Potent, selective and orally bioavailable Leucine-Rich Repeat Kinase 2 (LRRK2) Inhibitors

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Graphical Abstract

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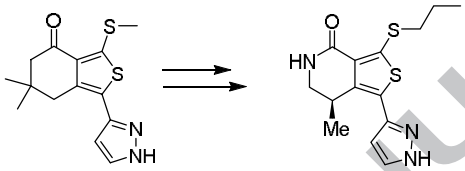

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ABSTRACT

Familial Parkinson's disease cases have recently been associated with the leucine rich repeat kinase 2 (LRRK2) gene. It has been hypothesized that inhibition of the LRRK2 protein may have the potential to alter disease pathogenesis. A dihydrobenzothiophene series of potent, selective, orally bioavailable LRRK2 inhibitors were identified from a high-throughput screen of the internal Merck sample collection. Initial SAR studies around the core established the series as a tractable small molecule lead series of LRRK2 inhibitors for potential treatment of Parkinson's disease. It was also found that incorporation of a lactam into the core drastically improved the CNS and DMPK properties of these small molecules.

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by the onset and gradual worsening of cardinal motor symptoms. Motor symptoms are attributed to the primary pathological hallmark of the disease, the loss of nigrostriatal dopamine neurons. There are several non-motor symptoms associated with the disease, as well as extra-nigral pathology, however, diagnosis is still made based on presence of motor symptoms and responsiveness to dopamine replacement therapies. There are no effective therapies that prevent neuron cell death and symptomatic treatments only partially ameliorate motor symptoms. Disease risk increases substantially with advancing age[1] and, with the aging of Western populations, incidence and prevalence rates are projected to increase.[2] These circumstances highlight the need to better understand disease pathogenesis and identify effective therapeutic strategies to lessen the impact of this disease.

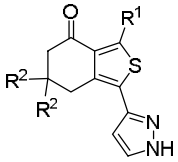
Though most PD cases are idiopathic, meta-analyses of genome-wide association studies of Parkinson's patients and controls in the United States and Europe identified 11 genetic loci associated with increasing PD risk. Mapping of these loci identified variants in several genes including α-synuclein, microtubule-associated protein Tau, and leucine rich repeat kinase 2 (LRRK2) that increased disease risk.[3] LRRK2 mutations are associated with both familial and sporadic disease and are the most common known cause of inheritable PD.[4] LRRK2 PD pathology is nearly indistinguishable from sporadic disease with respect to age of onset, motor symptoms and responsiveness to dopamine replacement therapies.[5] LRRK2 may therefore play a broad role in disease pathogenesis, and therapeutic intervention is likely to impact both familial and sporadic disease.

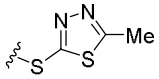
LRRK2 is a large (268 kDa) multidomain protein consisting of: N-terminal ankyrin repeats, a leucine-rich repeat region, the catalytic core comprised of a GTPase ROC (Ras of Complex) and COR (C-terminus Of ROC) domains, the serine/threonine kinase domain, and a C-terminal WD-40 domain. Over the last decade, several mutations have been identified throughout the LRRK2 gene. Six of these mutations (R1441G, R1441C, R1441H, Y1699C, G2019S, and I2020T) were deemed pathogenic,[5] and 5 of the 6 pathogenic mutations occur within the enzymatic cores of the protein. Additionally, the LRRK2 triple mutation N551K-R1398H-K1423K was identified as decreasing disease risk.[6] Together, these data support the hypothesis that pharmacological mainpulation of LRRK2 enzymatic activity can be of therapeutic value in treating PD.

The kinase domain of LRRK2 is of particular interest, as it represents a classically druggable target. There has been considerable success in developing relatively selective LRRK2 kinase inhibitors, and recently some of these have been publically disclosed.[7-17] Continued discovery of selective LRRK2 kinase inhibitors with "drug-like" properties is critical to enabling the long-term safety and efficacy studies needed to fully interrogate the therapeutic potential of this important target. To this end, we carried out a screen of the Merck sample collection using recombinant wt LRRK2 in a binding assay.[18] This led to identification of the potent LRRK2 inhibitor thiophene **3a** (Table

1) which showed inhibition of both wt LRRK2 and the G2019S mutant with K_i = 377 and 234 nM, respectively. The dihydrobenzothiophene series initially drew our attention as a result of its limited activity in other high-throughput screening (HTS) campaigns. For example, compound **3a** had been assayed in 274 HTS campaigns and had not been confirmed to be active in any of these screens.[19] Recognizing that other HTS campaigns had been conducted for kinases prior to the LRRK2 screen, we were attracted to the dihydrobenzothiophenes for their lack of activity against other kinases. In order to enhance our understanding of the general kinase activity properties of this series, **5c** was screened against a panel of 192 kinases. As seen in the heat map in Figure 1, minimal off-target activity was observed, further solidifying the selectivity profile of this series.

Table 1.



Compound	R^1	R^2	LRRK2 wt K_i (nM)	LRRK2 G2019S K_i (nM)
3a	SMe	Me	377	234
4a	SO_2Me	Me	7130	2793
5a	S(2-propyl)	Me	256	137
5b	SEt	Me	156	177
5c	S(cyclopentyl)	Me	389	94
5d	SCH_2(cyclopropyl)	Me	172	133
5e	SBn	Me	>10 uM	863
5f	SCH_2CH_2Ph	Me	1892	454
5g	SCH_2CH_2OH	Me	515	240
5h		Me	993	3994
3b	SMe	H	312	189
5i	S(cyclopentyl)	H	295	432
5j	NH(cyclopentyl)	H	>10 uM	160
5k	NH(cyclopentyl)	Me	2794	97
5l	O(cyclopentyl)	Me	475	301
5m	Propyl	Me	5293	2242

Compound **5c** was shown to have very high clearance in rats (Cl = 96 mL/min/kg) and did not achieve good CNS penetration with a measured CSF level of 10 nM at 1 h after systemic injection (5 mpk IP), demonstrating the need for optimization of its DMPK properties. This result is not entirely unexpected, as **5c** has a rather high cLogP value of 5.5 which is a key contributing factor to a poor CNS MPO score[20] of 3.8. High plasma protein binding (99.3%) is likely responsible for the low observed CNS exposure, as **5c** was not a substrate for P-glycoprotein, one of the major efflux pumps in the CNS (BA/AB Ratio = 0.6).

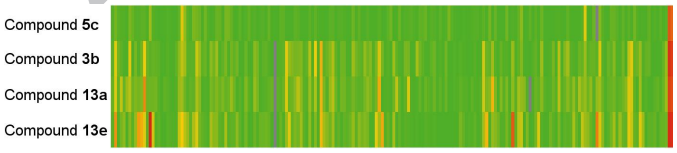
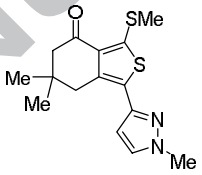
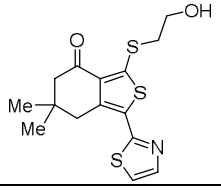


Figure 1. Heat map of kinase inhibition (reported as percent inhibition) across a panel of 192 kinases at a testing concentration of 10 μM. Red = 100% inhibition, yellow = 50% inhibition, green = 0% inhibition, gray = not available. Note: LRRK2 wt and G2019S on far right.

Initial SAR studies around **3a** showed that the oxidation state of the sulfur was important for activity as evidenced by the reduction in potency of sulfone **4a** (Table 1). Alkyl substituents on the sulfur were well-tolerated (**5a**–**5d**), as was an hydroxy ethyl substituent (**5g**). Aromatic residues including benzyl (**5e**), phenethyl (**5f**) and thiadiazole (**5h**) led to a reduction of LRRK2 activity. The gem-dimethyl group seemed to have little effect, see compound **3a** vs. **3b** and **5c** vs. **5i**. We then explored the requirement of a sulfur atom at R_1. Interestingly, a nitrogen substituent at R_1 (**5j** and **5k**) gave a large difference in activity between wt and mutant LRRK2 enzymes and were in fact equipotent to **5c** against the G2019S mutant, while significantly less potent against wt. An O linker (**5l**) was moderately potent, whereas the all carbon case led to poor activity (**5m**). The unsubstituted pyrazole substituent was found to be important for maintaining LRRK2 binding affinity (Table 2); N-methylation of the pyrazole led to an inactive compound (**6**) and replacement with a thiazole (**7**) also lowered activity compared to **5g**. The SAR around the pyrazole group, together with induced fit docking results based on a homology model of LRRK2, led us to conjecture that the pyrazole was involved in two hydrogen bonds with the backbone atoms of hinge residues Glu-1948 and Ala-1950 (Figure 2a).

Table 2.

Compound	Structure	LRRK2 wt K_i (nM)	LRRK2 G2019S K_i (nM)
6		5583	>10,000
7		nd	5464

The synthesis of keto-thiophenes **5a-m** commenced with thiocarbonylation of 1,3-cyclohexanedione **1** with carbon disulfide, followed by alkylation of the resultant dithioic acid with chloroacetone to produce a dithioester intermediate (Scheme 1).[21,22] Trapping of the dithioester *in situ* with MeI effected a condensation/aromatization sequence that smoothly provided the desired thiophenes **2** in good yields. Subsequent treatment of ketone **2** with DMF-DMA followed by hydrazine-mediated cyclization of the resultant vinylogous amide afforded pyrazoles **3**. The thiomethyl ether of **3** can be readily displaced to introduce alternative functionality by straighforward oxidation to the sulfone **4** with *m*CPBA followed by SnAr reaction with a corresponding nucleophile to give rise to the desired keto-thiophenes **5a-m**.

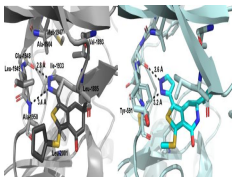
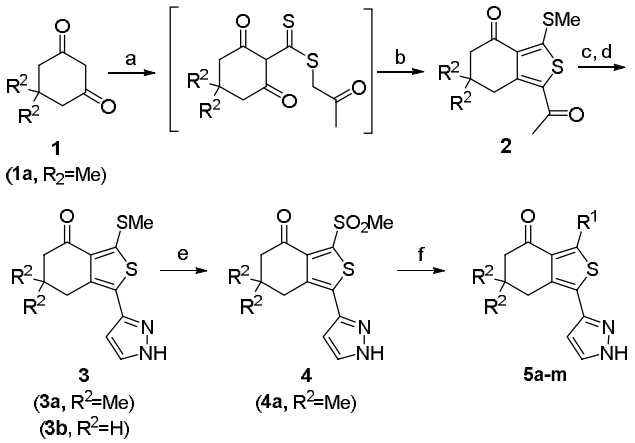


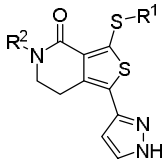
Figure 2. (a) Induced fit docking pose for compound **5c** in a LRRK2 homology model based on the TAK1 crystal structure (PDB ID 2EVA). Schrödinger's induced fit docking protocol was used to generate this pose. (b) Crystal structure of compound **13e** in TrkA (PDB code: 5I8A).[23]



Scheme 1. Synthesis. Reagents and conditions: (a) K_2CO_3, CS_2, DMF; $ClCH_2COCH_3$ (b) MeI; (c) DMF-DMA; (d) N_2H_4, EtOH; (e) mCPBA, CH_2Cl_2; (f) i. R^1SH, NaH, THF, 0 °C – rt; ii. R^1NH_2, DMSO, 100 °C; iii. R^1OH, NaH, THF, 60 °C; iv. R^1MgCl, CuBr-DMS, TMSCl, THF, -78 °C.

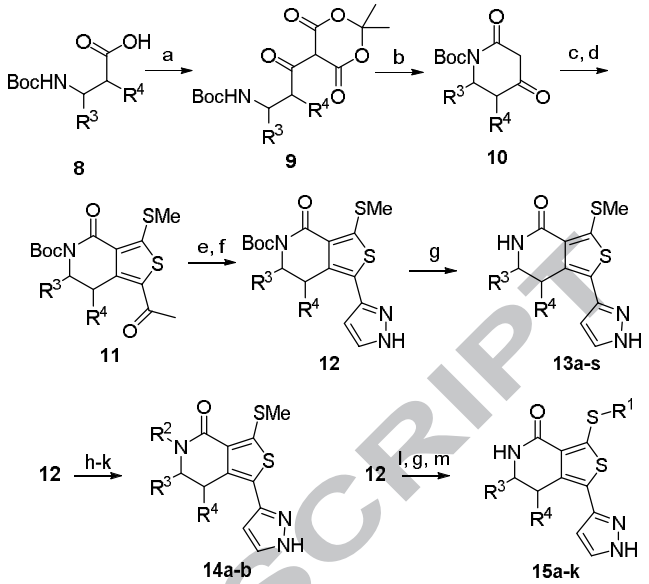
We then explored the possibility of replacing the cyclohexanone with a lactam as shown in Table 3. Compound **13a**, the direct lactam analog of **3c**, did not show very good activity, but larger alkyl substituents (**15a**–**15f**) were tolerated in this series. As in the cyclohexanone case, aromatic groups at R^1 led to a loss of potency (**15g**). Furthermore, substituents on the amide nitrogen were not tolerated (**14a** and **14b**).

Table 3.



Compound	R^1	R^2	LRRK2 wt K_i (nM)	LRRK2 G2019S K_i (nM)
13a	Me	H	3407	776
15a	Et	H	589	348
15b	1-propyl	H	572	355
15c	2-propyl	H	988	557
15d	cyclopentyl	H	459	286
15e	cyclohexyl	H	919	372
15f	CH_2(cyclopropyl)	H	614	305
15g	Ph	H	4458	1598
14a	Me	Me	5566	2535
14b	Me	Ph	4706	2219

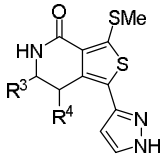
The requisite dioxopiperidines **10** for the preparation of lactam thiophenes **13a-s**, **14a-b**, **15a-k** (Scheme 2) were prepared as follows in a two-step sequence from various N-Boc-protected β-amino acids **8**.[22] EDC coupling of Meldrum's acid to β-amino acid **8** afforded tricarbonyl **9** which, upon subjection to thermolysis in ethyl acetate, the intermediate ketene produced was intramolecularly trapped by the Boc-amine to smoothly provide lactams **10** in good yields. Similarly to Scheme 1, dicarbonyls **10** were transformed to amido-thiophenes **11** via cyclization of the corresponding dithioester intermediate upon treatment with MeI. Subsequently, the pyrazole was introduced via standard vinylogous amide formation of the acetyl group of **11**, followed by cyclization with hydrazine to provide lactam **12**.



Scheme 2. Synthesis. Reagents and conditions: (a) Meldrum's Acid, EDC, DMAP, CH_2Cl_2; (b) EtOAc, reflux; (c) $ClCH_2COCH_3$, Cs_2CO_3, DMF; CS_2; (d) MeI; (e) DMF-DMA; (f) N_2H_4, EtOH; (g) TFA, CH_2Cl_2; (h) SEMCl, NaH, CH_2Cl_2; (i) TFA; (j) R^2I, NaH, DMF; (k) HCl, MeOH; (l) mCPBA, CH_2Cl_2; (m) R^1SH, NaH, THF, 0 °C – rt.

Thiophene **12** proved to be a useful intermediate to probe the SAR of the lactam series. Final compounds were prepared from **12** via the following three synthetic methods (Scheme 2): 1) Simple deprotection of the Boc group with TFA afforded lactams **13a-s**; 2) Protecting group manipulations of **12**, which included SEM protection of the pyrazole (NaH, SEMCl), followed by deprotection of the Boc group with TFA, allowed for selective functionalization of the lactam nitrogen. Alkylation of the lactam with alkyl halides, followed by subsequent deprotection of the SEM with HCl/MeOH afforded the desired tertiary lactams **14a-b**; and 3) Oxidation of the thiomethyl ether to the sulfone with *m*CPBA and deprotection of the Boc group under standard conditions produced the SnAr substrate, which was readily displaced with thiol nucleophiles to provide the lactams **15a-k** in excellent yields (NaH, RSH).[22]

Table 4.



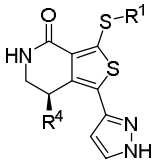
Compound	R^3	R^4	LRRK2 wt K_i (nM)	LRRK2 G2019S K_i (nM)
13b	Me *(rac)*	H	1238	561
13c	Bn *(rac)*	H	928	3564
13d	CF_3 *(rac)*	H	501	198
13e	Ph *(R)*	H	174	64
13f	Ph *(S)*	H	120	53
13g	3-F-Ph *(rac)*	H	3293	1640
13h	4-F-Ph *(rac)*	H	271	92
13i	3-pyridyl *(S)*	H	>10 uM	4226
13j	CH_2OMe *(rac)*	H		341
13k	cyclobutyl *(rac)*	H	1045	293
13l	spiro-cyclopropyl	H	>10 uM	1650
13m	spiro-cyclobutyl	H	233	143
13n	spiro-cyclopentyl	H	411	225
13o	H	Me *(R)*	319	186
13p	H	Me *(S)*	603	367

13q	H	Et *(R)*	82	46
13r	H	Et *(S)*	1443	700
13s	H	Bn *(S)*	342	159

These synthetic routes to the lactam compounds gave us the opportunity to access both the 6- and 7- positions of the bicyclic ring system for substitution by using readily available β-amino acid starting materials. Substitution at R^3 (Table 4) generally resulted in improvements in potency over the parent compound **13a** (see **13b** – **13n**). Specifically, introduction of phenyl and 4-fluorophenyl provided a 10-fold boost (**13e**, **13f**, **13h**). Smaller substitution, such as methyl and trifluoromethyl did not have as drastic effect, but were tolerated. Interestingly, spirocyclic substitution at C-6 resulted in potency increases in the spirocyclobutyl (**13m**) and spirocyclopentyl (**13n**) cases, but not with the spirocyclopropyl analogue **13l**.

In addition, it was found that substitution at the C-7 position of the lactam resulted in improvements in potency as seen with the methyl and ethyl analogues **13o**, **13p**, and **13q**. We next looked to see if SAR was additive in the lactam series by exploring thioether substituents as we had in the cyclohexanone series. Combining the optimal R^4 substituents *R*-Me and *R*-Et with two of our known potency enhancing S-alkyl substituents led to compounds **15h**–**15k** (Table 5), each of which showed <100 nM activity against both the wt and G2019S mutant LRRK2 variants.

Table 5.



Compound	R^1	R^4	LRRK2 wt K_i (nM)	LRRK2 G2019S K_i (nM)
15h	1-propyl	Me	84	39
15i	CH_2(cyclopropyl)	Me	83	34
15j	1-propyl	Et	68	30
15k	CH_2(cyclopropyl)	Et	58	28

The lactam series generally showed improved cLogP values and CNS MPO scores, with clogP being ~1 unit lower and the CNS MPO score being ~0.5 higher for paired molecules. Experimentally, these compounds also demonstrated improved *in vivo* intrinsic clearance values (Cl_{int}) versus the cyclohexanones. While the rat *in vivo* Cl_{int} values of the cyclohexanones were high (undefined for **5c**, 6,500 mL/min/kg for **3b**), the lactam series compounds **13a**, **13e**, and **15h** demonstrated improved values (1,300, 3,300, and 1,200 mL/min/kg, respectively).[24] The improved Cl_{int} values for the lactam series seems to correlate well with the higher observed bioavailabilities (%F) with PO dosing and improved CSF levels following IP dosing (Table 6). Compounds in the cyclohexanone series (**5c** and **3b**) showed relatively poor rat PK with high clearance and low bioavailability. The lactam series, on the other hand, gave improved rat PK with compound **15h** showing 98% bioavailability and low to moderate clearance.

In order to improve our structural understanding of this series, we considered employing our in-house TrkA crystallography effort as a surrogate for LRRK-2. To enable this, we submitted several dihydrobenzothiophenes to our in-house TrkA Caliper assay despite our selectivity panel data which indicated low activity against the Trk kinases. Our rationale for doing this was that the TrkA crystal system had already demonstrated its ability to structurally characterize weakly active compounds, and some inhibition observed in the Caliper assay in a full dose response might enable the use of TrkA as a surrogate for LRRK-2. Indeed, compound **13e** was shown to be ~8 uM vs. TrkA in the Caliper assay, so we leveraged our in-house TrkA crystal system to solve the structure of **13e** with TrkA (Figure 2b, PDB code: 5I8A).[23] This structure confirmed that our hypothesis about the binding mode of this series in LRRK-2 was reasonable, although it does not provide definitive proof that this mode is in fact correct for the LRRK-2 protein.

Nevertheless, what is immediately obvious from the TrkA structure, which is in good agreement with our structural hypothesis derived from induced fit docking to a homology model of LRRK-2, is that the gatekeeper+2 residue (G+2) in TrkA (Tyr-591) is in close proximity to the SMe group in **13e** (Figure 2a). The G+2 residue in LRRK-2 is Leu-1949 whereas most kinases have a larger residue at this position (Figure 3), so LRRK-2 can be expected to accommodate larger substitutions in the region of the SMe of **13e**, for instance the cyclopentyl of **5c**, with minimal loss of activity.

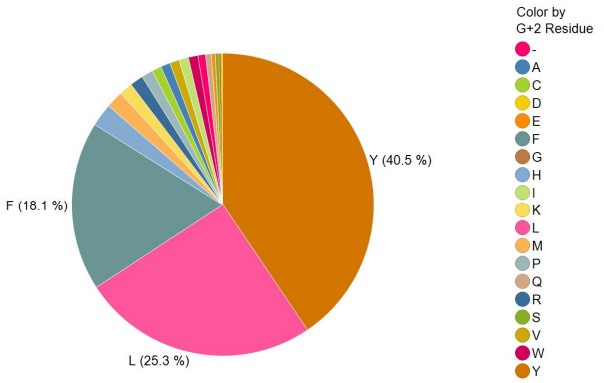


Figure 3. Pie chart showing the frequency of residue identity at the G+2 position. The kinase sequence alignment used was taken from www.kinase.com.

Indeed, of the compounds submitted for selectivity data **5c** showed the least inhibition of off-target kinases (Figure 1: top to bottom: **5c**, **3b**, **13a**, **13e**), consistent with the idea that larger substitutions in this region of the protein create destabilizing interactions with most kinases due to unfavorable interactions with larger residues that are homologous to Leu-1949 in LRRK-2 (e.g. Tyr-591 in TrkA). For instance, at a testing concentration of 1 uM, compounds **3b**, **13a**, and **13e** all inhibit Aurora A by ~40-65%, Aurora B by ~55%, and CHK2 by ~45-70%, whereas **5c** inhibits each of these proteins by <~10%. Aurora A and Aurora B both have Tyr at the G+2 position, supporting the hypothesis that the size of the G+2 residue and the substitution pattern of the inhibitor play an important role in the selectivity profile of our compounds, although CHK2 has Leu at this position so clearly other factors are at work as well.

Table 6. Rat pharmacokinetic parameters

Compound	Cl[a] (mL/min/kg)	$T_{1/2}$[a] (h)	%F	PO[b] C_{max} (μM)	PO[b] AUC (μM*h)	IP[c] [Plasma] (μM)	IP[c] [CSF] (μM)
5c	95.5	1.2	0.2	0.12	0.01	0.97 @ 1 h	0.01 @ 1 h
3b	56.0	6.5	33	1.6	3.8	2.33 @ 1 h	0.05 @ 1 h
13a	47.5	1.4	70	4.8	14.0	28.3 @ 1 h	0.50 @ 1 h
13e	22.0	4.3	84	5.1	19.7	5.65 @ 1 h	0.07 @ 1 h
15h	11.9	3.6	98	16.0	46.1	24.4 @ 1 h	0.15 @ 1 h

[a]IV: 2 mg/kg, n = 2, 50%PEG400/50%DMSO or 60%PEG200/30%DMA/10%water

[b]PO: 10 mg/kg, n = 2, PEG400; [c]IP: 5 mg/kg, n = 3, PEG400

In conclusion, a dihydrobenzothiophene series of potent, selective, orally bioavailable LRRK2 inhibitors were identified from a high throughput screen of the Merck sample collection. Initial SAR studies around the core established the series as a tractable small molecule lead series of LRRK2 inhibitors for potential treatment of Parkinson's disease. It was also found that incorporation of a lactam into the core drastically improved the CNS and DMPK properties of these small molecules. Further efforts to optimize the overall drug-like profile are ongoing and will be reported in due course.

References and notes

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