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Characterization of TAE684 as a potent LRRK2 kinase inhibitor

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Abstract

Leucine-rich repeat kinase 2 (LRRK2) is linked to Parkinson's disease and may represent an attractive therapeutic target. Here we report a 2,4-dianilino-5-chloro-pyrimidine, TAE684, a previously reported inhibitor of anaplastic lymphoma kinase (ALK), is also a potent inhibitor of LRRK2 kinase activity (IC_{50} of 7.8 nM against wild-type LRRK2, 6.1 nM against the G2019S mutant). TAE684 substantially inhibits Ser910 and Ser935 phosphorylation of both wild-type LRRK2 and G2019S mutant at a concentration of 0.1–0.3 μM in cells and in mouse spleen and kidney, but not in brain, following oral doses of 10 mg/kg.

Keywords

Parkinson's disease; LRRK2; TAE684

Parkinson's disease (PD) is a debilitating neurodegenerative disease that affects over one million Americans.[1,2] Recent genetic studies have revealed an underlying genetic cause in at least 10% of all PD cases,[3] which provides new opportunities for discovery of molecularly targeted therapeutics that may ameliorate neurodegeneration. Among the genes associated with PD, leucine-rich repeat kinase 2 (LRRK2) is unique because of a missense mutation, G2019S, is frequently found in not only familial but also sporadic Parkinson's disease cases.[4,5] The G2019S mutation enhances kinase activity, suggesting that small molecule LRRK2 kinase inhibitors may be able to block aberrant LRRK2-dependent signaling in Parkinson's disease.[6,7]

LRRK2 kinase inhibitors are being actively pursued and recently first-generation 'tool' inhibitors that exhibit excellent potency and selectivity for LRRK2 such as LRRK2-IN-1[8] and CZC-25146[9] have been reported. However, neither compound is able to achieve good exposure in mouse brains which limits their utility in murine PD models and eventual translation in human clinical trials.[8,9] Here we report that TAE684, a previously reported

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.084. These data include MOL files and InChiKeys of the most important compounds described in this article.

inhibitor of anaplastic lymphoma kinase (ALK),[10] is also a potent LRRK2 inhibitor which posses a favorable pharmacokinetic profile in mice.

In an effort to discover new compound classes that are capable of potently inhibiting LRRK2 kinase activity and that also have the ability to traverse the blood–brain barrier (BBB), we screened our in-house kinase targeted compound library using an LRRK2 enzymatic assay. TAE684, 5-chloro-N4-(2-(isopropylsulfonyl)phenyl)-N2-(2-methoxy-4-(4-(4-methyl-piperazin-1-yl)piperidin-1-yl)phenyl)pyrimidine-2,4-diamine, a previously reported ALK kinase inhibitor,[10] emerged as a potent LRRK2 inhibitor with biochemical IC_{50}s of 7.8 and 6.1 nM against wild-type LRRK2 and LRRK2[G2019S], respectively (Fig. 1). While the biochemical potency of TAE684 for inhibition of wild-type and G2019S LRRK2 is similar to LRRK2-IN-1, TAE684 maintains significant inhibition of the A2016T mutation which induces dramatic resistance to LRRK2-IN-1 (Fig. 1). Although both LRRK2-IN-1 and TAE684 share the aminopyrimidine pharmacophore, a molecular model of TAE684 built based on a previously published crystallographic structure with ALK,[11] suggests that isopropylsulfone moiety is able to avoid the steric clash that is likely between the anthranilic acid ring of LRRK2-IN-1 with the A2016T residue (Fig. 2a,b).[8,12,13] We next examined the ability of TAE684 to inhibit LRRK2 in a cellular context in comparison to LRRK2-IN-1. As there are no validated direct phosphorylation substrates of LRRK2, we monitored phosphorylation of Ser910 and Ser935, two residues whose phosphorylation is known to be dependent upon LRRK2 kinase activity[14] (Fig. 3). TAE684 induced a dose-dependent inhibition of Ser910 and Ser935 phosphorylation in both wild-type LRRK2 and LRRK2[G2019S] stably transfected into HEK293 cells (Fig. 3a). Significant dephosphorylation of Ser910 and Ser935 was observed at 0.1–0.3 μM of TAE684 for wild-type LRRK2 and at slightly higher doses for LRRK2[G2019S] (Fig. 3a), which is approximately 10-fold more potent compared to LRRK2-IN-1 (compare Fig. 3a to b). Consistent with the biochemical results, TAE684 also induced dephosphorylation of Ser910 and 935 at a concentration of 1–3 μM in the drug-resistant LRRK2[A2016T+G2019S] and LRRK2[A2016T] mutants (Fig. 3a), revealing that TAE684 has a different profile compared to LRRK2-IN-1 (compare Fig. 3a to b).

We next examined the effect of TAE684 on endogenously expressed LRRK2 in human lymphoblastoid cells derived from a control and Parkinson's disease patient homozygous for the LRRK2[G2019S] mutation (Fig. 4a). We found that increasing doses of TAE684 led to similar dephosphorylation of endogenous LRRK2 at Ser910 and Ser935, as was observed in HEK293 cells stably expressing wild-type LRRK2 or LRRK2[G2019S] (compare Figs. 3a to 4a). Moreover, endogenous LRRK2 was also more sensitive to TAE684 than LRRK2-IN-1, which is consistent with the trend we observed in HEK293 cells. We also found that TAE684 induced similar dose-dependent Ser910 and Ser935 dephosphorylation of endogenous LRRK2 in mouse Swiss 3T3 cells (Fig. 4b).

The mouse pharmacokinetic profile of TAE684 demonstrated excellent oral bioavailability (83% *F*), half-life of 11.3 h and excellent plasma exposure (6374 h*ng/mL, AUC_{last}) (Table 1). More importantly, TAE684 exhibited brain to plasma ratio of 2, indicating superior BBB property (Table 1). Based on these excellent pharmacokinetic properties, pharmacodynamic experiments examining inhibition of LRRK2 Ser910/Ser935 phosphorylation were conducted after oral gavage administration with 10 mg/kg or 50 mg/kg of TAE684. We observed complete Ser910 and Ser935 dephosphorylation of LRRK2 in the kidney and spleen at dose of 50 mg/kg, and slightly lower effect at dose of 10 mg/kg. These also demonstrated improved potency relative to LRRK2-IN-1 (Fig. 5).[8] Unfortunately, despite the significant exposure of TAE684 in the brain, no inhibition of LRRK2 Ser910 or Ser935 phosphorylation was observed in the brain (Fig. 5). We are currently investigating the reasons for this unexpected result.

The kinase selectivity of TAE684 was assessed using standard radioactivity-base enzymatic assays against a panel of 124 kinases (Dundee profiling).[15] At a concentration of 1 μM, TAE684 inhibited the kinase activities of CAMKKβ, CHK2, FGF-1R, NUAK1, PHKã1(PBK), and TSSK1 higher than 90% (detail results please see Supplementary data). Recently published Kinomescan binding data against a panel of 442 kinases revealed that TAE684 is a potent binder of many kinases with sub-100 nM K_ds reported for: CAMKKβ, CHK2, FGF-1R, NUAK1, PHKã1(PBK), and TSSK1.[16] These results show that TAE684 is a relatively broad-based kinase inhibitor and considerable less selective than LRRK2-IN-1 and CZC-25146.

In summary, we have discovered that TAE684 is a potent biochemical and cellular inhibitor of LRRK2 kinase activity. Detailed characterization of TAE684 using LRRK2-IN-1 as a bench mark revealed that TAE684 significantly inhibited phosphorylation of wild-type LRRK2 and LRRK2[G2019S] mutant at Ser910 and Ser935 at 0.1–0.3 μM in vivo, which is about 5–10-fold more potent than LRRK-IN-1. TAE684 is relatively insensitive to the A2016T mutation which suggests that this mutant will not be useful to validate whether the pharmacological effects of the compound are LRRK2-dependent. TAE684 achieves good exposure to mouse brain following oral administration but interestingly does not inhibit phosphorylation of Ser910 and Ser935 of LRRK2. Further characterization of clinical stage kinase inhibitors related to TAE684 may result in the identification of other compounds that might be relevant as pharmacological agents to investigate the impact of LRRK2 inhibition in animal models and eventually in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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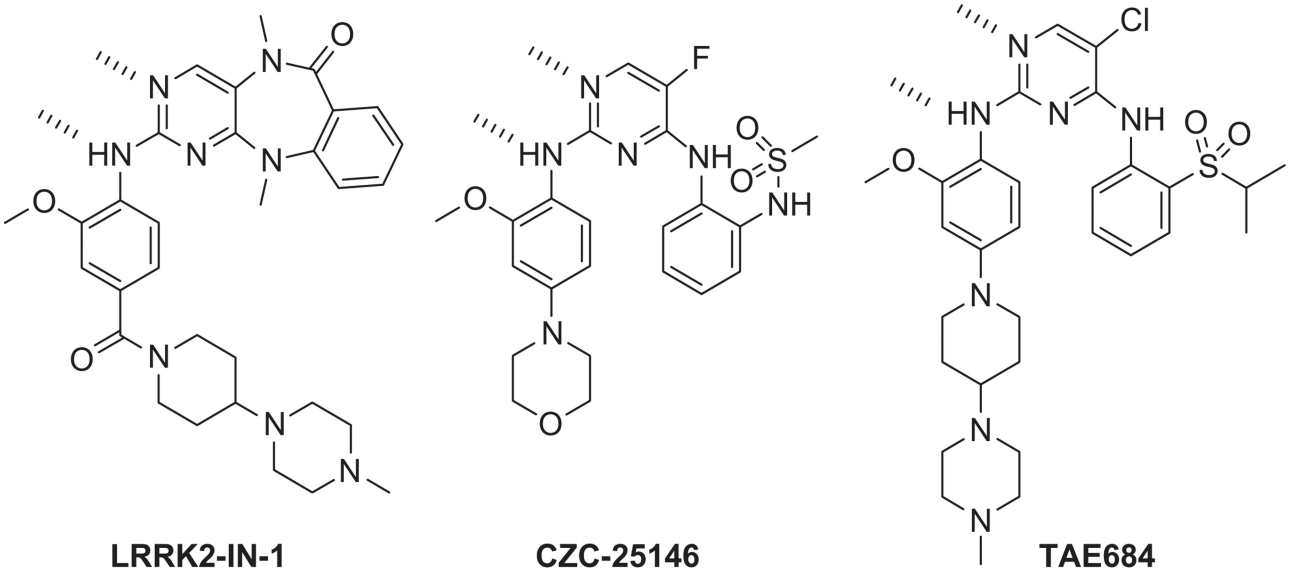

Compound ID	IC_{50} (nM)[a]			
	wild-type LRRK2	LRRK2-G2019S	LRRK2-A2016T	LRRK2-G2019S+A2016T
LRRK2-IN-1	13	6	2450	3080
TAE684	7.8	6.1	93.3	21.9

[a] GST-LRRK2(1,326-2,517), GST-LRRK2[G2019S](1,326-2,527), GST-LRRKT[A2016T](1,326-2,517) and GST-LRRK2[G2019S+A2016T](1,326-2,517) were assayed using 20 μM Nictide in the presence of 100 μM ATP. Results are average of duplicate experiments.

Figure 1.
LRRK2 inhibitors.

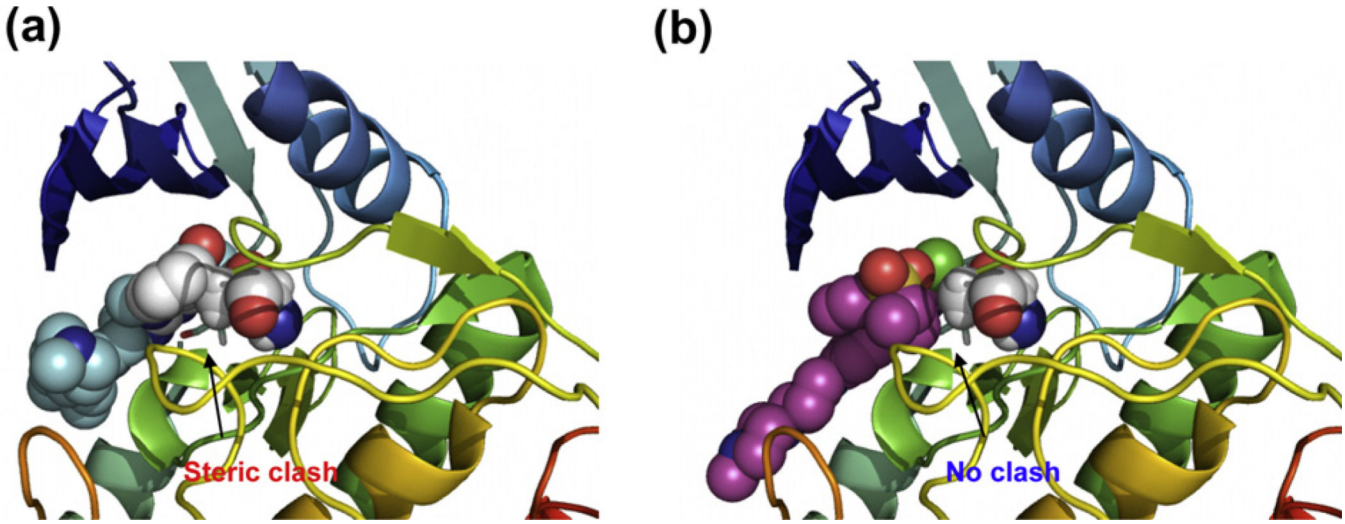

Figure 2.
Modeling study. (a) Molecular model of LRRK2-IN-1 with LRRK2[A2016]. Potential steric clash is indicated. (b) Molecular model of TAE684 with LRRK2[A2016T]. Small molecules are shown in space-filling mode and protein is depicted in ribbon format.

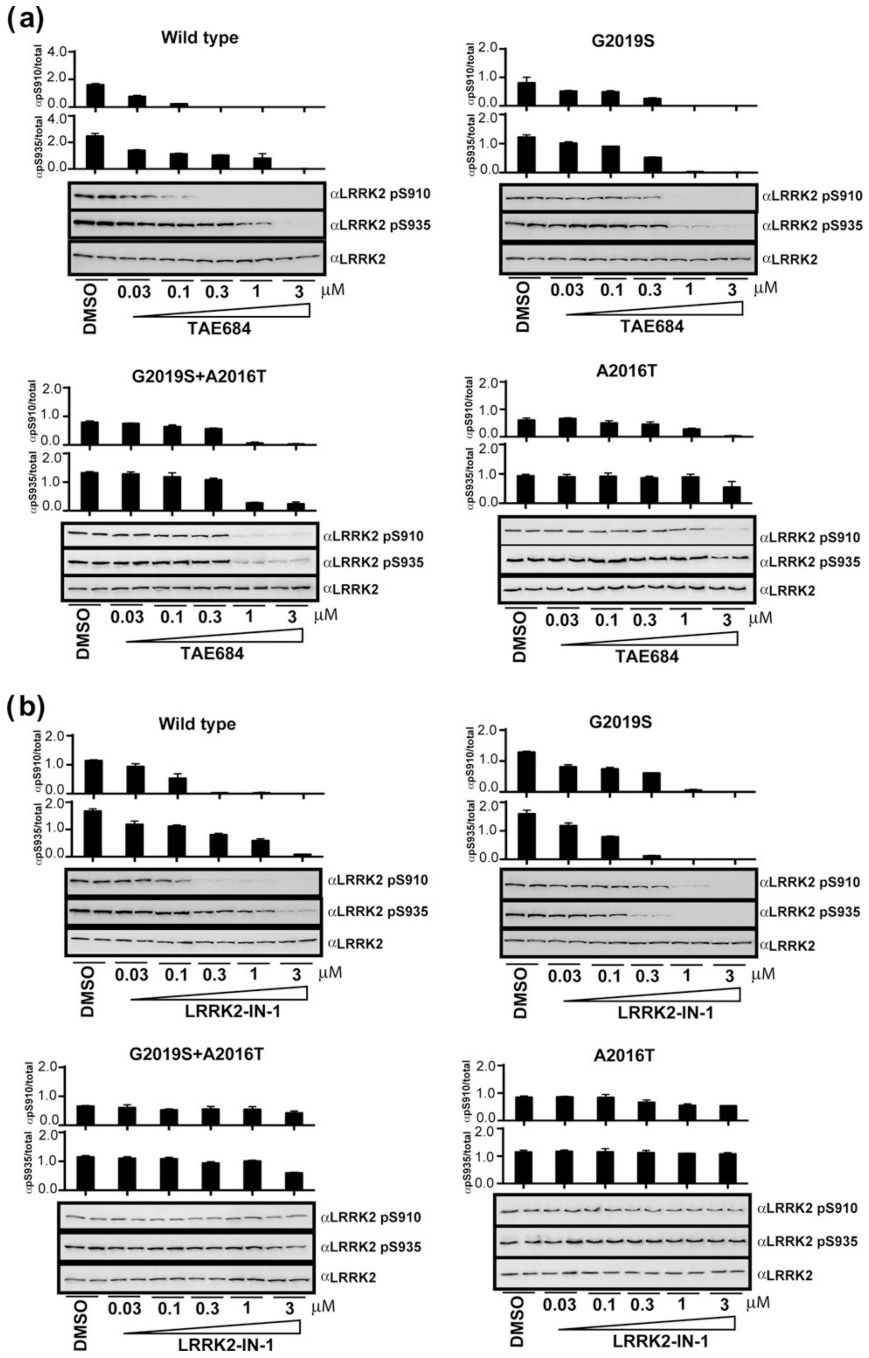

Figure 3.

Compound TAE684 inhibits LRRK2 in cells. (a) HEK 293 cells stably expressing wild-type GFP-LRRK2, GFP-LRRK2[G2019S], GFP-LRRK2[G2019S+A2016T], and GFP-LRRK2[A2016T] were treated with DMSO or increasing concentrations of TAE684 for 90 min. Cell lysates were subjected to immunoblotting for detection of LRRK2 phosphorylated at Ser910 and Ser935 and for total LRRK2. (b) As in (a) except LRRK2-IN-1 was used.

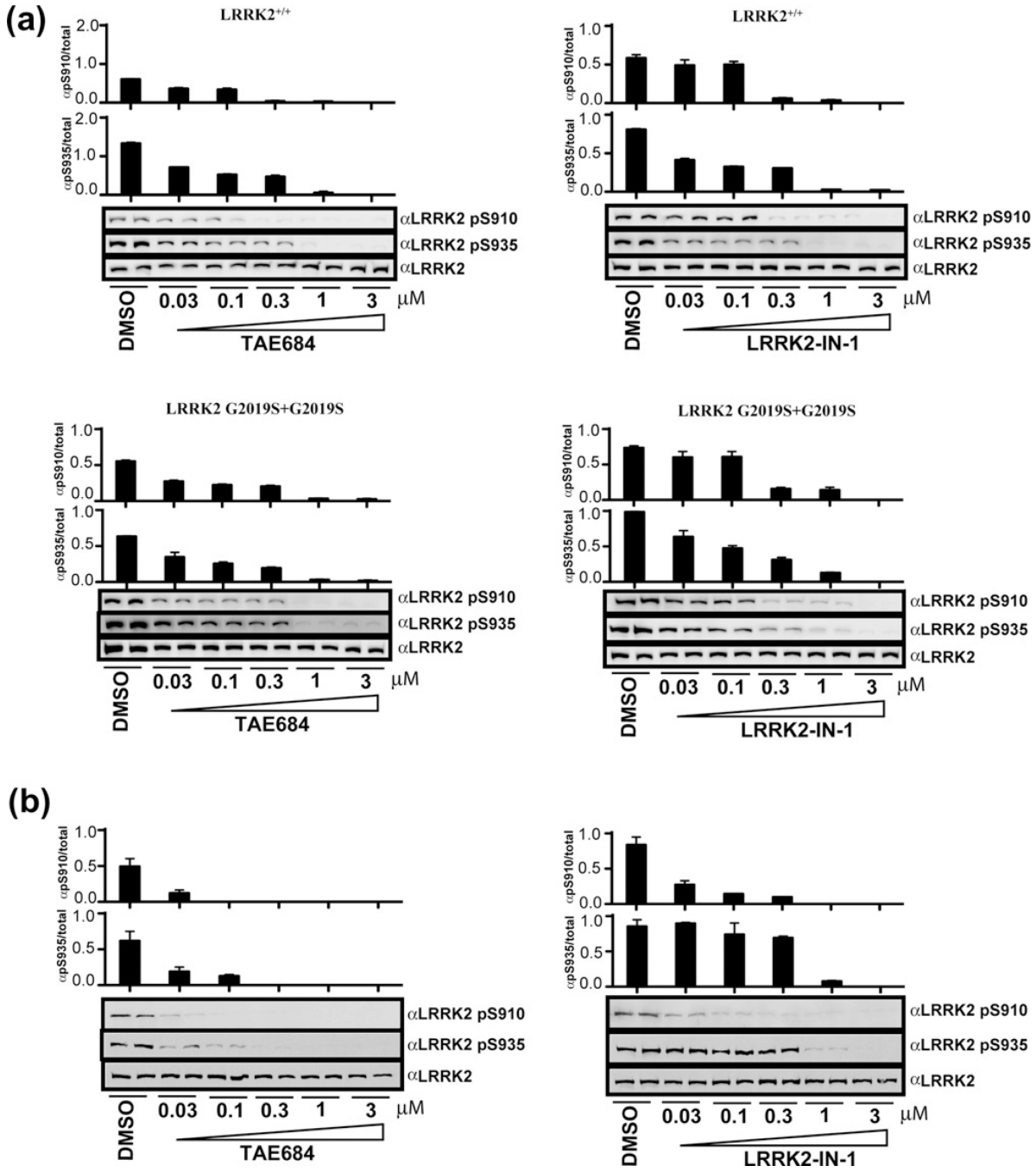

Figure 4.

Compound TAE684 inhibits endogenously expressed LRRK2. (a) Endogenous LRRK2 from EBV immortalized human lymphoblastoid cells from a control subject and a Parkinson's disease patient homozygous for the LRRK2[G2019S] mutation. After treatment of the cells with DMSO or the indicated concentration of TAE684 (or LRRK2-IN-1) for 90 min, cell lysates were subjected to immunoblot analysis with the purified indicated antibody for western analysis. Immunoblots were performed in duplicate, and results were representative of at least two independent experiments. (b) As in (a) except mouse Swiss 3T3 cells were used.

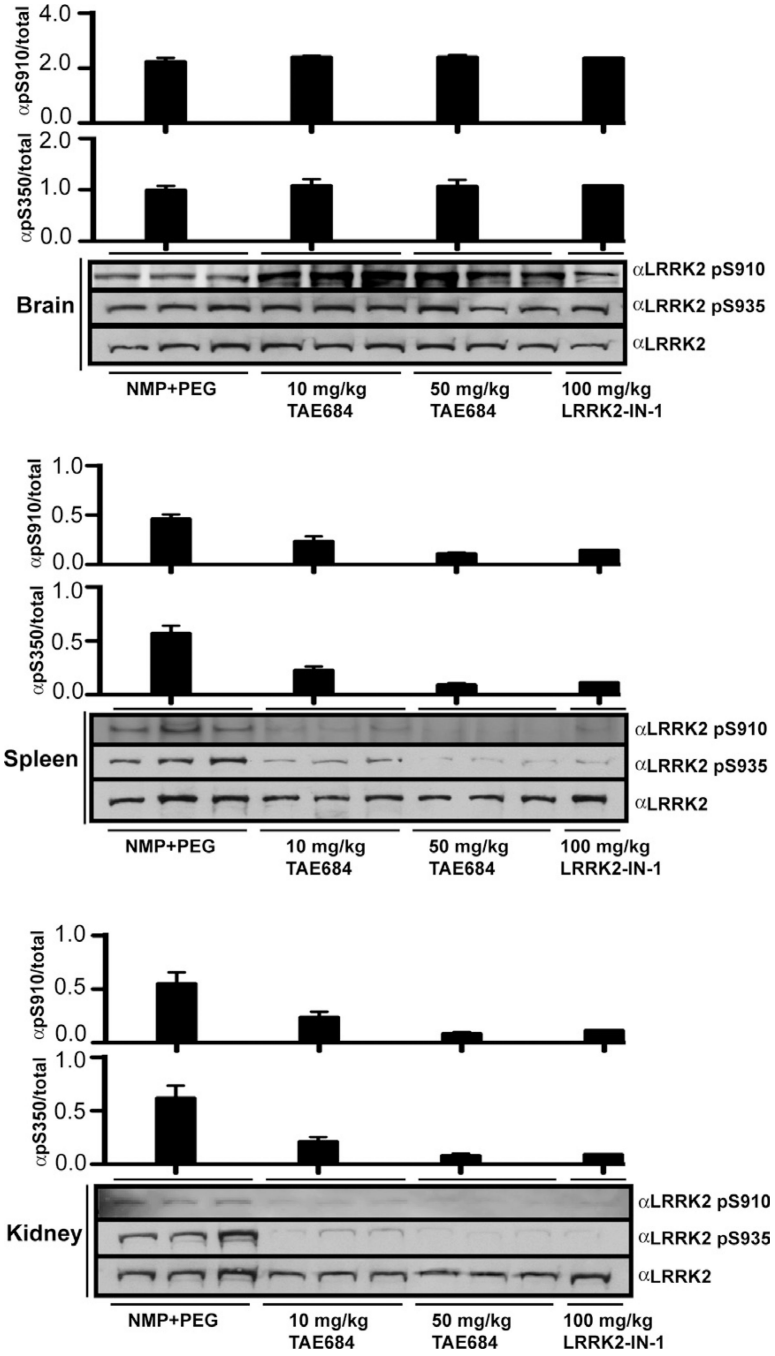

Figure 5.

Pharmacodynamic analysis for TAE684. Pharmacodynamic study of TAE684 from brain, spleen and kidney following oral administration at the indicated doses. Tissues were collected and endogenous LRRK2 was resolved by SDS–PAGE and blotted with a phospho-specific antibody directed against Ser910, Ser935 and total LRRK2.

Table 1

Pharmacokinetic parameters of TAE684[a]

Route	Matrix	AUC_{last} (h*ng/mL)	$T_{1/2}$ (h)	CL (mL/min/kg)	V_{ss} (L/kg)	Brain/plasma (AUC_{last}) ratio	%*F*
Iv	Plasma	772	11.32	17.14	14.50	1.7	82.6
	Brain	1318	—	—	—		—
Po	Plasma	6374	—	—	—	2.3	—
	Brain	14,430	—	—	—		—

[a]Experiments were done in male Swiss Albino Mice following single intravenous (iv, 1 mg/kg) and oral (po, 10 mg/kg) administration. AUC = area under the curve (measure of exposure), $T_{1/2}$ = half life, CL = plasma clearance, V_{SS} = volume of distribution, *F* = oral bioavailability.