, we turned our attention toward pyrazolopyrimidines and purine analogues (Table 3). We began by synthesizing compounds **21** and **22** with methylamino and methoxy substitution at the C-6 (R_2) position, respectively. Interestingly, compound **22** with a methoxy substituent at C-6 position showed greater potency in both

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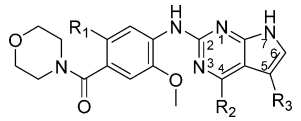
Table 2. Pyrrolopyrimidine Structure−Activity Relationship (SAR) with Enzyme IC_{50}s



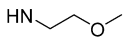
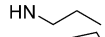
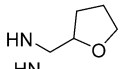
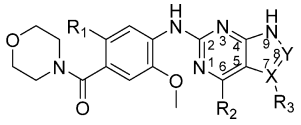
compd	R_1	R_2	R_3	Enzyme IC_{50} wt (nM)	Enzyme IC_{50} G2019S (nM)	MPO Score
8	H	NHMe	H	2.6 +/- 0.5	5.1 +/- 0.6	4.6
9	H	OMe	H	13.6 +/- 0.3	95.7 +/- 0.3	4.8
10	H	H	H	34 +/- 2.1	40 +/- 6.3	5.3
11	H		H	4 +/- 0.7	3 +/- 0.1	4.1
12	H		H	1.1 +/- 0.7	2.5 +/- 0.1	4.5
13	H		H	1.6 +/- 0.2	2.4 +/- 0.2	3.9
14	H		H	3.3 +/- 0.3	4.4 +/- 0.1	3.8
15	H		H	2.4 +/- 0.2	4.7 +/- 0.8	4.4
16	F	NHMe	H	2.1 +/- 0.1	7.1 +/- 0.4	4.6
17	H	H	Cl	159 +/- 0.6	108 +/- 1.7	4.9
18	H	NHMe	Cl	6.5 +/- 0.1	2.2 +/- 0.1	4.8
19	H		Cl	1.4 +/- 0.7	4.4 +/- 0.6	4.5
20	H		Cl	7.7 +/- 0.3	12.4 +/- 0.5	4.4

Table 3. Pyrazolopyrimidine and Purine SAR with Enzyme IC_{50}s

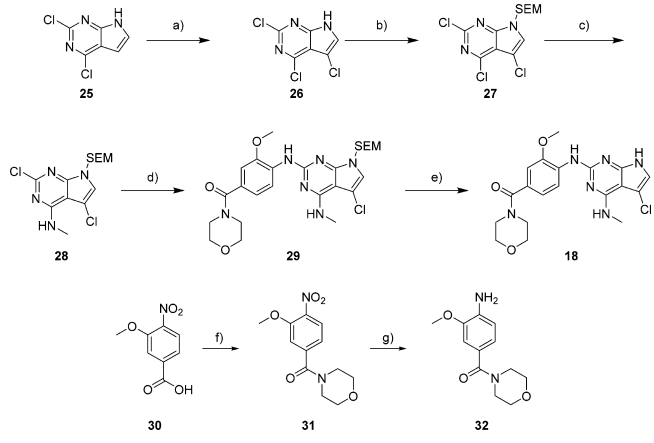


compd	R_1	R_2	R_3	X	Y	Enzyme IC_{50} WT nM	Enzyme IC_{50} G2019S nM	MPO Score
21	H	NHMe		N	CH	35 ± 0.6	44 ± 0.2	4.4
22	H	OMe		N	CH	3 ± 0.4	3 ± 0.6	4.7
23	H	NHMe	H	CH	N	11 ± 0.1	4 ± 0.3	4.3
24	H	OMe	H	CH	N	19 ± 2.1	18 ± 1.6	4.6

wild-type and G2019S enzymatic assays than the corresponding methylamino substituted compound **21**, which was the opposite of what was observed in the pyrrolopyrimidine series. We then turned our attention toward pyrazolopyrimidine analogues and made similarly substituted compounds **23** and **24**. In this case, the methylamino substituted compound was more potent than the corresponding methoxy substituted compound.

Compound **18** was prepared from commercially available 2,4-dichloropyrrolopyrimidine and 3-methoxy-4-nitrobenzoic acid (Scheme 1). 3-Methoxy-4-nitrobenzoic acid **30** was subjected to chlorination with thionyl chloride, followed by reaction with morpholine to generate the corresponding amide **31**, which was reduced by hydrogenation to yield aniline **32**. 2,4-Dichloropyrrolopyrimidine **25** was chlorinated using *N*-chlorosuccinimide (NCS) followed by 2-(trimethylsilyl)ethoxymethyl (SEM) protection to give the SEM-protected trichloropyrrolopyrimidine **27**. Compound **27** was regioselectively aminated with methylamine to afford the 2,5-dichloro-*N*-methylpyrrolopyrimidine-4-amine **28.** Compound **28** was aminated with aniline **32** under Buchwald coupling conditions followed by removal of the SEM group to furnish the desired compound **18**. Pyrazolopyrimidine and purine analogues were prepared in a similar manner starting from the corresponding dichloropyrazolopyrimidine or dichloropurine.

Scheme 1. Synthesis of JH-II-127[a]



[a]Reagents and conditions: (a) NCS, THF/DCM, 90 °C, 2.5 h, 93%; (b) NaH, SEMCl, 0 °C to RT 3 h, 90%; (c) 33% $MeNH_2$ in EtOH, EtOH, 70 °C 1 h, 93%; (d) **32**, $Pd_2(dba)_3$, XPhos, K_2CO_3, *sec*-BuOH 90 °C, 6 h; (e) (i) TFA, DCM RT, 2 h, (ii) $NaHCO_3$, THF, H_2O, 12 h, 58%; (f) (i) toluene, thionyl chloride, 120 °C, 2 h, (ii) DIEA, morpholine, THF, 0 °C to RT, 1 h, 92%; (g) 10% Pd/C, MeOH, RT, 12 h, 98%.

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A molecular docking study was then pursued based on a crystal structure of Roco kinase (PDB accession code: 4F1T), which revealed three hydrogen bonds to the hinge region between backbone M1949, A1950, and the pyrrolopyrimidine. Additionally, the docking study suggests the possibility of a halogen interaction[26] with M1947 and the chlorine at the 5-position of the pyrrolopyrimidine as shown in Figure 2.

We next examined the ability of the bicyclic compounds that showed the greatest potency in the enzymatic assay to inhibit LRRK2 in a cellular context in comparison to LRRK2-IN-1 (**1**). At the time this study was undertaken, there were no validated direct phosphorylation substrates of LRRK2; therefore, we monitored phosphorylation of Ser910 and Ser935, two residues whose phosphorylation is known to be dependent upon LRRK2 kinase activity[27] (Figure 3). Compound **18** was chosen as the lead

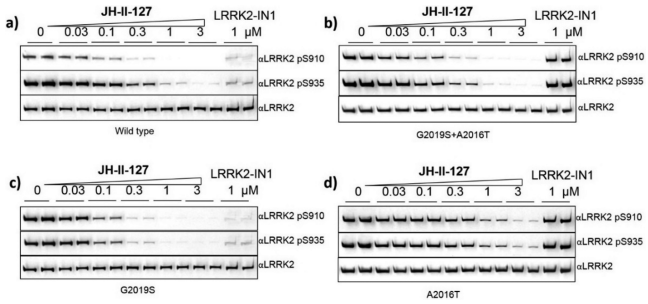

Figure 3. JH-II-127 (**18**) inhibits LRRK2 in cells. HEK293 cells stably expressing (a) wild-type GFP-LRRK2, (b) GFP-LRRK2[G2019S], (c) GFP-LRRK2[G2019S + A2016T], and (d) GFP-LRRK2[A2016T] were treated with DMSO or increasing concentrations of **18** for 90 min (1 μM LRRK2-IN-1 was used as a control). Cell lysates were subjected to immunoblotting for detection of LRRK2 phosphorylated at Ser910 and Ser935 and for total LRRK2.

compound due to the combination of potency and high MPO score.[28] Compound **18** induced a dose-dependent inhibition of Ser910 and Ser935 phosphorylation in both wild-type LRRK2 and LRRK2[G2019S] stably transfected into HEK293 cells (Figure 3a). Substantial dephosphorylation of Ser910 and Ser935 was observed at approximately 0.3 μM concentration of **18** for wild-type LRRK2 and LRRK2[G2019S] (Figure 3b), which is a similar potency to that observed for LRRK2-IN-1 (**1**). Consistent with the biochemical results, **18** also induced dephosphorylation of Ser910 and Ser935 at a concentration of 0.3−1 μM in the drug-resistant LRRK2[A2016T + G2019S] and LRRK2[A2016T] mutants, revealing that the A2016T mutation does not induce resistance to **18**.

We next examined the effect of **18** on endogenously expressed LRRK2 in human lymphoblastoid cells derived from a control and Parkinson's patient homozygous for the LRRK2[G2019S] mutation (Figure 4a). We found that increasing doses of **18** led to similar dephosphorylation of endogenous LRRK2 at Ser910 and Ser935, as was observed in HEK293 cells stably expressing wild-type LRRK2 or LRRK2[G2019S] (compare Figure 3a to Figure 4a). Moreover, endogenous LRRK2 was also more sensitive to **18** than LRRK2-IN-1 (**1**), which is consistent with the trend we observed in HEK293 cells. We also found that **18** induced similar dose-dependent Ser935 dephosphorylation of endogenous LRRK2 in mouse Swiss 3T3 cells (Figure 4b). IC_{50}s were calculated for **18** against wild-type LRRK2, G2019S, A2016T, and G2019S + A2016T mutants, which showed that compound **18** had an increase in potency against all of the

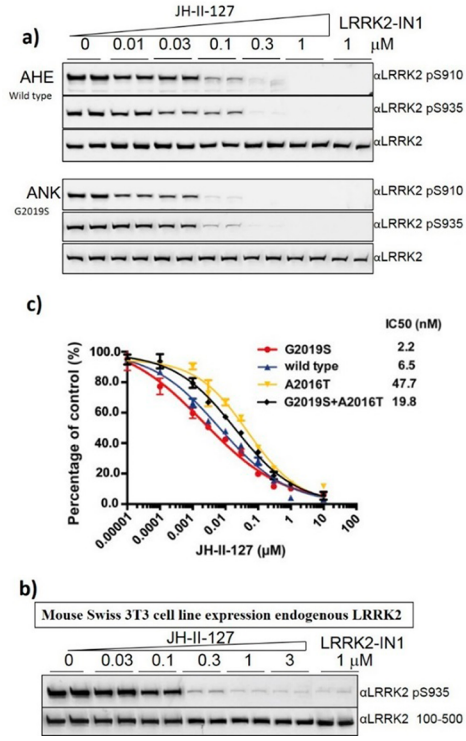

Figure 4. JH-II-127 (**18**) inhibits endogenously expressed LRRK2. (a) Endogenous LRRK2 from EBV immortalized human lymphoblastoid cells from a control subject and a Parkinson's disease patient homozygous for the LRRK2[G2019S] mutation. After treatment of the cells with DMSO or the indicated concentration of JH-II-127 (**18**) (or LRRK2-IN-1 (**1**)) for 90 min, cell lysates were subjected to immunoblot analysis with the indicated antibody for Western analysis. Immunoblots were performed in duplicate, and results were representative of at least two independent experiments. (b) Same as that in panel a except mouse Swiss 3T3 cells were used. (c) Enzyme activity of JH-II-127. GST-LRRK2(1326−2517), GST-LRRK2[G2019S](1326−2517), GST-LRRK2[A2016T](1326−2517), and GST-LRRK2[G2019S + A2016T](1326−2517) were assayed using 20 μM Nictide in the presence of 100 μM ATP. Results are the average of duplicate experiments.

mutants compared to previous compounds shown in Figure 1 (Figure 4c).

The mouse pharmacokinetic profile of **18** demonstrated good oral bioavailability (116% *F*), a half-life of 0.66 h, and a plasma exposure of 3094 (h·ng/mL, AUC_{last}) following 10 mg/kg p.o. dosing (Table 4). Additionally, following 2 mg/kg i.v. dosing, **18** showed a plasma exposure of 533 (h·ng/mL, AUC_{last}) and a brain exposure of 239 (h·ng/mL, AUC_{last}), which equates to a brain/plasma concentration ratio of 0.45. We then compared the pharmacodynamic properties of **18** with GNE7915 (**6**) by monitoring inhibition of LRRK2 Ser910/Ser935 phosphorylation in kidney, spleen, and brain following intraperitoneal delivery of each compound at 100 mg/kg (Figure 5). We observed near complete dephosphorylation of Ser935 of LRRK2 in all tissues including brain at this dose for both compounds. We then repeated the study at lower doses of 50, 30, and 10 mg/kg of **18** and **6**. With **18**, we observed near complete inhibition in all tissues at 30 mg/kg but only partial inhibition in brain at the 10 mg/kg dose. However, with **6**, complete inhibition in brain was only observed at 100 mg/kg. These results indicate that **18** is a promising chemotype for achieving dephosphorylation of Ser935 in the brain. Interestingly, a recent study found that kinase

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Table 4. Pharmacokinetic Parameters for JH-II-127 (18)

matrix	route	dose (mg/kg)	T_{max} (h)	C_0/C_{max} [a] (ng/mL)	AUC_{last} [b] (h·ng/mL)	AUG_{INF} (h·ng/mL)	$T_{1/2}$ (h)	CL (mL/min/kg)	V_{ss} (L/kg)
plasma	i.v.	2		1604.47	532.67	535.57	0.66	62.24	1.73
	p.o.	10	1	802.72	3094.58	3867.07			
brain	i.v.	2		1343.6	239.31	246.47	0.23	135.24	1.7
	p.o.	10	1	247.35	688.21	762.38			

[a] C_0, back extrapolated conc. for i.v. group. [b] AUC_{last} was considered for calculating bioavailability.

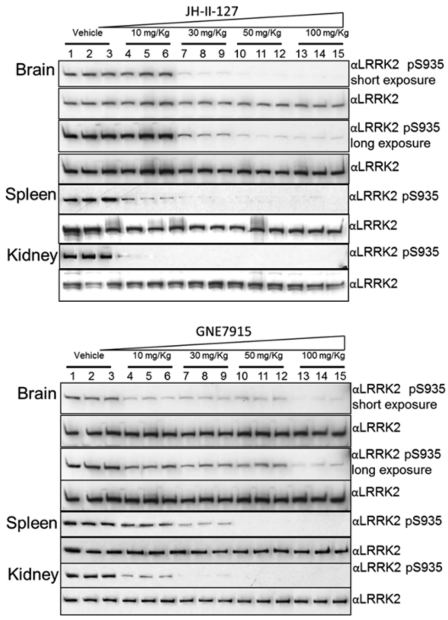

Figure 5. Pharmacodynamic analysis for JH-II-127 (**18**) and GNE7915. Pharmacodynamic study of JH-II-127 (**18**) and GNE7915 from brain, spleen, and kidney following oral gavage administration at the indicated doses. Tissues were collected, and endogenous LRRK2 was resolved by SDS-PAGE and blotted with a phospho-specific antibody directed against Ser935 and total LRRK2.

inhibition reduced total LRRK2 expression causing a phenotype similar to LRRK2 KO mice;[29] however, neither of these compounds reduced total LRRK2 expression. Additionally, pharmacodynamic studies using our previously reported inhibitor, HG-10-102-01, did not reveal a reduction in total LRRK2 expression even after 6 h (see Supporting Information)

The kinase selectivity of **18** was assessed using standard radioactivity-base enzymatic assays against a panel of 138 kinases (Dundee profiling).[30] At a concentration of 1 μM, compound **18** only inhibited the kinase activities of SmMLCK and CHK2 to greater than 90% of the DMSO control (complete results presented in the Supporting Information). Dose−response analysis revealed inhibition of SmMLCK with an IC_{50} = 81.3 nM and CHK2 with an IC_{50} = 27.6 nM. KinomeScan analysis against a near comprehensive panel of 451 kinases at a concentration of 1 μM resulted in no interactions with kinases other than LRRK2[G2019S] with the exception of TTK and RPS6KA4, demonstrating the outstanding selectivity of this inhibitor (complete profiling results provided in the Supporting Information).[31]

These results suggest that **18** is a highly selective LRRK2 inhibitor; however, further profiling of additional kinases and other ATP-dependent enzymes is still warranted.

In summary, we have discovered that **18** is a potent biochemical and cellular inhibitor of LRRK2 kinase activity. Detailed characterization of **18** using LRRK2-IN-1 as a benchmark revealed that **18** significantly inhibited phosphorylation of wild-type LRRK2 and LRRK2[G2019S] mutant at Ser910 and Ser935 at 0.3−1.0 μM in cell culture, which is approximately the same potency as LRRK2-IN-1 (**1**). Compound **18** is relatively insensitive to the A2016T mutation, which suggests that this mutant will not be useful to validate whether the pharmacological effects of the compound are LRRK2-dependent. Compound **18** exhibits excellent kinase selectivity as assessed by recombinant kinases (138) and KinomeScan (451 kinases). Compound **18** can inhibit phosphorylation of Ser935 of LRRK2 in brain and peripheral tissues following intraperitoneal doses of 30 mg/kg. Further optimization of this chemotype especially in regards to *in vivo* half-life will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, compound characterization, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

[‖]These authors contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

LRRK2, leucine-rich repeat kinase 2; PD, Parkinson's disease; PK, pharmacokinetics; *F*, bioavailability; AUC, area under the concentration time curve; CL, clearance; C_{last}, last measured concentration; C_{max}, maximum concentration observed; $T_{1/2}$, elimination half-life; V_{ss}, volume in steady state

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