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### **Graphical Abstract**

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5-Substituted-N-pyridazinylbenzamides as Potent and Leave this area blank for abstract info. Selective LRRK2 Inhibitors: Improved Brain Unbound **Fraction Enables Efficacy** Xiao Ding<sup>a</sup>, Luigi Piero Stasi<sup>a</sup>, Xuedong Dai<sup>a</sup>, Kai Long<sup>a</sup>, Cheng Peng<sup>a</sup>, Baowei Zhao<sup>a</sup>, Hailong Wang<sup>a</sup>, Changhui Sun<sup>a</sup>, Huan Hu<sup>a</sup>, Zehong Wan<sup>a</sup>, Karamjit S. Jandu<sup>b</sup>, Oliver J. Philps<sup>b</sup>, Yan Chen<sup>a</sup>, Lizhen Wang<sup>a</sup>, Qian Liu<sup>c</sup>, Colin Edge<sup>d</sup>, Yi Li<sup>c</sup>, Kelly Dong<sup>c</sup>, Xiaoming Guan<sup>a</sup>, F. David Tattersall<sup>a</sup>, Alastair D. Reith<sup>b</sup>, Feng Ren<sup>\*, a</sup> **18** LRRK2 HTRF pIC<sub>50</sub> = 7.4 PFI = 7.1  $F_{u,bl}$  = 13%,  $F_{u,br}$  = 4.6%  $K_p = 1.44, K_{p,uu} = 0.51$ ~50% inhibition of pS935 of LRRK2 in rat brain COR



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### 5-Substituted-*N*-pyridazinylbenzamides as Potent and Selective LRRK2 Inhibitors: Improved Brain Unbound Fraction Enables Efficacy

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#### ARTICLE INFO

ABSTRACT

We describe the discovery and optimization of 5-substituted-N-pyridazinylbenzamide Article history: Received derivatives as potent and selective LRRK2 inhibitors. Extensive SAR studies led to the Revised identification of compounds 18 and 23, which demonstrated good in vitro pharmacokinetic profile and excellent selectivity over 140 other kinases. Both compounds demonstrated high Accepted Available online unbound fractions in both blood and brain. Compound 18 proved to be brain penetrant, and the high unbound fraction of compound 18 in brain enabled its in vivo efficacy in CNS, wherein a significant inhibition of LRRK2 Ser935 phosphorylation was observed in rat brain following Keywords: intravenous infusion at 5 mg/kg/h. CNS penetration LRRK2 Parkinson's disease 2009 Elsevier Ltd. All rights reserved. PFI unbound fraction

Parkinson's disease (PD) is a common and complex neurological disorder which affects approximately 2-3% of the population greater than 60 years of age<sup>1-3</sup>. Currently available treatments can only address the dopamine deficiencies to relieve the symptoms<sup>4</sup>, and a disease modifying therapy to halt the progression of disease remains a major unmet medical need <sup>5</sup>. Over the past several years, great progress has been made in the field of PD genetics and several common variants have been identified by genome-wide association studies (GWAS)<sup>6</sup>. Among them, mutations of Leucine-rich repeat kinase 2 (LRRK2) genes have been identified as the most common known genetic cause of PD, accounting for 4% of familial cases and 1% of sporadic cases<sup>7-10</sup>. Several LRRK2 mutations, with the most frequent one G2019S located in the activation loop of the kinase domain<sup>10-11</sup>, have been demonstrated to increase kinase activity both in vitro and in vivo. Hence, smallmolecules that inhibit LRRK2 kinase activity could be a new class of disease-modifying agents for the treatment of PD. Several structurally diverse LRRK2 inhibitors have been reported <sup>12-13</sup> in the literature including 7*H*-pyrrolo[2,3-d]pyrimidin-2-amines<sup>14-15</sup>, indazoles<sup>16-17</sup>, indolinones<sup>18</sup> and 2,4diaminopyrimidines<sup>19</sup>.

We recently reported the discovery of a series of 5substituent-*N*-arylbenzamide derivatives as LRRK2 inhibitors exemplified by 1 and 2 (Scheme 1)<sup>20-21</sup>. Given the structural novelty, high potency and good selectivity across the kinome, compound 1 (GSK2578215A) has been widely used as a tool for the exploration of biological roles of LRRK2<sup>22-23</sup>. However, despite its high exposure in brain, no significant inhibition against phosphorylation of Serine 935 (Ser935) of LRRK2 was observed. We hypothesized its extremely low unbound fraction in brain could be accountable for its lack of target engagement in CNS. Besides, both compounds 1 and 2 showed poor developability profile (e.g. solubility). Herein, we describe the optimization starting from compound 2 towards the identification of 5-substituted-N-pyridazinylbenzamides as potent and selective LRRK2 kinase inhibitors with improved developability profile. Representative compounds 18 and 23 demonstrated good in vitro pharmacokinetic profile and excellent kinase selectivity. More importantly, these compounds exhibited much improved free fractions in both blood and brain in rodent species as well as in human serum. The high brain unbound fraction of compound 18 enabled its in vivo efficacy, wherein a significant inhibition of Ser935

phosphorylation of LRRK2 was observed in rat brain following intravenous infusion at 5 mg/kg/h.



Figure 1. Structures of compounds 1 and 2

Our work was initiated with re-evaluation of different substitutions on the N-aryl group  $(\mathbf{R}^1)$  and benzyl ether moiety (R<sup>2</sup>) of compound 2 to improve physicochemical properties and developability profile. Chromlog  $D_{74}$  and property forecast index (PFI)<sup>24</sup> served as key indicators of developability since they have been reported to show good relationship with hydrophobicity, solubility and other developability parameters<sup>24</sup>. As described previously<sup>20</sup>, compound **2** displayed high potency against LRRK2 inhibition (HTRF  $pIC_{50} = 7.7$ ), good in vitro and in vivo pharmacokinetic profile but high tissue binding in mouse brain which was proposed to be responsible for the lack of in vivo efficacy. Further profiling revealed low solubility (11  $\mu$ M), high ChromlogD<sub>7.4</sub> (6.3) and PFI (9.3) of the compound. Previous SAR showed only limited substituents could be tolerated at  $R^1$  position since this group was proposed to be buried into the conservative pockets close to the gatekeeper<sup>20</sup>. As illustrated in Table 1, replacing the pyridine group (2 and 3) with 3-chlolo-phenyl (4 and 5) or 3fluoro-phenyl (6) substituents on R<sup>f</sup> resulted in similar potencies but around 2 units higher of PFI values. The corresponding nitrile analogue (7) demonstrated increased potency (pIC<sub>50</sub>= 8.1) and lower PFI (10.5) compared to that of the fluoro analogue (6). Compounds with 4-pyridazinyl substituent at the  $R^1$  position gave the improved PFI (8.4) and comparable potency as compound 2. However, no improvement in solubility was observed for compound 8 even though the lipophilicity and PFI were reduced. Nevertheless, the 4-pyridazinyl group was identified as the optimal R<sup>1</sup> moiety in terms of the balance of potency and PFI.

We then focused our optimization on exploring substituents on the 5-position of the benzamide  $(R^3)$ , aiming to further reduce PFI for improved solubility and developability profile (Table 2). A variety of different groups were synthesized and their potencies, physicochemical properties and in vitro pharmacokinetic profile were determined. Not surprisingly, the HRTF potency against LRRK2 inhibition for these compounds was retained with O-, N- and C-linked substituents, consistent with the previous predicted binding mode that R<sup>3</sup> group was pointed to the solvent-exposed area of LRRK2 kinase<sup>20</sup>. Replacing the high lipophilic group CF<sub>3</sub> with ether group such as methoxy (9) gave a comparable potency (pIC<sub>50</sub> = 7.8) and much reduced lipophilicity. However, O-linked compounds suffered from high human liver microsome clearance<sup>24</sup> which was probably due to the oxidative de-alkylation of the alkoxy group. Changing  $\mathbb{R}^3$  to N-linked moieties, such as cyclic-amine substituents with different ring size (compound 10-26) provided improved PFI values compared with compound 8 except for compounds 12 and 13. As a result, the solubility of these compounds was improved significantly with compounds 10 and 17 as exceptions. Compared with the O-linked analogue 9, the metabolic stabilities were slightly improved for the 4membered and 5-membered ring analogues (compounds 10, 11, and 12). A more significant improvement in metabolic stability was observed for the 6-membered ring analogue 14. Further reduction of the ChromLogD7,4 by introducing the hydroxy group resulted in compounds (15 and 16) with excellent metabolic stability. However, both compounds started to show Pgp/BCRP efflux liability with permeability ratio (PR) > 2.0, raising concerns for CNS penetration. The Pgp/BCRP efflux of both compounds might be due to the additional hydrogen bond donor from the hydroxy group. Changing the hydroxy group to the cyano group (17) mitigated the Pgp/BCRP efflux liability, whereas the metabolic stability was still above our cut-off value of 3 mL/min/g thus was not acceptable. A balanced profile was observed for the morpholine analogue 18, which demonstrated acceptable metabolic stability (2.1 mL/min/g) in human liver microsome, free of Pgp/BCRP efflux liability (PR = 1.3), good permeability (500 nm/s), and decent solubility (68  $\mu$ M). Introducing of methyl group(s) to the morpholine moiety didn't further improve the overall profile.

 Table 1. LRRK2 inhibition activities for benzamide compounds 2–8.

 CF2

R <sup>1</sup> NH O					
Cpd	$\mathbf{R}^1$	$\mathbb{R}^2$	HTRF pIC <sub>50</sub>	ChromlogD <sup>a</sup> (PFI) <sup>b</sup>	Solubility <sup>c</sup> (uM)
2	<b>N</b> *	F	7.7	6.3 (9.3)	11
3	×	Н	7.9	6.3 (9.3)	<1
4	CI *	F	7.9	8.7 (11.7)	$\mathbf{ND}^{d}$
5	CI *	Н	7.6	8.8 (11.8)	7
6	F *	F	7.5	8.2 (11.2)	<1
7	CN *	F	8.1	7.5 (10.5)	<1
8	N N	Н	7.8	5.4 (8.4)	<1

<sup>a</sup>Measured ChromLogD<sub>pH7.4</sub>. <sup>b</sup>Property Forecast Index = ChromLog  $D_{pH7.4} + # Ar. c$ Kinetic solubility. <sup>d</sup>Not determined.

We then started to explore the piperazine substituents, and all piperazine derivatives (21–23) demonstrated good HTRF potency against LRRK2 inhibition. Not surprisingly, with one more basic center, the ChromlogD<sub>7.4</sub> and PFI values were reduced and solubility was increased. In addition, the metabolic stabilities of these analogues were even better than the morpholine analogues (18 and 20), which could be partially attributed to their lower ChromLogD<sub>7.4</sub> values. The free piperazine analogue 21 showed significant Pgp/BCRP efflux liability, likely attributed to its additional hydrogen bond donor. Masking the N-H bond with a methyl group (22) mitigated the Pgp/BCRP efflux however with the borderline PR (1.8). Further increasing the size of the side chain led to compound 23 with no concern of Pgp/BCRP efflux (PR = 1.3).

The solubility of **23** was measured high (373  $\mu$ M) and its clearance was measured low (1.3 mL/min/g). The fused amine (**24**) and spiro-amines (**25** and **26**) were also evaluated. Despite their good potencies and solubility, they suffered from either high clearance or Pgp/BCRP efflux liability thus was

unlikely to be progressed as CNS compounds. Further, changing the piperazine moiety of compounds 22 and 23 to piperidine moieties gave *C*-linked analogs 27 and 28 with increased Pgp/BCRP efflux liabilities (PR = 6.9 and 2.5, respectively), which might be due to the higher basicity of the piperidine compared with piperazine.

Table 2. LRRK2 inhibition activities and *in vitro* profile of benzamide compounds 8–28.



<sup>a</sup>Measured ChromLogD<sub>pH7.4</sub>, <sup>b</sup>Property Forecast Index = ChromLogD<sub>pH7.4</sub> + # Ar. <sup>c</sup>Human liver microsome clearance. <sup>d</sup>MDCKII-MDR1 transduced with BacMam2-BCRP cell line. <sup>e</sup>Permeability ratio,  $A \rightarrow B$  (apical to basolateral) with GF120918/ $A \rightarrow B$  without GF120918. <sup>f</sup>Passive permeability,  $A \rightarrow B$  with GF120918. <sup>g</sup>Kinetic solubility. <sup>h</sup>Pyridyl analogue instead of pyridazinyl. <sup>h</sup>Not determined. <sup>j</sup>MDCKII-MDR1 cell line.

#### Table 3. Developability profile of compounds 18 and 23

	18	23
ANK/AHE pIC <sub>50</sub> <sup>a</sup>	7.0 / 6.8	6.6 / 6.4
Kinase selectivity <sup>b</sup>	1/140	1/140
F <sub>u</sub> % in blood/brain <sup>c</sup>	13/4.6	11/6.4
F <sub>u</sub> % in HSA <sup>d</sup>	12.5	13.6
CYP inhibition pIC <sub>50</sub> (3A4, atorvastatin)	5.2	5.0
hPXR pEC <sub>50</sub>	<4.3	<4.3
OATP1B1 pIC50	4.8	4.4
hERG IonWorks pIC50	<4.2	5.1

<sup>*a*</sup>Human lymphoblastoid cells derived from a control (AHE) and Parkinson's patient (ANK) homozygous for the LRRK2[G2019S] mutation. <sup>*b*</sup>Standard radioactivity-based enzymatic assays against a panel of 140 kinases at 1 uM, quoted as numbers of kinase displaying >50% activity. <sup>*c*</sup>Free fraction in rat brain and blood. <sup>*d*</sup>Free fraction in human serum albumin.

Compound 18 and 23 were then selected for further progression (Table 3) given their high potencies, decent physicochemical properties (ChromlogD7,4, PFI, and solubility), good metabolic stabilities, and Pgp/BCRP efflux profile. The in vitro pharmacology of compounds was examined on endogenous LRRK2 in human lymphoblastoid cells derived from a healthy subject (AHE) and a Parkinson's disease patient with homozygous LRRK2[G2019S] mutation (ANK).<sup>26</sup> Compound **18** demonstrated pIC<sub>50</sub> values of 6.8 in AHE and 7.0 in ANK cell assays. Relatively lower potencies were observed for compound 23 with pIC<sub>50</sub> values of 6.4 and 6.6 in AHE and ANK assays, respectively. Both compounds were progressed to kinase selectivity assessment using standard radioactivity-based enzymatic assays at the concentration of 1 µM. They both demonstrated excellent selectivity against a panel of 140 kinases among which both compounds hit only one kinase, 84% inhibition against mitogen-activated protein kinase kinase 1 (MKK1) for compound 18 and 53 % inhibition against AMPKrelated protein kinase 5 (ARK5) for compound 23. The unbound fractions were also determined in rat blood and brain tissue. Compounds 18 and 23 demonstrated good free fractions in both blood (13% and 11%, respectively) and brain (4.6% and 6.4%, respectively). Consistently, good unbound fractions were also observed in human serum albumin (HSA) with 12.5% and 13.6 % for compounds 18 and 23, respectively. Further, the developability profile was evaluated for both compounds. They demonstrated moderate inhibition against human CYP3A4 and low inhibition on OATP1B1, and no meaningful activity against human PXR (pIC50 < 4.3), indicating low concerns for drugdrug interactions. In addition, the preliminary cardiac safety was evaluated by conducting the hERG binding assay, and the compound18 was determined to have a lower risk of QT interval prolongation (*h*ERG pIC<sub>50</sub> < 4.2) compared with compound 23 (hERG pIC<sub>50</sub> = 5.1). In this regard, compound **18** demonstrated a superior developability profile than compound 23.

As reported previously, the benzamide analogue (1) suffered from the lack of *in vivo* pharmacological efficacy in CNS, which was hypothesized to due to its low free fraction in brain (0.3%).<sup>20</sup> Discovery of compound **18** with significantly improved free unbound fraction in brain (4.6%) provided a good tool to test the hypothesis. In order to ensure sufficient exposure of compound **18** in brain at steady state, an intravenous (iv) bolus followed by intravenous infusion administration was applied in the *in vivo* pharmacodynamics study, wherein the inhibition of LRRK2 Ser935 phosphorylation in brain, kidney and spleen was determined.



Figure 2.Pharmacodynamic study of 18 at 4 h after intravenous infusion at dose 5 mg/kg/h.

To our delight, ~50% reduction on phosphorylation of LRRK2 Ser935 in brain was observed at 4 h after iv infusion (5 mg/kg/h) in rats (Figure 2). In addition, ~60% and ~70% reductions on phosphorylation of LRRK2 Ser935 were observed in kidney and spleen, respectively. Pharmacokinetics (PK) analysis revealed drug concentrations in blood, brain, kidney, and spleen at steady state were 1126 ng/mL, 1627 ng/g, 3967 ng/g, 1680 ng/g, respectively. Compound 18 proved to be brain penetrant with good brain-to-blood ratios in both total drug concentration ( $K_p =$ 1.44) and free drug concentration ( $K_{p,uu} = 0.51$ ). The drug concentration of compound 18 in brain correlated well with its in vivo CNS efficacy. This result, comparing with the previous report of the lack of correlation between CNS efficacy and drug exposure for compound 1, suggested that the free unbound fraction of compound 18 in brain enabled its in vivo efficacy in CNS.

In conclusion, through extensive SAR studies and careful modifications of ChromLogD7,4 and PFI starting from compound 5-substituted-N-2. we discovered а series of pyridazinylbenzamide derivatives with high potencies of LRRK2 properties. inhibition. improved physicochemical and developability profile. Compounds 18 and 23 were identified with good in vitro PK profile and excellent selectivity over 140 other kinases. Most importantly, both compounds exhibited high free unbound fractions both in blood and brain. Compound 18 proved to be brain penetrant with good brain-to-blood ratios in both total drug concentration ( $K_p = 1.44$ ) and free drug concentration ( $K_{p,uu} = 0.51$ ). In vivo pharmacology study of compound 18 demonstrated significant inhibitions of LRRK2 Ser935 phosphorylation in brain, kidney and spleen following iv infusion (5 mg/kg/h) in rats. We concluded that the in vivo efficacy in CNS of compound 18 was enabled by its high free unbound fraction in brain. The desirable in vitro and in vivo activity and developability profile of compound 18 made it a

good tool for the exploration of the biological roles of LRRK2 in Parkinson's disease.

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#### **Supplementary Material**

Supplementary data associated with this article can be found in the online version.

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