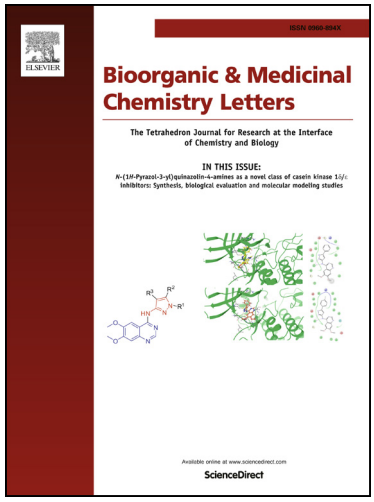

Discovery of 5-substituent-*N*-arylbenzamide derivatives as potent, selective and orally bioavailable LRRK2 inhibitors

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Graphical Abstract

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Discovery of 5-substituent-*N*-arylbenzamide derivatives as potent, selective and orally bioavailable LRRK2 inhibitors

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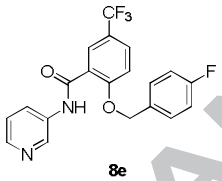

8e

LRRK2 HTRF pIC_{50} = 7.7
Oral bioavailability = 91.6%
$DNAUC_{Br}/DNAUC_{BI}$ = 1.3

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ABSTRACT

Abstract: Leucine-rich repeat kinase 2 (LRRK2) has been suggested as a potential therapeutic target for Parkinson's disease. Herein we report the discovery of 5-substituent-*N*-arylbenzamide derivatives as novel LRRK2 inhibitors. Extensive SAR study led to the discovery of compounds **8e**, which demonstrated potent LRRK2 inhibition activity, high selectivity across the kinome, good brain exposure, and high oral bioavailability.

Parkinson's disease (PD), a slowly progressive neurodegenerative disorder mainly affecting the motor function, has been recognized as the second most prevalent neurodegenerative diseases.[1] It was reported that PD affected ~4 million people over age 50 worldwide in 2005 and the number was estimated to be doubled in 2030.[2] Current therapies for PD are limited to alleviating the symptoms of the disease in early stage,[3] and development of disease-modifying therapies to slow down disease progression is therefore profoundly needed.[4] Leucine-rich repeat kinase 2 (LRRK2) gene mutations have been considered as the most common genetic cause of familial and sporadic PD.[5] Among the several mutations presented in PD patients carrying LRRK2 mutations, G2019S is the most frequent one identified which results in aberrant kinase activity, suggesting small molecules inhibiting LRRK2 activity could be potential therapeutic treatments for PD.[6]

A number of structurally diverse small molecule LRRK2 inhibitors have been disclosed in the past several years (Figure 1),[7] including LRRK2-IN-1 (**1**), [8] GNE-7915 (**2**),[9] PF-06447475 (**3**),[10] Nov-LRRK2-11 (**4**),[11] MLi-2 (**5**),[12] and compounds with quinoline[13] and triazolopyridazine[14] core structures. Some of these compounds demonstrated good pharmacokinetic profile, acceptable CNS penetration, and the ability to inhibit LRRK2 activity both *in vitro* and *in vivo*. However, no LRRK2 inhibitor has yet been reported to be progressed to clinical development. We have previously reported GSK2578215A,[15] a novel 5-substituent-*N*-arylbenzamide analogue, as a potent LRRK2 inhibitor with high selectivity across the kinome and good CNS penetration (brain to plasma exposure ratio = 1.4) in mice. The compound was observed to substantially inhibit the phosphorylation of S910 and S935 of LRRK2 in spleen and kidney after intraperitoneal injection (100 mg/kg) to normal mice. The compound's potent LRRK2 activity, high kinase selectivity, and good PK profile have made it a versatile tool for the exploration of the biological roles of LRRK2 including roles in autophagy,[16] receptor trafficking,[17] protein degradation,[18] and inflammation,[19] as well as definition of physiologic substrates of LRRK2[20] and pharmacodynamic markers assays[21]. The 5-substituent-*N*-arylbenzamide pharmacophore is a distinct structure scaffold for LRRK2 small molecule inhibitors. In this report, we describe the detailed SAR studies of 5-substituent-*N*-arylbenzamide series leading to the discovery of the potent, selective and orally bioavailable LRRK2 inhibitor **8e**.

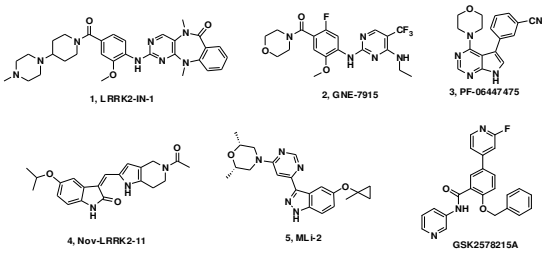


Figure 1. Structures of reported LRRK2 inhibitors

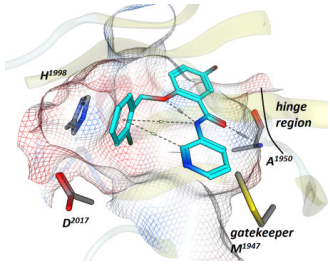
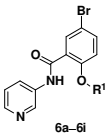


Figure 2. The predicted docking pose of KCS Hit (**6a**, cyan) in the LRRK2 homology model.

A KCS (kinase-focused set of compounds for lead discovery) screening of the GSK in-house compounds using a homogeneous time-resolved fluorescence (HTRF) assay resulted in the identification of several LRRK2 inhibitor hits including the 5-substituent-*N*-arylbenzamide compound **6a** with a pIC_{50} of 7.0. Given its structural novelty and low molecule weight, this hit series was selected for further optimization. Docking of compound **6a** to a LRRK2 homology model[22] suggested that **6a** bound to the LRRK2 ATP pocket (Figure 2). The amide carbonyl group of **6a** formed a hydrogen bond interaction with A1950 in the hinge region and was proposed as the key interaction with LRRK2. The bromide group of **6a** pointed toward the solvent-exposed area of the kinase. 2-Cl-phenyl ring and the pyridine ring were both buried in the binding pocket: the former was closer to D2017 and the latter to the gatekeeper residue, M1947.

Table 1. LRRK2 HTRF potency for compounds **6a**–**6i**.

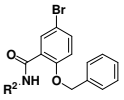


Compd	R^1	HTRF pIC_{50}[a]
6a	2-Cl-$C_6H_4CH_2$-	7.0
6b	2-F-$C_6H_4CH_2$-	8.1[b]
6c	3-F-$C_6H_4CH_2$-	7.8
6d	4-F-$C_6H_4CH_2$-	8.1
6e	4-OCH_3-$C_6H_4CH_2$-	7.4
6f	4-CN-$C_6H_4CH_2$-	7.2
6g	$C_6H_5CH_2$-	7.9
6h	3,4-di-F-$C_6H_3CH_2$-	7.7[b]
6i	C_6H_5-$CH(CH_3)$-	7.4

[a] HTRF assay data is the average of at least two determinations. [b]One determination.

SAR exploration of the 5-substituent-*N*-arylbenzamide series started with different substitutions on the benzyl ether moiety (R^1) in order to improve potency of the hit compound **6a** (Table 1). Replacing the chloro group of **6a** with a smaller fluoro substitution (**6b**) provided more than 10 fold improvement in potency (pIC_{50} = 8.1). Changing the position of fluorine from *ortho* (**6b**) to *meta* (**6c**) or *para* (**6d**) resulted in similar potency. Because *para*-substituted benzyl alcohols are readily available, we thus focused our SAR exploration on *para*-substitutions. Either electron-donating group (methoxy, **6e**) or electron-withdrawing group (cyanide, **6f**) led to reduced LRRK2 potency by ~10 folds. On the other hand, the un-substituted analogue (**6g**) demonstrated good potency comparable to that of **6d**. These data suggested only small substitutions such as hydrogen and fluorine were well tolerated at the *para*-position of the phenyl ring, while bulkier substitutions resulted in decreased potency. The 3,4-difluoro analogue (**6h**) showed slightly decreased potency which, again, might due to steric effect. Furthermore, introducing a methyl group to the benzylic position provided compound (**6i**) with lower potency.

Table 2. LRRK2 HTRF potency for compounds **7a**–**7l**.



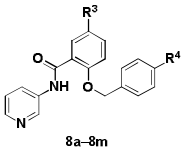
Compd	R^2	HTRF pIC_{50}[a]
6g		7.9
7a		7.7
7b		7.3
7c		5.7
7d		<4.6
7e		<4.6
7f		7.2
7g		6.6
7h		7.6
7i		6.9
7j		4.8
7k		5.6
7l		<4.6

[a] HTRF assay data is the average of at least two determinations.

Having identified the un-substituted benzyl group as one of the best R^1 moieties, attention was then turned to optimizing the heteroaryl (R^2) group (Table 2). Several different heteroaromatic analogues were synthesized and their LRRK2 HTRF potency evaluated. Among them, the 3-pyridinyl (**6g**) and the 4-pyridazinyl (**7a**) groups proved to be the most optimal fragments. Changing the position of the nitrogen atom of **7a** or adding substitutions such as chlorine to **6g** resulted in decreased LRRK2 activities to different extents (**7b**–**7e**). The predicted binding model showed the pyridine group of R^2 bounds in a shallow pocket closer to M1947 and E1948 residues of LRRK2. Introduction of a N atom or a Cl substitution to the 5-position of the pyridine introduces an electrostatic or steric repulsion, which

could result in inactive compounds (**7d** and **7e**). Replacing 3-pyridinyl with 3-methyl phenyl (**7f**) or 3-fluoro phenyl (**7g**) groups resulted in decreased potency. However, the corresponding chloro analog (**7h**) demonstrated comparable potency (pIC_{50} = 7.6) to that of the pyridine analogue (**6g**). When electron-donating groups such as methoxy (**7i**) or dimethylamino (**7j**) groups were introduced, the potency decreased dramatically. Changing the six-membered heteroaryl ring to the five-membered ring (**7k**) yielded a much-decreased activity. Furthermore, the saturated ring such as cyclohexyl led to totally inactive compound (**7l**), which might be caused by the twisted dihedral angle between the amide group and the cyclohexyl ring.

Table 3 LRRK2 HTRF potency for compounds **8a**–**8m**.



8a–8m

Compd	R^3	R^4	HTRF pIC_{50} [a]	Cli (mL/min/g) [b]
6g	-Br	H	7.9	2.8
8a	-F	H	7.4	18.3
8b	-Cl	H	7.8	ND[d]
8c	$-CH_3$	H	7.9	16.2
8d	$-OCH_3$	H	7.8	22.6
8e	$-CF_3$	F	7.7	0.8
8f	$-CON(CH_3)_2$	H	6.7	0.7
8g	$-SO_2N(CH_3)_2$	H	5.9[c]	ND[d]
8h		H	8	5.1
8i		H	8	1.2
8j		H	7.9	2.3
8k		H	8.1	1.2
8l		F	8	1.5
8m		F	8	0.6

[a]HTRF assay data is the average of at least two determinations. [b] *In vitro* human liver microsome clearance. [c]One determination. [d]Not determined.

From the preliminary SAR exploration, compound **6g** was discovered with high potency and good "drug likeness" profile (low MW, cLogP, etc.) based on Lipinski's rule.[23] However, the *in vitro* clearance profile of **6g** in human liver microsome[24] (2.8 mL/min/g) was observed to be sub-optimal which precluded its further progression. We then focused our efforts on SAR exploration of R^3 to improve this series' metabolic stability and physicochemical properties at the same time to maintain potencies. A variety of functional groups was well tolerated at the R^3 position, including electron-withdrawing groups such as fluorine and chlorine (**8a** and **8b**) as well as electron-donating groups such as methyl and methoxyl (**8c** and **8d**). Even though these compounds demonstrated similar potency as **6g**, their clearance in human liver microsome was still high. To our delight, introduction of a trifluoromethyl group to the R^3 position provided the compound (**8e**) with good potency (pIC_{50} = 7.7) and metabolic stability in human liver microsome (Cli = 0.8 mL/min/g). In addition, the solubility of **8e** (11 μM) was also improved compared to that of **6g** (<1 μM). We further evaluated more polar groups such as amide (**8f**) and sulfonamide (**8g**). However, both compounds turned out to be less potent.

Heteroaromatic rings were also explored including pyridines (**8h** and **8i**) and pyrazoles (**8j**–**8m**), and all compounds demonstrated high potency and improved metabolic stability (except for **8h** and **8j**).

Table 4. Mouse PK profile of **8e**, **8i**, and **8l**.[a]

	$T_{1/2}$ (h)	CL_b (mL/min/kg)	Vss (L/kg)	$DNAUC_{0-t}$ ((ng·h/mL)/(mg/kg))	F%	Br/Bl ratio
8e[b]	1.2	27.7	2.7	597	91.6	1.3
8i[c]	1.1	30	2.3	520	12.2	1.4
8l[b]	0.4	69.9	1.6	253	10	ND[d]

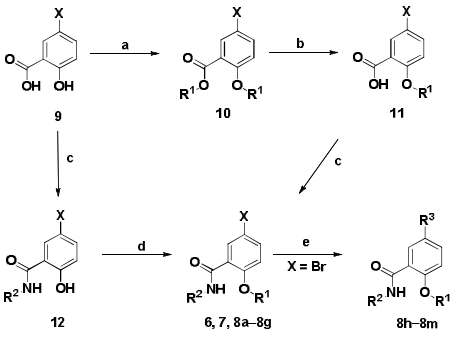
[a]Experiments were done in male Swiss Albino Mice. DNAUC = dose normalized area under the curve (measure of exposure) after i.v., $T_{1/2}$ = half-life, CL_b = blood clearance, Vss = volume of distribution, F = oral bioavailability. [b]1 mg/kg i.v. and 2 mg/kg p.o. [c]1 mg/kg i.v. and 10 mg/kg p.o. [d]Not determined.

Representative compounds **8e**, **8i** and **8l** were selected for further progression. They all demonstrated good passive permeability (> 100 nm/s) and proved not to be Pgp efflux transporter substrates in the Polarized Madin-Darby canine kidney (MDCKII) cells heterologously expressing human Pgp (MDCKII-MDR1 cell line)[24]. They were then progressed to *in vivo* to evaluate their brain penetration and pharmacokinetic properties in the mouse crossover PK study[25] (i.v. and p.o.). As shown in Table 4, compound **8e** was observed to be brain penetrable in mice with high brain to blood ratio (Br/Bl = 1.3) in terms of total drug exposure. In addition, compound **8e** also demonstrated excellent oral bioavailability (F = 91.6%), and it was the first compound in the 5-substituent-*N*-arylbenzamide series observed to be orally bioavailable. In comparison, the previously reported compound **8i** (GSK2578215A) had very low oral bioavailability (F = 12.2%) even though its brain penetration was good (Br/Bl = 1.4).[15] Both compounds **8e** and **8i** showed low clearance in mice (27.7 and 30 mL/min/kg, respectively). On the other hand, compound **8l** had much higher clearance (69.9 mL/min/kg) thus a short half-life ($T_{1/2}$ = 0.43 h), and its oral bioavailability was low (F = 10%). Compound **8e** was further evaluated for its selectivity over 300 other kinases using the HotSpot assay platform.[26] The results indicated **8e** is a highly selective inhibitor of LRRK2. Only LRRK2 and one other kinase (MSSK1-STK23) had inhibition levels of over 40% at 1μM compound concentration. Subsequent dose-response titrations for LRRK2 and MSSK1-STK23 gave pIC_{50} of 8.1 and 6.8, respectively.

As reported previously, compound **8i** (GSK2578215A)[15b] showed strong inhibition against phosphorylation of S910 and S935 of LRRK2 in mouse spleen and kidney, whereas no significant inhibition in mouse brain despite its high brain exposure[25]. Mouse brain tissue binding was measured for both compound **8e** and **8i**. Not surprisingly, both compounds showed very high tissue binding in mouse brain (99.6% and 99.7% for **8e** and **8i**, respectively). The low free unbound drug concentration of **8i** in mouse brain might account for its lack of efficacy in CNS.

Efficient syntheses of 5-substituent-*N*-arylbenzamide derivatives **6**–**8** were developed (Scheme 1). Treatment of commercial benzoic acids **9** with various alkylation reagents in the presence of a base (K_2CO_3) in acetone provided benzoic esters **10**. Hydrolysis of intermediates **10** using LiOH in the mixed solvent of THF and water resulted in benzoic acids **11**. Treatment of **11** with different amines in the presence of coupling reagents such as EDCI/HOBT or HATU in DMF yielded target compounds **6a**–**6h**, **7**, **8a**–**8g**. Compounds **6i** could be obtained by alkylation of phenols **12** under basic conditions (KOH) in methanol. Intermediates **12** were prepared from commercial

benzoic acids **9** through amide bond formation. Suzuki coupling reactions of **6d** or **6g** with various boronic acids in the presence of Pd catalysts at elevated temperatures provided compounds **8h–8m**.



Scheme 1. Synthesis of 5-substituent-*N*-arylbenzamide derivatives **6–8**. Reagents and conditions: (a) R^1Br, K_2CO_3, acetone; (b) LiOH, THF/water; (c) R^2NH_2, EDCI/HOBT or HATU, DMF; (d) R^1Br, KOH, MeOH; (e) $R^3B(OH)_2$, $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$, DME/water.

In summary, we discovered a novel series of 5-substituent-*N*-arylbenzamide derivatives as potent LRRK2 inhibitors. Extensive SAR studies led to the identification of compounds **8e** and **8i** with high potencies of LRRK2 inhibition and good selectivity over hundreds of kinases. Compound **8e** demonstrated good *in vitro* and *in vivo* pharmacokinetic profile with high exposure in both brain and blood (Br/Bl ratio = 1.3). **8e** was orally bioavailable (F = 91.6%) in comparison with the previously reported compound **8i** (GSK2578215A, F = 12.2%). Both compound **8e** and **8i** were observed to be highly tissue bound (99.7% and 99.6%, respectively) in mouse brain. The low free unbound drug concentration of **8i** in brain was proposed to be accountable for its lack of efficacy in CNS (no significant inhibition against phosphorylation of S910 or S935 in mouse brain). Further efforts will be focused on improving the series' free unbound fraction in brain.

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Supplementary Material

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

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24. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.
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26. In vitro profiling of the 300 member kinase panel was performed at Reaction Biology Corporation (www.reactionbiology.com, Malvern, PA) using the "HotSpot" assay platform (1 μM, n = 2) (http://www.reactionbiology. xcom/webapps/site/KinaseDetail.aspx).