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Discovery of novel indolinone-based, potent, selective and brain penetrant inhibitors of LRRK2

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ABSTRACT

Mutations in leucine-rich repeat kinase-2 (LRRK2) are the most common genetic cause of Parkinson's disease (PD). The most frequent kinase-enhancing mutation is the G2019S residing in the kinase activation domain. This opens up a promising therapeutic avenue for drug discovery targeting the kinase activity of LRRK2 in PD. Several LRRK2 inhibitors have been reported to date. Here, we report a selective, brain penetrant LRRK2 inhibitor and demonstrate by a competition pulldown assay in vivo target engagement in mice.

Parkinson's disease (PD) is the second most common progressive neurodegenerative movement disorder affecting 1% of people over the age of 60. Currently, no disease-modifying therapies are available. Human genetics have identified manifold causes of PD that have opened up new possibilities for therapeutic targets. Mutations in leucine-rich repeat kinase-2 (LRRK2) cause late-onset autosomal dominant PD that is clinically indistinguishable from idiopathic PD and account for approx. 5% of familial and 1–2% of sporadic PD.[1–8]

LRRK2 is a large multi-domain protein containing several potential protein–protein interaction domains, as well as a GTPase and a serine/threonine kinase domain. The most prominent mutation associated with PD resides in the activation loop of the catalytic kinase domain and encodes for a glycine-to-serine substitution (G2019S). Several studies have shown that this mutation increases kinase activity[9,10] and induces toxicity,[11–13] hence opening the possibility for therapeutic kinase inhibition. Several putative substrates have been put forward,[14–17] but a robust physiological substrate for LRRK2 is still missing.[18–20] In addition, despite the genetic link for the causal role of LRRK2 mutations in late-onset PD the underlying patho-mechanism and molecular pathways are still uncertain and remain to be elucidated.[21] In the brain, LRRK2 expression is not restricted to the nigro-striatal pathway. Outside the nervous system, several peripheral organs and cell types express LRRK2 including kidney, lung and B cells suggesting that LRRK2 mutations may impact physiology and exert disease-relevant roles.[22–29]

Several phosphorylation sites in LRRK2 have been discovered and extensively analyzed, like the serine at position 935 and 910 before the LRR domain[30–37] and as well as others.[38,39] Importantly, all of these sites are sensitive to LRRK2 inhibitors and can thus be used to monitor LRRK2 activity. These discoveries are vital in drug discovery as they provide the basis for cellular assays and in vivo pharmacodynamics to find suitable candidates for further development. To date, several groups have reported potent and brain penetrant LRRK2 inhibitors.[30,39–44] Here, we report a LRRK2 inhibitor with high potency and good PK properties and demonstrate its target engagement in vivo.

The first low molecular weight compounds reported to inhibit LRRK2 kinase activity (mainly staurosporine-related compounds, maleimides or indolinones)[45,46] typically had low potency and selectivity, and were tool-like in structure. Improved second

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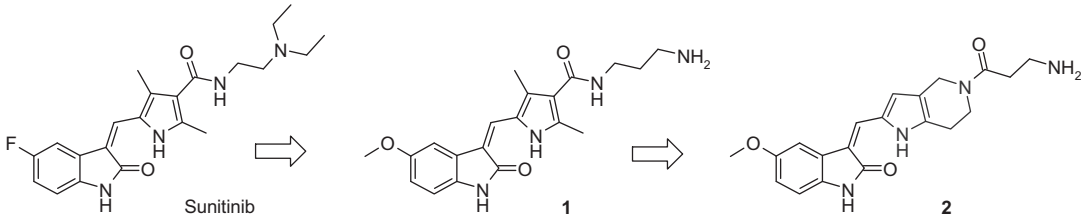


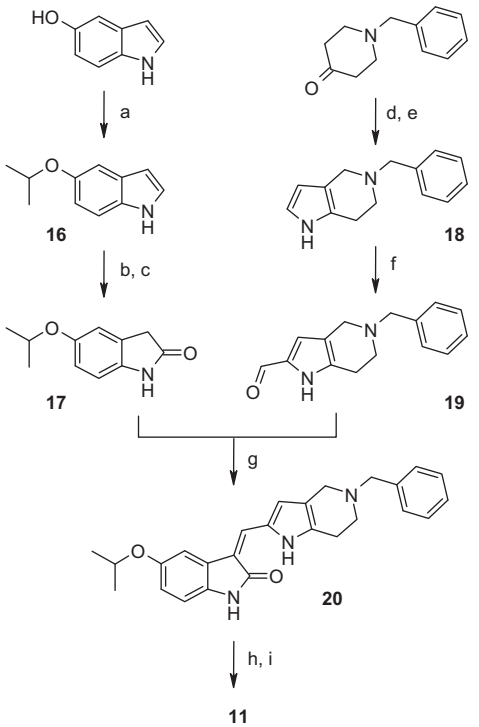
Figure 1. Discovery of 1,4,6,7-tetrahydro-pyrrolo[3,2-*c*]pyridine containing LRRK2 inhibitors.

generation inhibitors like the 2,4-diaminopyrimidines LRRK2-IN-1[47] or CZC-25146[48] showed excellent potency and selectivity for LRRK2, but lacked sufficient brain exposure to be used in rodent models of PD. In contrast to this, the dual ALK/LRRK2 inhibitor TAE684 has been shown to achieve significant mouse brain exposure, but failed to inhibit LRRK2 phosphorylation in the brain after oral dosing.[36] More recently, further optimized 2,4-diaminopyrimidines with improved brain penetration have been disclosed by several groups.[30,40,41,44] Some of them were shown to significantly reduce LRRK2 autophosphorylation in the brain of G2019S LRRK2 transgenic mice after oral dosing.[30,44] In addition to these 2,4-diaminopyrimidines based compounds, cinnolines,[42] triazolopyridazines[49] and 3-cyanoquinolines[43] have been published that potently inhibit wild-type and mutant LRRK2 in vitro.

Early on in our LRRK2 kinase inhibitors program, a medicinal chemistry effort was initiated with two main goals: (i) identification of a potent, reasonably selective LRRK2 kinase inhibitor with an attachment point (preferably a primary or secondary amine) remote from the kinase interaction part that allows for crosslinking to a solid support for pulldown experiments,[50] and (ii) identification of a proprietary starting point for a derivation program with the potential for high potency, selectivity and brain penetration. For the first goal, we decided to start from the indolinone Sunitinib, a broad-band kinase inhibitor that was found to also inhibit LRRK2[51] (Fig. 1). In our biochemical assay, Sunitinib inhibited LRRK2 kinase with an IC_{50} of 0.028 μM, and, as expected, was found to be highly unselective in a kinase selectivity panel (inhibition of 20 out of 54 kinases with an IC_{50} <1 μM). A limited derivation program around Sunitinib revealed that the ethylene linker at the amide can be extended in length, and the tertiary amine at its end be replaced with a primary amine without loss of activity in the biochemical assay. In addition, replacement of the fluorine in position 5 of the indolinone core with a methoxy considerably improved kinase selectivity, while retaining full activity on LRRK2. Hence, derivative **1** inhibited LRRK2 with an IC_{50} of 0.046 μM, and blocked only 5 out of 36 other kinases in the selectivity panel with an IC_{50} <1 μM. This cross-linkable compound could successfully be coupled to sepharose solid support (see Supplementary data), and turned out to be a highly versatile tool for pull-down experiments.

In order to obtain proprietary starting points for a drug development program, options were considered on how to morph the 2,4-dimethyl-3-carboxamide substituted pyrrole into a novel, hitherto unknown moiety. Docking studies of **1** in a LRRK2 homology model (vide infra) suggested that forming an additional ring between the 2-methyl group and the amide nitrogen, with concomitant inversion of the amide, should be tolerated by the kinase. Indeed, 4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine derivative **2**, the corresponding analog of **1**, showed an IC_{50} of 0.011 μM on LRRK2, nicely confirming this hypothesis. Although some of the kinase selectivity of **1** was lost by incorporation of this new moiety (20 out of 32 other kinases inhibited with IC_{50} <1 μM), it was decided, based on the high initial potency and the favorable IP position for this novel moiety, to initiate a derivation program around **2** with the main goals of improving kinase selectivity and demonstrating favorable PK properties, including brain penetration.

Derivatives **2**–**14** were prepared by condensation of an appropriately substituted indolinone (as exemplified by **17**) with the benzyl protected tetrahydropyrrolo-pyridine-2-carbaldehyde building block **19**, followed by debenzylation and introduction of the final substituent at the tetrahydropyridine nitrogen (e.g., acetyl for derivative **11**, Scheme 1). 5-substituted indolinones were either commercially available, or were prepared from the corresponding indole by a bromination/hydrolysis sequence (e.g., **16**–**17**, Scheme 1). Aldehyde building block **19** was prepared as described by Voskressensky et al.[52] from 1-benzyl-piperid-4-one oxime (KOH, acetylene gas, DMSO), followed by selective formylation in position 2 of the pyrrole derivative **18** ($POCl_3$, DMF/Et_2O) (Scheme 1). Derivative **7** bearing a chloro substituent in position 3 of the pyrrole moiety was prepared starting from **18** by de-benzylation (H_2, Pd/C), acetylation (AcCl, Et_3N, DCM), formylation in position 2 ($POCl_3$, DMF/Et_2O) and chlorination in position 3 (NCS, benzoylperoxide, CCl_4), followed by condensation with 5-methoxyindolinone (EtOH, cat. piperidine, reflux). Compound **15** (Fig. 2) carrying a methyl



Scheme 1. Synthesis of LRRK2 inhibitor **11**. Reagents and conditions: (a) *i*-PrOH, PPh_3, DIAD, THF, rt, 16 h (51%); (b) Br_2, DMF, 0°, 30′ (quant.); (c) phosphoric acid, $MeOCH_2CH_2OH$, 100°, 2 h (28%); (d) $NH_2OH{\cdot}HCl{\cdot}K_2CO_3$, EtOH, 80°, 1 h (98%); (e) acetylene gas, KOH, DMSO, 90°, 6 h (36%); (f) $POCl_3$, DMF, Et_2O, rt, 1.5 h (71%); (g) EtOH, cat. piperidine, 95°, 4 h (92%); (h) ammonium formate, Pd/C, MeOH, rflx, 2 h (97%); (i) ClCOMe, DIEA, DCM, rt, 1 h (75%).