Inhibitors of LRRK2 Kinase Activity To Probe the Treatment Option in Parkinson's Disease

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In the past decade, genetic variations in the leucine-rich repeat kinase 2 (LRRK2) gene have been linked to increased LRRK2 activity and risk for the development Parkinson's disease (PD).[1] In particular, the specific G2019S mutation of LRRK2 has been associated with both familial and idiopathic PD.[2,3] LRRK2 is a multidomain protein containing several protein interaction motifs as well as dual enzymatic domains of GTPase and protein kinase activities. Although the detailed physiological function and effectors of the LRRK2 are largely unknown, numerous recent in vitro and in vivo studies have demonstrated that the kinase activity of LRRK2 is involved in PD pathophysiogenesis.[4,5] However, because of the lack of kinase selectivity of compounds used in the efficacy studies, the biological effects of LRRK2 kinase inhibition remain to be elucidated. Therefore, there is a demand for highly selective and potent brain penetrant small molecule inhibitors of LRRK2 to study the detailed role LRRK2 in PD.

In the study by Estrada et al.,[6] the discovery of small molecule inhibitors that possess an ideal balance of LRRK2 cellular potency, broad kinase selectivity, metabolic stability, and brain penetration across multiple preclinical species is described. Their strategy is based on the use of property- and structure-based drug design to carry out a hit-to-lead optimization of a highly ligand efficient but nonselective 2,4-diaminopyridine lead compound **1** discovered earlier.

The structure of the kinase domain of LRRK2 is not yet known, so a JAK2-based LRRK2 homology model was used in conjunction with binding site sequence analysis and MMP activity cliff analysis to identify residues that could potentially impart general kinase selectivity. One such residue is an amino acid Leu1949 in LRRK2. By incorporation of small substituents on an otherwise nonselective kinase inhibitor to reach the small selectivity pocket created by Leu1949, broad kinase selectivity can be achieved. This finding led to identification of **1** (Figure 1), the starting compound of the study by Estrada et al.[6] **1** has a LPPK2 biochemical potency at the nanomolar scale (3 nM)

and a JAK2 biochemical selectivity index of >1067✕, and it only inhibits wild-type and G2019S mutant LRRK2 at greater than 50% inhibition at 1 μM in a panel of 63 kinases.

The first series of compounds in the lead optimization efforts were modified at C-4 and C-5 positions of the aminopyrimidine structure (Figure 1). Their cellular activity was determined in LRRK2 cellular assay by inhibition of LRRK2 autophosphorylation in HEK293 cells. The C-4 aminomethyl and C-5 trifluoromethyl substituted aminopyridimine **7** proved to provide the best inhibition of cellular pLRRK2 with an IC_{50} of 9 nM, while the corresponding value for the lead compound **1** was 29 nM. While the initial kinase selectivity profiling demonstrated that **7** is likely to be a highly selective inhibitor for LRRK2, in a subsequent inhibition assay it inhibited TTK activity by 55% at 0.1 μM and 98% at 1 μM. Because LRRK2 and TTK share some structural homology, more selective LRRK2 inhibitors were still pursued.

Comparison of the inhibitor binding site residue differences between a published TTK crystal structure and the LRRK2 homology model suggested that the Ser1954 (LRRK2) and Asp608 (TTK) residue difference can be exploited in the design of more selective LRRK2 inhibitors. To this end, the second series of inhibitors contained substituents at C-5′ of the phenyl ring to introduce unfavorable steric and electrostatic interactions with the bulkier, negatively charged Asp608 side chain. Desirable selectivity index over TTK was achievable with chlorine, fluoro, and methoxy substituents at C-5′. Further varying aminoalkyl substitution at C-4 position resulted in low nanomolar LRRK2 cellular activities (9 nM for aminoethyl **18** and 4 nM for aminocyclopropyl **19**). Expanded kinase profiling (187 kinases) at 0.1 μM for both **18** (100-fold over LRRK2 K_i) and **19** (250-fold over LRRK2 K_i) resulted in only TTK showing greater than 50% inhibition. Although further optimization of structures were attempted, their DMPK profile precluded progression of these inhibitors.

Both **18** and **19** demonstrated excellent in vitro DMPK and in vivo rat PK profiles including minimal turnover in human hepatocytes and low total and unbound clearance values as predicted by rat hepatocytes, long half-lives, good oral exposure, high passive permeability, no human P-gp efflux, and good brain penetration in rats. Both compounds also showed concentration-dependent knockdown of pLRRK2 in the brain of BAC transgenic mice expressing human LRRK2 protein with the G2019S PD mutation. A pharmacodynamic inhibition model had a calculated in vivo unbound brain IC_{50} of 7 and 27 nM for **18** and **19**, respectively. In studies with cynomolgus monkey, **18** had the oral bioavailability of 17% at 1
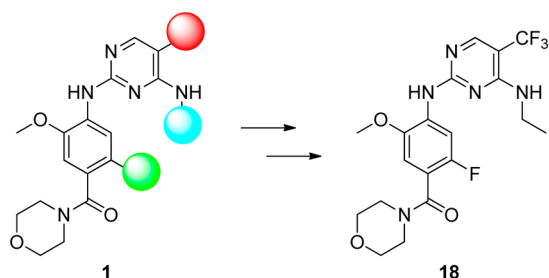

Figure 1. Lead optimatization toward highly potent, selective, and brain penetrable LRRK2 inhibitors (**1**; red = Cl, blue = NHMe, green = H).

mg/kg dose and 24% at 20 mg/kg dose. Additionally, terminal brain concentration 24 h after the last dose on day 24 in cynomolgus monkey resulted in good brain exposure with a Bu/Pu ratio of 0.6. In toxicity studies in both rats and cynomolgus monkeys **18** was well-tolerated. These results indicate that **18** is a selective and brain penetrant small molecular inhibitor of LRRK2 kinase activity.

PD is the second most common neurodegenerative disease in the world, and it affects 1.5% of the global population over 65 years of age. Despite nearly 200 years since its initial description, a definitive understanding of disease etiology and a preventive therapeutic approach are still missing. The discovery of LRRK2 mutations in PD has proved to be monumental to the field because, for the first time, gene mutations have been found to be a determinant of both familial and idiopathic PD. It is clear that further knowledge of LRRK2 is necessary to appreciate fully the role that this multidomain, dual enzyme plays in both normal neuronal function and PD pathogenesis. Therefore, there is a high demand for potent, selective, and bioavailable small molecule inhibitor of LRRK2 which could serve as a useful tool to examine the function of LRRK2 in PD in a more detailed manner. In the best case scenario, the inhibitors of LRRK2 may offer an alternative option in the treatment of PD. However, several critical issues need to be addressed before potential clinical development of LRRK2 inhibitors. For example, what is the normal function of LRRK2 as it is expressed, for example, in kidney, lung, and spleen? What functional effect do LRRK2 PD-associated mutations have on neurons? And why are dopaminergic neurons particularly sensitive to LRRK2 mutations when the protein has a widespread expression pattern?

The study by Estrada et al.[6] is a paragon for a comprehensive property and structure-based approach for the successful design and optimization of small molecular weight inhibitors of LRRK2. The optimized LRRK2 inhibitor has a promising outlook to be utilized as a preclinical tool assessing the consequences of inhibiting LRRK2 kinase activity in the brain. Consequently, it will certainly be interesting to learn whether the inhibition of LRRK2 kinase activity is a potentially viable treatment option in PD.

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Notes

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