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Kinase domain inhibition of leucine rich repeat kinase 2 (LRRK2) using a [1,2,4]triazolo[4,3-*b*]pyridazine scaffold

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ABSTRACT

Leucine rich repeat kinase 2 (LRRK2) has been genetically linked to Parkinson's disease (PD). The most common mutant, G2019S, increases kinase activity, thus LRRK2 kinase inhibitors are potentially useful in the treatment of PD. We herein disclose the structure, potential ligand–protein binding interactions, and pharmacological profiling of potent and highly selective kinase inhibitors based on a triazolopyridazine chemical scaffold.

Parkinson's disease (PD) is the most common movement disorder and the second most common neurodegenerative disorder after Alzheimer's disease (AD).[1] Significant excitement has been generated by recent genome-wide association studies (GWAS) that linked the PARK8 mutation, encoding the leucine rich repeat kinase 2 (LRRK2) protein, to PD.[2] LRRK2 is a very large protein (2527 amino acids; 286 kDa MW) comprised of multiple domains (Fig. 1).[3] The most common LRRK2 mutation is G2019S (GS) which is in the activation loop (DFG motif-DYG in LRRK2) of the kinase domain.[4] This mutation has been reported to increase the kinase activity of LRRK2, thus the identification of potent, selective, brain penetrant kinase inhibitors could dampen this hyperactivity and be of value in the treatment of PD.[5]

LRRK2 linkage to PD is a very recent discovery and a significant amount of LRRK2 biology relating to PD has yet to be elucidated.[6] One key gap is our lack of understanding of the exact role of LRRK2 in PD. In an effort to gain insight into its PD role, various groups have explored LRRK2 biology with re-purposed kinase inhibitors.[7] Other groups sought to identify novel chemical tools with a goal of improving kinase specificity.[8] Utilization of modestly selective tool compounds to probe the biology of LRRK2 can be problematic in that it generates data that is difficult to interpret, as one needs to de-convolute on-target from off-target pharmacology.[9] Although the discovery of potent and selective LRRK2 kinase inhibitors may be challenging and require significant investment of resources, they should allow for a robust understanding of the biological consequences of LRRK2 inhibition. Thus, in our efforts to develop LRRK2 kinase inhibitors, potency, selectivity, in vivo efficacy and brain penetration were all tracked in an effort to generate

Abbreviations: AD, Alzheimer's disease; ANK, ankyrin repeat domain; ARM, armadillo repeat domain; AUC, area under the curve; BA, brain availability; BBB, blood brain barrier; BCRP, breast cancer related protein; BI, brain impairment; CSD, Cambridge Structural Database; CNS, central nervous system; COR, C-terminal of ROC domain; ER, efflux ratio; GS, LRRK2 G2019S mutation; GWAS, genome-wide association studies; HLM, human liver microsome; HTS, high throughput screening; KO, knockout; $K_{p,uu}$, unbound brain to unbound plasma concentration ratio; KSS, kinase selectivity screening; LE, ligand efficiency; LipE, lipophilic ligand efficiency; LRR, leucine rich repeat domain; LRRK2, leucine rich repeat kinase 2; MDR1, multi-drug resistance protein 1 (human *P*-gp); MPO, multi-parameter optimization; PD, Parkinson's disease; PDB, protein data bank; PK/PD, pharmacokinetic/pharmacodynamic; ROC, ras of complex domain; SAR, structure activity relationship; WCA, whole cell assay based on pS935 readout; WD40, WD40 repeat domain; WT, wild type.

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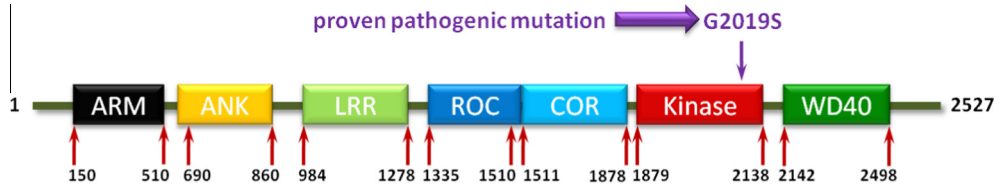
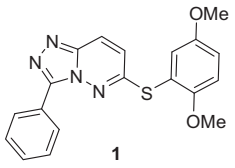


Figure 1. Multi-domain structure of LRRK2.



50 μM ATP LRRK2 (WT) IC_{50} = 40 nM
LE = 0.39; LipE = 3.6; CNS MPO = 4.82
1 mM ATP LRRK2 (WT) IC_{50} = 173 nM
1 mM ATP LRRK2 (GS) IC_{50} = 82 nM
HLM $CL_{IA,S}$ = 159 mL/min/kg
P_{app} AB = 12 x 10^{-6} cm/sec
MDR1 ER (BA/AB) = 1.77
KSS = 0/39 hits at 1 μM

Figure 2. Data summary of HTS hit **1**.

the most efficient inhibitors for potential treatment of PD. In this Letter, we disclose our optimization of a triazolopyridazine scaffold targeting the LRRK2 kinase domain.

A high throughput screen (HTS) of about 750,000 compounds allowed us to identify multiple scaffolds including triazolopyridazine **1** as a potent inhibitor of LRRK2, which bears high similarity to a hit recently described by scientists at Elan (Fig. 2).[8i] It exhibited double-digit nM potency in a LANTHA screen assay at the ATP K_M (50 μM) of LRRK2.[10] Shifting the ATP concentration to one more physiologically relevant (1 mM) resulted in a modest rightward shift in potency for both the WT and the GS mutation.[10] Along with its desirable in vitro potency, **1** exhibited physicochemical properties consistent with CNS drug space (e.g. high LE, LipE and good CNS MPO score).[11] This observation was buttressed by in vitro data showing excellent kinase selectivity,[12] high passive permeability, and a lack of *P*-gp transporter-mediated membrane asymmetry (good potential for brain availability). The HLM clearance was found to be high, as one might predict for a compound susceptible to an *O*-demethylation metabolic clearance pathway.

As part of our hit-to-lead approach, strict adherence to designing compounds with an eye to physicochemical properties was followed. Given that treating PD would involve a long-term, chronic regimen, minimizing daily dose and maximizing the safety profile were critical attributes that could be readily achieved, at the design stage, using this strategy. The CNS MPO score was a relatively straightforward yardstick to monitor our ability to align drug-like properties and all compounds presented in this Letter met or exceeded the cut-off for CNS drug-like space.[11c] In examining the sources of potential diversity within the di-substituted triazolopyridazine scaffold, 4 points of diversity were readily apparent; the 2 substituent moieties (at C_3 and C_6), the S-linker atom, and the hinge interaction core itself.

Our SAR optimization began with the C_3 substituent. Table 1 provides a summary of some of the compounds prepared in the evaluation of this position. While small alkyls (**7**), saturated hetero-cycles (**8**–**9**), and 6-membered ring heteroaromatics (**2**–**6**) showed varying degrees of potency, the 5-membered ring heteroaromatics (**11**–**16**) proved to be optimal for this diversity vector. In particular, methyl-pyrazole **15** became an obvious standout. Not only does this compound have improved potency (and LipE) but it also is the only compound that significantly reduced clearance whilst maintaining potency. Adding a methylene spacer to this moiety (**10**) resulted in a dramatic loss in potency. While not formally presented in the tables, G2019S IC_{50} values were determined and found to be roughly equipotent with the wt isoform (within ±3×). For all compounds tested in this series, we found a 5–10× rightward shift in potency in going from the cell free to whole cell assays[10] despite the good potency at cellularly relevant ATP concentrations and excellent passive permeability.

While our hit-to-lead efforts were ongoing, we were very interested in better understanding the potential binding interactions of these compounds. In the absence of LRRK2 crystallographic information, we employed a surrogate crystallography approach based on kinase similarity and cross-over of compound activity. Though LRRK2 only has ~30% residue identity and ~50% similarity in the overall kinase domain to its closest neighbors, the residues in its ATP-binding site pocket have greater similarity to a number of other kinases. For instance, tyrosine kinase 2 (Tyk2) ATP-binding site residues are 74% similar to those in LRRK2. In addition, there was some cross-over activity of this series of triazolopyridazine compounds with the JAK family of kinases (vide infra), suggesting Tyk2 is a reasonable surrogate crystallographic system for LRRK2.

With crystals of Tyk2 readily available from a previous project, we pursued soaking studies of **15** with Tyk2 to see if it could act as a model system and provide some insight into the potential binding interactions. Figure 3 shows the X-ray crystal structure of **15** in Tyk2 (PDB ID: 4PY1) highlighting several protein-ligand interactions. Compounds of this scaffold appear to make a single point interaction with kinase hinge via the C_1 *N*-atom of the triazole moiety, with the Me-pyrrazole occupying the ribose pocket (towards solvent) and not a position adjacent to gatekeeper, in contrast to a binding mode previously suggested for this chemotype.[8i]

Having identified optimal C_3 substituents, we next focused attention on the C_6-S substituent as it was a potential metabolic soft spot and safety liability (potential for quinone formation). Table 2 provides a sampling of substituents explored at this position. Compounds **17** and **18** provided evidence that the two methoxy groups of the phenyl ring were having a synergistic effect, in that, independently they provided weak potency to the scaffold but together, as in **1**, resulted in a dramatic increase in kinase inhibition. This is somewhat in contrast to the SAR reported by Elan, whereby they demonstrated LRRK2 potency with solely a *meta* substituted aryl at this position.[8i] The binding pose (Fig. 3) allows one to speculate if an interaction of the two methoxys with the *P*-loop and floor of the ATP site is required for good potency (observed with tofacitinib). This bioactive conformation could be favored by the *ortho*-OMe twisting the aryl ring orthogonal to the plane of the triazolopyridazine core. In support of this hypothesis, the *ortho*-OMe was found to be required, whereas, small substituents could be tolerated at the *meta*-position, for example, **19** and **20**. While **20** maintained good predicted brain availability, this change did increase clearance compared to **15**. Conversely, **19** showed a modest decrease in clearance but now had the potential for *P*-gp efflux susceptibility. Clearance could be greatly improved as **23** demonstrated, by modulating the potential phenyl metabolic soft spots, but could not be coupled with potency.

We were concerned the *S*-linker may be a metabolic liability and explored options for its replacement. While all of the compounds examined were predicted to be in good CNS space (MPO > 4), all linker replacements examined (ether **24**, amino **25**,