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Design and synthesis of highly selective, orally active Polo-like kinase-2 (Plk-2) inhibitors

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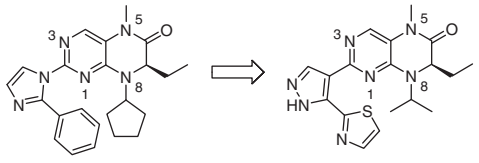
ABSTRACT

Polo-like kinase-2 (Plk-2) is a potential therapeutic target for Parkinson's disease and this Letter describes the SAR of a series of dihydropteridinone based Plk-2 inhibitors. By optimizing both the N-8 substituent and the biaryl region of the inhibitors we obtained single digit nanomolar compounds such as **37** with excellent selectivity for Plk-2 over Plk-1. When dosed orally in rats, compound **37** demonstrated a 41–45% reduction of pS129-α-synuclein levels in the cerebral cortex.

Parkinson disease (PD) is a devastating neurological disorder characterized by a progressive loss of dopaminergic neurons with the subsequent lack of dopamine causing the classical motor symptoms of bradykinesia, rigidity and resting tremors.[1] The major histological marker of PD is the formation of neuronal intracellular protein inclusions known as Lewy bodies.[2] Lewy bodies are characterized by the accumulation of α-synuclein possessing a number of protein modifications with the major modification being phosphorylation at Ser-129.[3] Consequently, the kinases responsible for this phosphorylation have been the subject of intense investigation. Recent discoveries from both our laboratories[4] and others[5] have implicated Polo-like kinase 2 (Plk-2) as a major contributor to the formation of pS129 α-synuclein and thus, inhibitors of Plk-2 have the potential to be useful therapeutics for the treatment of PD and related Lewy body diseases.

Five mammalian Plk family members have been identified (Plk-1–5)[6] with Plk-1 being the most characterized kinase in the group. Plk-1 plays an essential role during mitosis and is over expressed in human cancer tissues which has lead to an extensive search for Plk-1 inhibitors for the treatment of cancer.[6] Plk-2 is required for centriole duplication near the G1 to S phase transition,[7] while Plk-3 directs centrosome localization.[8] Plk-4 is the most structurally divergent family member and, similar to Plk-2, also controls centriole duplication.[6] Plk-5 is expressed mainly in differentiated tissues such as the cerebellum and lacks the kinase domain in



1

Plk2 IC_{50} = 0.005 uM
Plk1 IC_{50} = 0.008 uM
Plk3 IC_{50} = 94 uM
Plk1/Plk2 = 1.6
OxMet % = m 0, r 0, h 0
Perm. nm/s = 114
P-gp efflux = 3.2
Solubility = 100 uM

2

Plk2 IC_{50} = 0.06 uM
Plk1 IC_{50} = 0.77 uM
Plk3 IC_{50} = 12.4 uM
Plk1/Plk2 = 14
OxMet % = m 33, r 38, h 38
Perm. nm/s = 183
P-gp efflux = 1.2
Solubility = 100 uM

Figure 1. Typical dihydropteridinone Plk inhibitors.[11,12]

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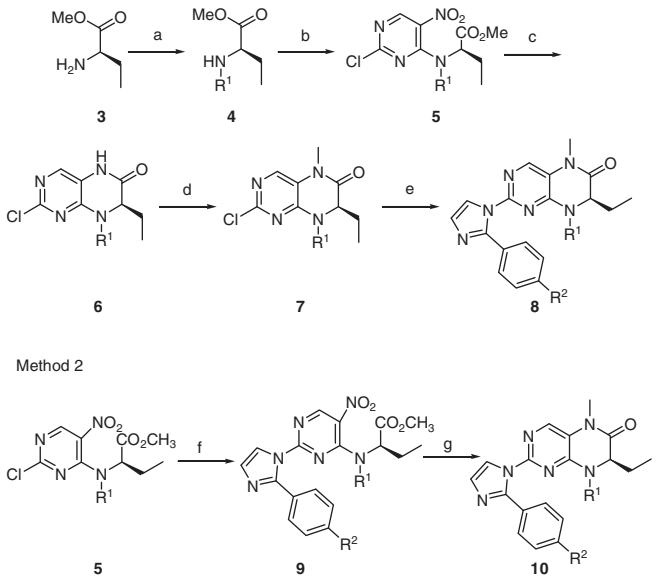


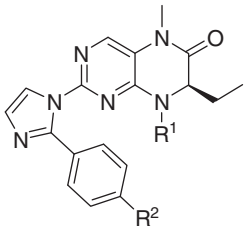
Scheme 1. Reagents and conditions: (a) aldehyde or ketone, $NaBH(OAc)_3$, NaOAc, CH_2Cl_2, rt, 18 h; (b) 2,4-dichloro-5-nitropyrimidine, $NaHCO_3$, hexane, Et_2O, 70 °C, 18 h; (c) Fe, AcOH, 90 °C, 2 h; (d) trimethylphosphate, K_2CO_3, dioxane, 100 °C, 18 h; (e) 2-(4-R^2-phenyl)-1*H*-imidazol-1-yl, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, 140 °C, μwave, 1 h; (f) 2-(4-R^2-phenyl)-1*H*-imidazol-1-yl, DMSO, 120 °C, 1 h; (g) (i) Fe, AcOH, 90 °C, 2 h; (ii) trimethylphosphate, K_2CO_3, dioxane, 100 °C, 18 h.

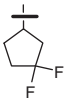
humans.[6b] While Plk-1 knockout mouse phenotypes are embryonically lethal, Plk-$2^{-/-}$ mice are viable[6] and therefore, to avoid potential toxicity, Plk-2 inhibitors should possess exquisite selectivity over Plk-1.

We have previously reported our efforts to improve the isoform selectivity and pharmacokinetic properties of a series of dihydropteridinone based Plk inhibitors which occupy the ATP-binding site of Plk.[9–11] Modification of the biaryl region of compounds such as **1** resulted in compound **2** (Fig. 1), which has good Plk-2 potency (Plk-2 IC_{50} = 55 nM), good passive permeability (Papp = 183 nm/s), low P-gp efflux (P-gp efflux ratio = 1.2) and improved microsomal stability compared to compound **1**. Unfortunately, the isoform selectivity of this compound is less than desirable and we describe in this Letter the continued optimization of these analogs with an emphasis on improving the isoform selectivity and metabolic stability.

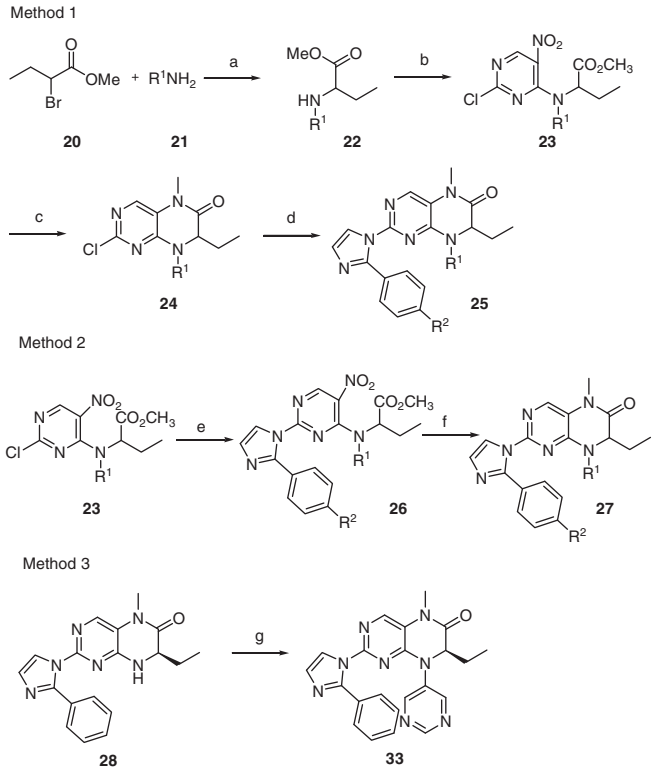
Metabolite identification studies identified both the substituent at the N-8 position of the pteridine and the phenyl ring of the biaryl system as the major sites of metabolism. It was envisioned that either the incorporation of fluorine substituents[13] or lowering the lipophilicity of the inhibitor would improve the metabolic stability of the compounds.[14] The first series of analogs that were prepared were based on the imidazole biaryl scaffold as this series has historically provided excellent Plk isoform selectivity.[9] These analogs were prepared using one of two methods outlined in Scheme 1. In Method 1, reductive amination of the appropriate aldehyde or ketone with amino ester **3**, followed by reaction with 2,4-dichloro-5-nitropyrimidine gave intermediate **5**. One-pot nitro reduction and

Table 1
SAR of N-8 aliphatic modifications



Compds	R^1	R^2	Plk-2 IC_{50}[a] (μM)	Plk-1 IC_{50}[a] (μM)	Plk-1/Plk-2	log*P*	OxMet %[b] (m, r, h)
11	*i*Pr	H	0.025	0.87	36	na	12, 0, 14
12		H	0.009	0.25	30	3.1	2, 0, 0
13		F	0.042	3.55	85	3.1	32, 30, 27
14[c]		F	0.013	0.32	25	3.1	15, 8, 6
15		F	0.012	9.39	80	3.0	30, 14, 19
16		F	1.04	>50	—	1.6	74, 80, 74
17		H	0.008	0.38	48	1.9	51, 13, 17
18		H	0.021	0.48	23	1.8	27, 0, 10
19		F	0.033	7.53	230	2.0	63, 67, 58

[a] See Ref. 12.
[b] See Ref. 12. Percent remaining after 30 min incubation with liver microsomes (m = mouse, r = rat and h = human).
[c] Compound is racemic.



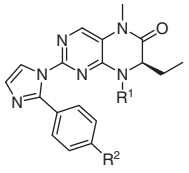
Scheme 2. Reagents and conditions: (a) K_2CO_3, KI, acetonitrile, 90 °C, 18 h; (b) 2,4-dichloro-5-nitropyrimidine, $NaHCO_3$, hexane, Et_2O, 70 °C, 18 h; (c) (i) Fe, AcOH, 90 °C, 2 h, (ii) trimethylphosphate, K_2CO_3, dioxane, 100 °C, 18 h; (d) 2-(4-R^2-phenyl)-1*H*-imidazol-1-yl, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, 140 °C, μwave, 1 h; (e) 2-(4-R^2-phenyl)-1*H*-imidazol-1-yl, DMSO, 120 °C, 1 h; (f) (i) Fe, AcOH, 90 °C, 2 h, (ii) trimethylphosphate, K_2CO_3, dioxane, 100 °C, 18 h; (g) 5-bromopyrimidine, Pd_2dba_3, Xantphos, NaO^{t}Bu, toluene, 110 °C, 18 h.

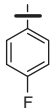
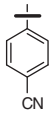
cyclization gave compound **6**, which was methylated with trimethylphosphate to give the key intermediate, chloropteridine **7**. Intermediate **7** could then be coupled to imidazoles[11] under palladium catalysis to give the analogs shown in Table 1. In some cases, the final imidazole coupling proceeded in low yield, and the alternative synthesis, Method 2, was used. Chloropyrimidine **5** was reacted with the imidazole and this intermediate, **9**, was then elaborated into the pteridine using similar conditions to those in Method 1.

Our efforts to replace the N-8 substituent are outlined in Table 1. Replacement of the isopropyl N-8 substituent (**11**) with a 3,3,3-trifluoropropyl group (**12**) resulted in a threefold improvement in Plk-2 potency but provided no significant change in the compounds in vitro metabolic stability. Interestingly, the addition of a fluorine substituent in the biaryl phenyl region of the inhibitor (**13**) resulted in an increase in metabolic stability along with an increase in the Plk-2 selectivity over Plk-1. The addition of gem-difluoro substituents to the cyclopentyl (**14**) and cyclobutyl rings (**15**) provided similar microsomal stability compared to 13 but offered no clear advantage. Lowering the log*P* of the inhibitors by incorporating heteroatoms into the N-8 substituent may also provide an increase in the stability of the compounds,[14] and a series of tetrahydrofurans and oxetanes were prepared. Oxetane 16 (log*P* = 1.6) has excellent metabolic stability but loses Plk-2 activity compared to the alkyl and fluoroalkyl analogs. Tetrahydrofuran **17** retains the excellent Plk-2 potency of the alkyl analogs and has a slightly improved metabolic stability, but when combined with the 4-fluorophenyl imidazole (**19**) a significant improvement in metabolic stability was achieved. Consistent with previous results, the additional fluorine substituent provided a significant increase in the isoform selectivity of the inhibitor compared to hydrogen at the R^2-position.

Metabolite identification studies with compound **19** indicated that N-dealkylation of the N-8 substituent is a major metabolic

Table 2
SAR of N-8 aryl modifications



Compds	R^1	R^2	Plk-2 IC_{50}[a] (μM)	Plk-1 IC_{50}[a] (μM)	Plk-1/Plk-2	log*P*	OxMet %[b] (m, r, h)
30[c]	Ph	H	0.017	2.83	164	3.3	28, 5, 21
31[c]		F	0.015	2.34	156	3.4	41, 11, 47
32[c]		F	0.031	3.76	122	2.7	49, 48, 63
33[d]		H	0.044	7.76	175	1.3	60, 76, 55
34		F	0.012	8.67	744	1.8	66, 82, 73
35[c]		F	0.009	3.42	380	2.2	67, 65, 72

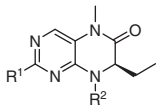
(*continued on next page*)

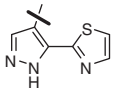
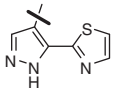
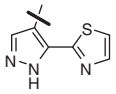
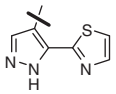
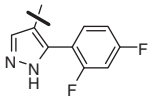
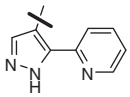
Table 2 (*continued*)

Compds	R^1	R^2	Plk-2 IC_{50}[a] (μM)	Plk-1 IC_{50}[a] (μM)	Plk-1/Plk-2	log*P*	OxMet %[b] (m, r, h)
36		F	0.021	3.29	155	2.0	50, 86, 69
37		F	0.007	1.35	193	2.4	25, 26, 63
38[b]		F	0.532	28.8	54	1.9	72, 80, 74

[a] See Ref. 12.
[b] See Ref. 12. Percent remaining after 30 min incubation with liver microsomes (m = mouse, r = rat and h = human).
[c] Compound is racemic.
[d] Prepared using Method 3.

Table 3
SAR of the imidazole replacements



Compds	R^1	R^2	Plk-2 IC_{50}[a] (μM)	Plk-1 IC_{50}[a] (μM)	Plk-1/Plk-2	OxMet %[b] (m, r, h)
41			0.036	0.25	7	44, 39, 47
42			0.039	1.41	37	47, 39, 51
43			0.092	3.03	33	57, 48, 84
44[c]			0.130	1.82	14	na
45			0.103	1.26	12	5, 13, 48
46[c]			0.168	1.82	11	64, 87, 88

[a] See Ref. 12.
[b] See Ref. 12. Percent remaining after 30 min incubation with liver microsomes (m = mouse, r = rat and h = human).
[c] Compound is racemic.

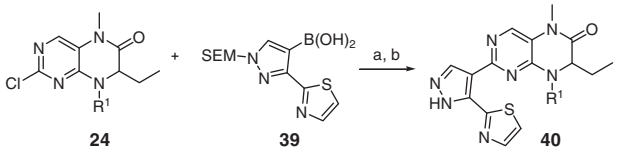
pathway for this series of compounds. In an effort to stabilize these analogs, the alkyl N-8 substituents were replaced with aromatic rings. These analogs were prepared using one of three methods outlined in Scheme 2. Anilines or aminoheterocycles were reacted with methyl 2-bromobutanoate (**20**) to give **22** which was elaborated into the pteridines in a similar manner to that described in Scheme 1. Alternatively, chloropyrimidine **23** was reacted with an appropriate 2-phenyl imidazole to give nitropyrimidine **26**, which was then converted into pteridine **27** in a two-step procedure. Chiral HPLC separation of the racemic products provided the analogs describef in Tables 2 and 3. Pyrimidine analog **33** was prepared by palladium catalyzed N-arylation of **28** (Method 3). Unfortunately, this method was not general and rapid access to a wide variety of heterocycles using this methodology was not possible.

The initial analog in the series, aniline **30** (Table 2), demonstrates that aromatic substituents at the N-8 position of the pteridine are tolerated (Plk-2 IC_{50} = 17 nM) and provide excellent

selectivity over Plk-1 (164-fold). The addition of fluorine to both the N-8 substituent and the phenyl imidazole (**31**) provided a modest increase in metabolic stability while maintaining excellent Plk-2 potency (Plk-2 IC_{50} = 15 nM). In order to increase the microsomal stability further, previous examples (Table 1) suggested that the log*P* of the inhibitor would need to be below **3**. The addition of a nitrile to the N-8 phenyl ring reduced the log*P* to 2.7 (**32**) but only offered a slight increase in the microsomal stability. Replacement of the phenyl group at the N-8 position with heterocycles should further reduce the log*P* of the inhibitors and, in turn, increase their metabolic stability. Pyrimidine **33** (log*P* = 1.3), possessed excellent metabolic stability and good Plk-2 potency (Plk-2 IC_{50} = 44 nM). Encouraged by this result with a 6-membered heterocycle, the SAR of a series of 5-membered heterocycles was explored with pyrazoles **34**–**38** (Table 2). Both 4-aminopyrazole (**34**) and 3-aminopyrazole (**35**) were among the most potent Plk-2 inhibitors in this series and both had excellent in vitro microsomal stability. Furthermore, these compounds had excellent isoform selectivity, with **34** being 744-fold selective for Plk-2 over Plk-1. *N*-Methyl pyrazoles **36** and **37** had similar potency to the NH pyrazoles but with slightly attenuated isoform selectivity and, as expected, slightly lower metabolic stability.

As we have reported previously,[10] by replacing the imidazole at the 2-position of the pteridinone with pyrazoles, analogs were obtained with much improved P-gp efflux and permeability (**2**).[10] In order to determine if these improvements could be combined with the improved N-8 substituents, which had more favorable in vitro metabolic properties a series of analogs were prepared. These compounds could be prepared by coupling chloropteridine **24** with an appropriate boronic acid **39**[10] (Scheme 3).

As shown in Table 3, the Plk-2 activity was slightly lower in this series of compounds compared to the imidazole C-2 pteridinones, and, as observed with **2**, the Plk- isoform selectivity was also much lower. The metabolic stability for the pyrazoles was generally similar to that of the imidazoles, and it appeared that the N-8 substituent had negligible benefits in this series.



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 100 °C, 18 h; (b) HCl/dioxane, MeOH, 70 °C.

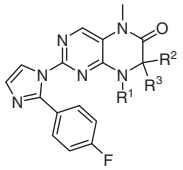
Metabolite identification studies also indicated that the ethyl group attached to the 7-position of the pteridine core was a metabolic liability, and a small set of analogs was prepared in an attempt to prevent this metabolic pathway (Table 4). These analogs were prepared in a manner analogous to Scheme 1.

Replacement of the ethyl group with a 2,2,2-trifluoroethyl substituent (**47**) had little effect on the metabolic stability of the compound compared to ethyl analog **13** (Table 1). Similarly, replacement of the hydrogen at the 7-position of the pteridine with a methyl group (**48**) had no effect on the metabolic stability of the inhibitors. Both spirocyclobutyl (**49**) and gem-dimethyl (**50**) also did not provide any advantages in terms of potency or metabolic stability compared to ethyl at this position, and no further analogs in this area were prepared.

In order for these compounds to reach their target they must be able to pass through the blood–brain barrier which requires a high rate of permeability and a low P-gp efflux. Table 5 contains the in vitro permeability and P-gp efflux for a select group of compounds that displayed good in vitro metabolic stability and good Plk-2 potency. For the C-2 imidazole analogs with alkyl N-8 substituents (**13** and **19**) the P-gp efflux ratios were low to moderate. Generally, the aromatic N-8 substituents (**33**–**37**) had greater P-gp efflux compared to the alkyl substituents, but, interestingly, removal of the hydrogen bond donor in pyrazoles **34** and **35** by methylation (**36** and **37**) resulted in a significant reduction in the P-gp efflux, with **37** having excellent permeability and essentially not being a substrate for P-gp (efflux ratio = 1.1). Previously, we have reported that the replacement of the imidazole with a pyrazole results in lower P-gp efflux ratios,[10] and for the alkyl N-8 (**41**–**42**) substituents this was the case. Unfortunately, this effect was not observed with the aryl N-8 analogs, with **46** possessing significant P-gp liability. The ability of these analogs to reduce α-synuclein phosphorylation in Plk-2 transfected HEK-293 cells is shown in Table 5.[12] Generally, the cellular IC_{50} tracks well with the enzyme IC_{50}, with an approximate 50–80 fold shift in the cellular activity.

Compound **37** demonstrated a favorable combination of good Plk-2 potency (Plk-2 IC_{50} = 7 nM), good selectivity over Plk-1 (193-fold), low P-gp efflux, good permeability and moderate microsomal stability, and was chosen for further evaluation. Compound **37** was screened against 308 kinases and had an exception-

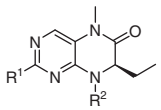
Table 4
SAR of the 7-position of the pteridine

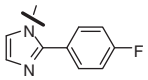
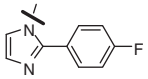
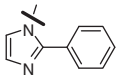
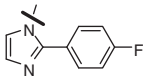
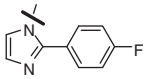
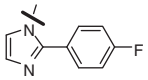
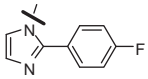
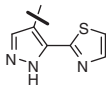
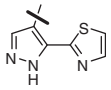
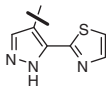
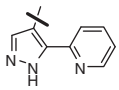
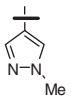


Compds	R^1	R^2	R^3	Plk-2 IC_{50}[a] (μM)	Plk-1 IC_{50}[a] (μM)	Plk-1/Plk-2	OxMet %[b] (m, h)
47[c]		CH_2CF_3	H	0.12	3.48	28	42, 30, 15
48[c]		Et	Me	0.03	7.16	233	29, 34, 17
49	Isopropyl	Spirocyclobutyl	Spirocyclobutyl	1.15	25.3	22	7, 1, 4
50	Isopropyl	Me	Me	2.71	>100	—	25, 10, 54

[a] See Ref. 12.
[b] See Ref. 12. Percent remaining after 30 min incubation with liver microsomes (m = mouse, r = rat and h = human).
[c] Compound is racemic.

Table 5
In vitro P-gp, permeability and metabolic stability data for select analogs



Compds	R^1	R^2	Plk-2 IC_{50}[a] (μM)	293 cell IC_{50}[a] (μM)	P_{app}[a] (nm/s)	P-gp efflux[a]
13			0.042	2.56	158	1.7
19			0.033	2.05	234	1.5
33[b]			0.044	3.87	122	4.2
34			0.012	1.87	87	8.6
36			0.021	0.78	212	2
35[b]			0.009	0.74	132	4.9
37			0.007	0.48	185	1.1
41			0.036	2.99	189	0.8
42[c]			0.039	3.19	168	1.1
43			0.092	5.82	66	8.0
46[c]			0.168	–	101	3.4

[a] See Ref. 12.
[b] Compound is racemic.
[c] See Ref. 14.

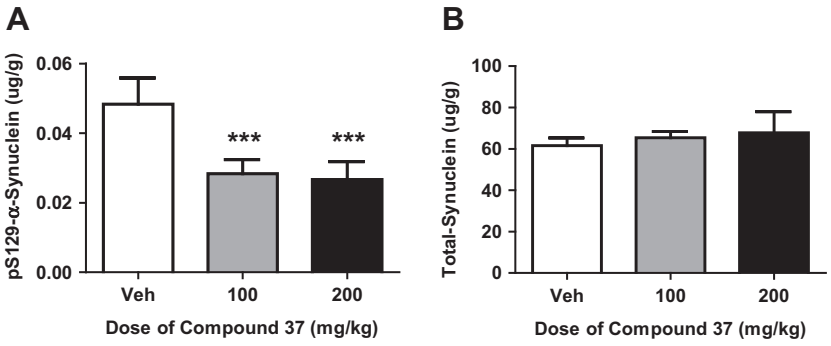


Figure 2. A single oral dose of **37** significantly reduces pS129-α-synuclein levels in rat cerebral cortex (A) whereas total-α-synuclein levels are unchanged (B). The data represents the mean ± SD with $n = 6$ for each group. Significant reduction ($^{***}p < 0.001$) versus vehicle control group (Veh) was determined by one way ANOVA with Dunnett's post hoc test.

Table 6
Plasma and brain levels of **37** at 6 h after a single oral dose

Dose (mg/kg, PO)	Time (h)	Plasma levels mean ± SD (ng/ml)	Brain levels mean ± SD (ng/ml)	Brain/plasma (B/P)
100	6	6286 ± 2594	2252 ± 1051	0.36
200	6	4881 ± 3239	1382 ± 768	0.28

ally clean profile, with only two non-Plk- kinases inhibited at 50% at 10 μM compound concentration.[12] The ability of **37** to reduce Plk-2 mediated phosphorylation of α-synuclein at Ser-129 in the brain was evaluated after single oral doses (100 and 200 mg/kg) in male Sprague–Dawley rats. A 41–45% reduction (p <0.001) of pS129-α-synuclein levels was measured by ELISA in the cerebral cortex 6 h after dosing (Fig. 2A). Similar reductions in pS129-α-synuclein levels were also noted in other brain regions including the olfactory bulb, striatum, ventral midbrain and hippocampus (not shown). As expected in vehicle-treated rats (5% DMSO/1% methylcellulose), total α-synuclein reached 60–70 μg/g with pS129-α-synuclein representing only about 0.1% of the total. The levels of total α-synuclein were unaffected by treatment with **37** (Fig. 2B).

The plasma and brain exposures of **37** at 6 h after oral dosing in rats showed good brain-to-plasma ratios (Table 6) in general agreement with the in vitro PK properties of the compound (Table 5). The 6-h brain concentrations of **37** at the 100 and 200 mg/kg doses were 5.2 and 3.2 μM,[15] respectively, which were well above the Plk-2 cellular IC_{50} value (IC_{50} = 0.485 μM) obtained from Plk-2-transfected HEK-293 cells.

In conclusion, we have expanded the SAR of our Plk- inhibitors. By optimizing both the N-8 substituent and the biaryl region of the inhibitor, we obtained single digit nanomolar compounds with excellent selectivity for Plk-2 over Plk-1, which, when dosed orally, demonstrated a robust reduction in pS129-α-synuclein without affecting total synuclein levels. Compound **37** will be a valuable tool to help understand the role of α-synuclein phosphorylation in the pathophysiology of Parkinson-like symptoms.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.065.

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15. The large variability in the individual data points are most likely due to the poor solubility of **37** at high doses when dosed as a suspension.