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Article

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Mitigation of Acetylcholine Esterase Activity in the 1,7-Diazacarbazole Series of Inhibitors of Checkpoint Kinase 1

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Abstract

Checkpoint kinsase 1 (ChK1) plays a key role in the DNA damage response, facilitating cell-cycle arrest to provide sufficient time for lesion repair. This leads to the hypothesis that inhibition of ChK1 might enhance the effectiveness of DNA-damaging therapies in the treatment of cancer. Lead compound **1** (GNE-783), the prototype of the 1,7-diazacarbazole class of ChK1 inhibitors, was found to be a highly potent inhibitor of acetylcholine esterase (AChE) and unsuitable for development. A campaign of analog synthesis established SAR delineating ChK1 and AChE activities, and allowing identification of new leads with improved profiles. *In silico* docking using a model of AChE permitted rationalization of the observed SAR. Compound **19** (GNE-900) and compound **30** (GNE-145) were identified as selective, orally bioavailable ChK1 inhibitors offering excellent *in vitro* potency with significantly reduced AChE activity. In combination with gemcitabine, these compounds demonstrate an *in vivo* pharmacodynamic effect and are efficacious in a mouse p53-mutant xenograft model.

Introduction

The eukaryotic cell cycle describes the sequential phases of growth, DNA replication, organization and mitosis that a proliferating cell must successfully navigate. Progression through the phases of the cell cycle is controlled by cell cycle checkpoints, biological safety mechanisms that monitor and verify the fidelity of the cell and readiness for the next phase.[1] Activation of checkpoints can occur in response to the detection of DNA damage, resulting in cell cycle arrest to facilitate repair of the damage. As such, the proper function of cell cycle checkpoints enable a mechanism for tumor cells to recover from DNA damage that occurs as a result of radio- or chemotherapeutic intervention, and evade cell death resulting from these therapies.[2]

Checkpoint kinase 1 (ChK1) is a serine/threonine kinase that plays a central role in mediating G2/M cell cycle arrest in response to DNA damage. Detection of damage results in activation of ChK1 through the ATR checkpoint pathway[2] and the initiation of repair mechanisms, or in the event of irreparable damage, induction of apoptosis. Successful repair results in the inactivation of ChK1, allowing resumption of the cell cycle and progression into the mitotic phase.

Tumor cells suffering certain genetic deficiencies, notably mutation in the p53 gene, lack functional G1/S checkpoint control early in the cell cycle.[3] As a result, cells with damaged DNA are able to progress through the G1 phase unabated, and are disproportionately reliant on ChK1-mediated checkpoint control later in the cycle. Failure of ChK1 signaling to halt progression into mitosis with damaged DNA would result in mitotic catastrophe and cell death. This opens the possibility of preferentially sensitizing these p53-disfunctional tumor cells to the effects of DNA damage by disabling ChK1. The potential therapeutic benefit of inhibiting ChK1 has attracted significant interest and a number of preclinical programs have been reported.[4] Several molecules are undergoing clinical evaluation in combination with chemotherapies, and three programs having progressed into Phase II.[5]

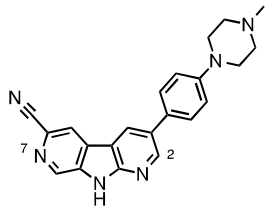
We embarked on a program to discover a small molecule inhibitor of ChK1, with the goal of improving the therapeutic benefit of existing DNA-damaging chemotherapeutic agents used in the treatment of cancer. We envisaged the ChK1 inhibitor would be co-administered and sensitize tumor cells to the cytotoxic effects of the agent. In order to maximize the flexibility of treatment regimen, in combination with both current and future standard of care chemotherapies, we focused our efforts on the discovery of an inhibitor that could be dosed orally. We recognized that a short period of efficient ChK1 inhibition was likely to be sufficient to release damaged cells from checkpoint arrest, and elicit a maximal effect. Our target profile allowed for little or

no cytotoxic activity as a single agent, and minimal toxicity that might be additive to or exacerbate the dose-limiting toxicities associated with chemotherapy.

We previously described the discovery of the 1,7-diazacarbazole series of inhibitors[6] and the identification of **1** (GNE-783)[7] (Figure 1a), an attractive ATP-competitive lead compound suitable for further optimization. Compound **1** demonstrated high biochemical and cellular potency, synergy with chemotherapeutics by isobologram analysis, a pharmacokinetic profile suitable for oral dosing and good kinase selectivity. This molecule has been successfully utilized as a biological tool compound for further investigation of ChK1 as a therapeutic target.[8]

The binding mode of **1** was revealed through X-ray crystallographic analysis of the complex with the ChK1 kinase domain (Figure 1b),[6] indicating the involvement of all nitrogen atoms in the tricyclic core in polar interactions with either the hinge residues (Glu85 & Cys87) or the highly conserved triad of waters in the kinase back-pocket. In addition, the proximity of C2-H to a hinge carbonyl (Cys87) suggests a productive, non-canonical interaction. The water network is such that the ligand (N7) must accept a hydrogen bond from the nearest of the three waters (Figure 1c). The 6-cyano function accepts a hydrogen bond from the protonated catalytic lysine (Lys38). Further binding affinity is gained from hydrophobic interactions with either face of the core tricycle (Leu15, Val23, Leu137) and the 3-phenyl substituent (Gly90) in the 'H2 pocket'.[9] The cationic piperazine moiety makes no direct contact with the protein, but protrudes from the ATP-binding pocket to allow maximal solvation.

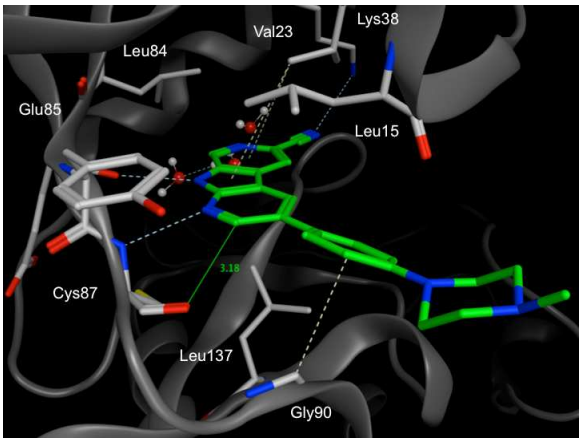
(a)



Compound **1**

ChK1 IC_{50}	1.3 nM
pHH3 EC_{50}	98 nM
Isobologram EC_{10fs}	47 nM
HT-29 GI_{50}	>25 μM
AChE IC_{50}	18 nM

(b)



(c)

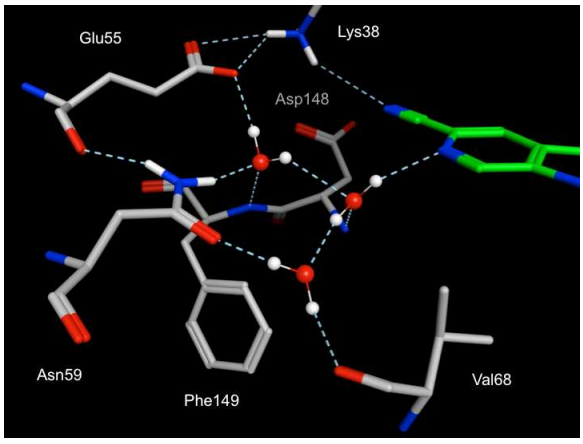


Figure 1. (a) Structure of compound **1**; (b) High resolution (1.9 Å) co-crystal structure of **1** with the kinase domain of ChK1 (PDB code: 4QYH); (c) Key water interactions in the back pocket.

Here we describe the structure-activity relationships we developed through lead optimization, following the identification of undesirable, non-kinase activity in **1** and related analogs. Further, an explanation is provided with the aid of computational modeling, allowing mitigation of this activity and selection of candidates for *in vivo* efficacy evaluation.

Results and Discussion

With potent cellular activity, good pharmacokinetic properties and a good kinase selectivity profile, compound **1** has been successfully employed as an *in vitro* and *in vivo* tool compound for the investigation of ChK1 biology.[8] Compound **1** also represented an excellent lead compound for establishing SAR around this novel kinase scaffold. However, during expanded pharmacokinetic evaluation, a significant undesired activity was discovered. Upon dose-escalation in rodents, muscle fasciculations were observed in the test subjects in a dose-dependent manner, implicating the engagement of neurological targets. Evaluation in a panel of ion channel and receptor targets,[10] designed to reveal pharmacological activities of significant clinical concern, identified potent binding to acetylcholine esterase (AChE) as the key finding. This unexpected activity was considered to represent an unacceptable risk and halted further development of this compound as a therapeutic agent. We therefore began investigation of the SAR of the 1,7-diazacarbazole scaffold, based on our lead compound **1**, with the goal of reducing AChE activity while maintaining the otherwise promising profile.

Inhibition of ChK1 kinase activity *in vitro* (ChK1 IC_{50}) was determined using an AlphaScreen peptide phosphorylation assay.[11] In parallel, we monitored cellular activity in HT-29 cells, a p53-mutant human colorectal adenocarcinoma cell line.[12] Following induction of mitotic arrest by treatment with SN-38,[13] release of G2/M checkpoint control and progression into mitosis was evidenced by phosphorylation of histone H3 (pHH3 EC_{50}).[14] We targeted a cellular potency superior to that of compound **1** (EC_{50} 98 nM). *In vitro* inhibition of human AChE was also monitored,[15] with the goal of significantly improving the margin observed for lead compound **1**, which was found to be highly potent in this assay (IC_{50} 18 nM).

Inspection of the chemical structure of **1** clearly implicated the presence of the exposed basic amine as responsible for the observed AChE activity, ionization of which could mimic the cationic moiety of acetylcholine (ACh). Our initial investigations therefore centered on establishing the SAR related to the 1,7-diazacarbazole core 3-aryl substituent, and the importance of the basic amine function.

Initially a series of analogs was prepared including both phenyl replacements and non-basic phenyl substitutions (Table 1). Significant ChK1 biochemical potency could be achieved with simple non-aromatic substituents (compounds **3**–**5**), or even no substituent (compound **2**), translating to exceptionally high ligand efficiencies.[16] These data indicate the tricyclic 1,7-diazacarbazole core to be highly optimized ligand for the ATP-binding pocket of ChK1, and responsible for much of the affinity of the diverse set of elaborated analogs.

The synthesis of a library of 3-aryl analogs was undertaken, including a comprehensive screen of phenyl substitutions, however no analogs exhibited potency comparable to **1**. The morpholine analog **6**, maintaining comparable hydrophobic contacts to compound **1** but lacking the protonated amine, lost biochemical and cellular potency. The most potent analogs were those containing a phenyl substituent bearing a hydrogen-bond donor (**7**–**10**), suggesting productive polar contacts with residues surrounding the H2 pocket. Examination of X-ray co-crystal structures of the ChK1 kinase domain with these ligands confirmed a common binding mode similar to compound **1**. The co-crystal structure with compound **7** revealed the phenolic hydroxyl was engaging the carboxylate sidechain of Glu91 (Figure 2a). The co-crystal with compound **8** indicated a hydrogen bond with hinge residue Tyr86, but with the acetamide carbonyl acting in the role of acceptor (Figure 2b). Otherwise polar contacts were not observed, and in all cases cellular potency was poor. Several heteroaromatic 3-substituents offered promising biochemical

potency, but in all cases lacked sufficient cellular activity to warrant further progression (**11**–**17**). In general, the poor aqueous solubility of these non-basic analogs was assumed to be limiting both activity in the cellular assay and the correlation with biochemical potency.

The likely role of the basic amine in promoting AChE binding was supported by the lack of AChE inhibitory activity observed in several non-basic 1,7-diazacarbazoles. Compound **5** and 3-aryl analogs **11** and **12** were assayed for AChE inhibition and found to elicit no activity up to 25 μM.

(a) (b)

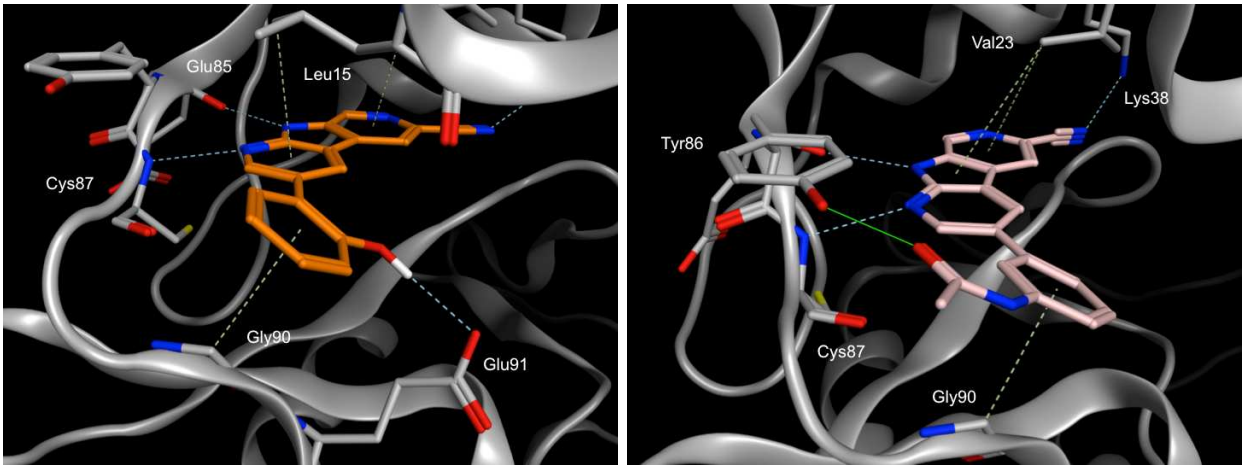
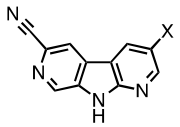
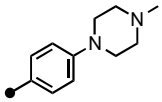
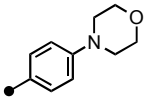
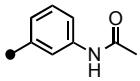
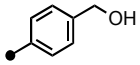
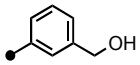


Figure 2. High resolution co-crystal structures of (a) compound **7** (1.85 Å) and (b) compound **8** (1.85 Å) with ChK1 kinase domain indicating polar and hydrophobic contacts (PDB codes: 4RVL & 4RVK).

Table 1. A basic 3-substituent is required for optimal ChK1 potency



Compound	X	ChK1 IC_{50} (nM)	pHH3 EC_{50} (nM)	ChK1 LE	cLogD (pH 7.4)
1		1.3	97.7	0.44	2.8
2	H	414	-	0.60	1.4
3	Br	140	-	0.60	2.4
4	OH	38.1	>5000	0.65	1.0
5	NHEt	56.6	1792	0.56	2.1
6		78.7	219	0.37	3.2
7		41.7	>5000	0.47	3.0
8		13.5	425	0.44	2.6
9		11.4	938	0.48	2.5
10		27.1	>5000	0.46	2.5
11		16.6	180	0.54	2.4
12		20.2	478	0.54	3.1
13		33.8	2495	0.49	2.2
14		14.7	>5000	0.55	1.5
15		127	>5000	0.46	2.3
16		76.5	>5000	0.47	2.2
17		42.7	>5000	0.49	2.1

These data suggested that an ionized basic amine substituent was needed to achieve optimal ChK1 potency. We therefore investigated alternative amine substitutions hoping to establish SAR delineating ChK1 potency from AChE inhibition, and to maintain desirable drug-like properties we targeted amines with a calculated pK_a below 9.[17]

This goal was quickly realized with our initial analogs. In fact, evaluation of a variety of basic analogs demonstrated consistently high ChK1 potency, while dramatically modulating inhibition of AChE (Table 2). These observations are consistent with the amine function being necessary for optimal affinity for ChK1, but having a more critical and intimate role in interaction with AChE.

Compared to **1**, isomer **18** demonstrates a more than 250-fold drop in potency in the AChE assay with only a small loss in ChK1 activity. However this represented the highest potency achievable among meta-substituted analogs (data not shown). Benzylic amine **19** demonstrated comparable ChK1 potency to **1** and excellent cellular activity, and with AChE binding potency reduced by 19-fold. This modification additionally eliminated the undesirable aniline moiety. Methyl substitutions around the benzylic position and piperidine moiety of compound **19** proved effective in modulating AChE activity, while maintaining ChK1 potency (compounds **20**–**24**). The effect on AChE was unpredictable. For example, isomeric dimethyl-derivatives **20** and **21** demonstrate similar ChK1 potency but a 30-fold difference in AChE inhibition. Geminally disubstituted isomers **22** and **23** demonstrate a more than 300-fold difference in AChE inhibition.

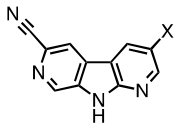
Electron withdrawing substitutents on the piperidine of **19** reduced the basicity of the amine and tended to further reduce AChE inhibition with limited effect on cellular potency (compounds **25**–**29**). Weakly basic morpholine **29** demonstrated the greatest attenuation of AChE activity,

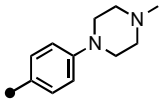
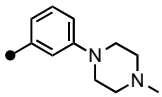
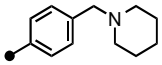
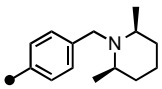
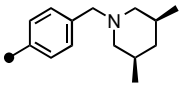
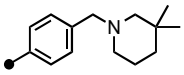
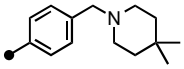
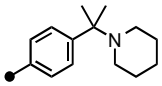
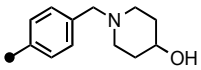
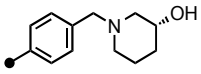
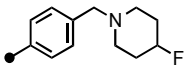
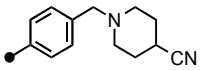
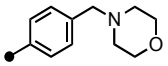
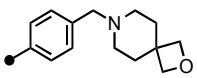
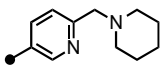
being more than 700-fold less potent than **1** and 40-fold less potent than the piperidine analog, compound **19**.

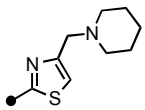
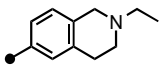
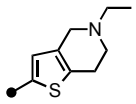
Differences in AChE activity not readily explained by either steric or electronic considerations were also observed. Dimethylpiperidine compound **23** demonstrated the most potent AChE activity observed (AChE IC_{50} 11 nM). In contrast, the spirocyclic oxetane **30**, which differed by the addition of a single oxygen atom, demonstrated a 220-fold reduction in activity (AChE IC_{50} 2.42 μM) while maintaining good potency in the pHH3 cellular assay.

Heterocyclic and bicyclic analogs (compounds **31**–**34**) were investigated but offered no further improvements versus compound **19**. In agreement with the unsubstituted heteroaryl derivatives, pyridine and thiazole analogs bearing a basic amine substituent (compounds **31** and **32**) exhibited reduced biochemical and cellular potency. Bicyclic thiazole **34** was the most effective heterocyclic analog, being comparable to **19** in cellular potency, but with greater activity versus AChE.

Table 2. Basic amine structure acutely modulates AChE activity

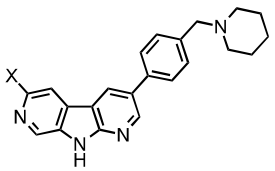


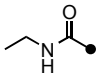
Compound	X	ChK1 IC_{50} (nM)	pHH3 EC_{50} (nM)	AChE IC_{50} (μM)	cLogD (pH 7.4)	cpK_a
1		1.3	97.7	0.018	2.8	7.89
18		1.9	133	4.77	2.8	7.89
19		1.0	53.2	0.333	3.0	8.49
20		2.1	99.1	0.135	3.6	8.90
21		4.2	209	4.10	3.7	8.38
22		9.4	128	3.33	3.7	8.38
23		4.4	172	0.011	3.4	8.71
24		10.2	228	0.507	4.2	8.71
25		1.8	144	0.378	2.2	8.21
26		1.9	118	0.708	2.6	7.72
27		5.0	95.8	0.890	3.4	7.75
28		11.6	339	2.69	2.9	7.23
29		13.9	216	13.3	2.6	6.73
30		2.5	72.8	2.42	2.4	8.26
31		5.3	540	0.187	2.0	8.28

32		13.6	658	0.018	2.9	7.84
33		2.3	152	0.160	2.4	8.52
34		0.78	50.9	0.259	2.1	8.62

We next explored replacement of the 6-cyano function as a potential means to reducing AChE potency (Table 3). With a promising improvement in AChE activity compound **19** was chosen as the parent compound for this series. Unlike the 3-aryl substituent, which does not form a discreet contact with the protein, the 6-cyano is involved in a polar interaction with the charged sidechain of Lys38 and is likely to be less amenable to modification if ChK1 potency is to be maintained. Removal of the cyano entirely yielded compound **35** that demonstrated a relatively modest 19-fold drop in potency. This further supports the optimal nature of the tricyclic 1,7-diazacarbazole core, providing much of the affinity through multiple polar contacts to the hinge and conserved back pocket waters.

Table 3. Effects of 6-subtitution on AChE activity.



Compound	X	ChK1 IC_{50} (nM)	pHH3 EC_{50} (nM)	AChE IC_{50} (μM)	cLogD (pH 7.4)
19		1.0	53.2	0.333	3.0
35		18.6	1922	-	3.2
36		4.3	292	0.217	2.5
37		102	2234	-	3.4
38		19.8	986	-	3.9
39		18.3	447	-	2.3
40		863	-	-	0.7
41		0.46	23.9	0.323	3.4
42		1.3	62.7	0.496	4.1
43		0.95	103	0.450	2.6
44		1.1	145	0.659	3.6
45		0.31	9.0	0.874	3.7
46		0.95	75.1	0.493	4.1
47		9.1	1573	0.777	4.1
48		2.3	168	1.213	3.2
49		2.9	656	1.037	3.1
50		5.1	163	0.266	3.4

As previously observed in the discovery of **1**,[6] the 6-carboxamide analog **36** offered good biochemical potency but weak cellular activity, and was not amenable to substitution (compound **37**). The 6-methoxy and 6-hydroxymethyl analogs (**38** and **39**) offered no great improvement over 6-H (**35**). The far lower potency of the 6-hydroxy (**40**) versus 6-methoxy (**38**) provided an interesting insight, as we may infer that the pyridone tautomer predominates. While the carbonyl offers a good acceptor at the 6-position, this necessarily generates a hydrogen bond donor (N7-H), which cannot satisfy the preference of the rigidly held back pocket water network (Figure 1b).

Replacement of 6-cyano with heteroaromatic rings bearing an appropriately placed acceptor proved highly effective (Table 3). Azole analogs were especially potent, however modulation of AChE activity was limited and the improvement over **19** was modest at best (compounds **41**–**45**). Pyrazole **45** was the most potent analog in biochemical and cellular assays discovered in this series and provided the greatest improvement in AChE inhibition, however reduced selectivity in a kinase screening panel[18] relative to **19** prevented further advancement. Azine derivatives (compounds **46**–**50**) offered a greater improvement, with 3-pyridyl most effective (**46**), but with reduced cellular potency in all cases.

In an effort to rationalize the observed AChE activities we examined correlations with various physical properties. Comparison with either calculated pK_a of the basic amine substituent or the basicity-dependent calculated logD showed no significant correlation with potency and no predictive potential. We therefore sought to use computational modeling of the AChE binding site to rationalize the relative activities of the diazacarbazoles.

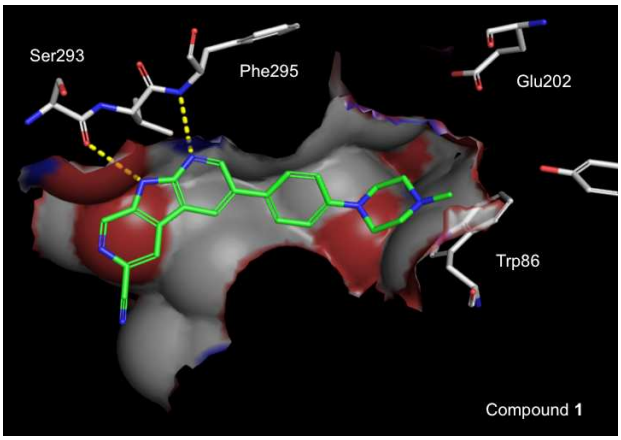
An *in silico* docking model of AChE was prepared utilizing an available high resolution murine protein X-ray crystal structure (PDB code 2HA3, 2.25 Å).[19] This provided the most

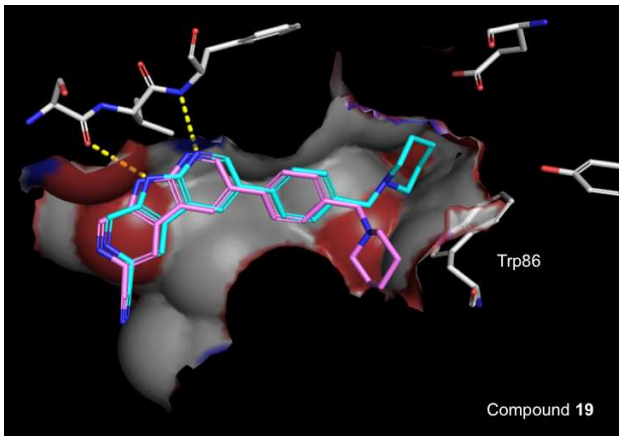
suitable basis structure in which the ACh binding pocket was not occluded, due to the presence of bound choline. The high sequence homology of the murine protein to human in the vicinity of the ACh binding site was further improved by mutation of Ile294 to Val294 in our model.[20]

Docking of **1** using the AChE model revealed a striking prediction (Figure 3a). The kinase hinge-binding 1,7-diazacarbazole core is predicted to form two hydrogen-bonded polar contacts with the Ser293 and Phe295 residues near the mouth of the ACh-binding pocket. These kinase hinge-like contacts ideally orient the 3-aryl substituent into the narrow, hydrophobic channel so as to place the basic amine in a position very similar to that occupied by the ACh quaternary ammonium cation. The close proximity of the cation to an electron-rich indole (Trp86) results in stabilization through a π-cation interaction.[21] This binding mode would be predicted to be of high affinity and highly sensitive to variations in substitution around the basic amine.

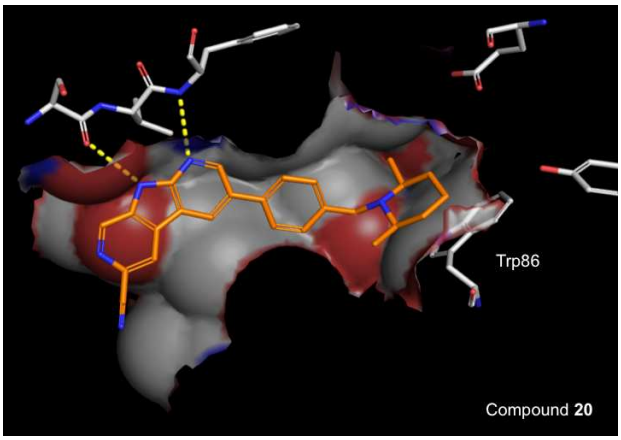
Contrasting with **1**, compound **19** is predicted to bind similarly and is accommodated in the cation-binding pocket in two conformations (Figure 3a). However due to the lesser proximity to Trp86, neither conformation can benefit from π-cation stabilization, and experimentally a 19-fold loss in binding affinity is observed.

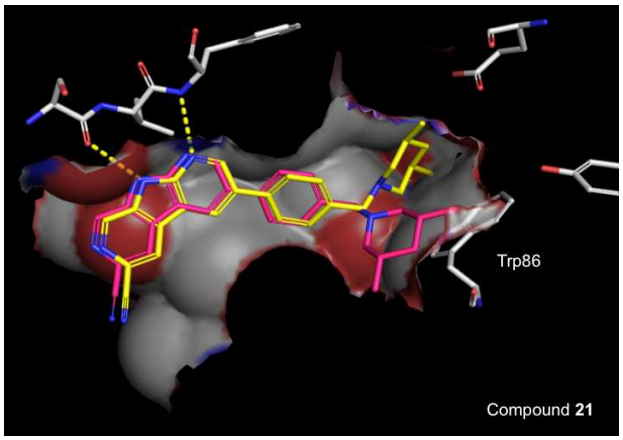
(a)


Ser293
Phe295
Glu202
Trp86
Compound 1

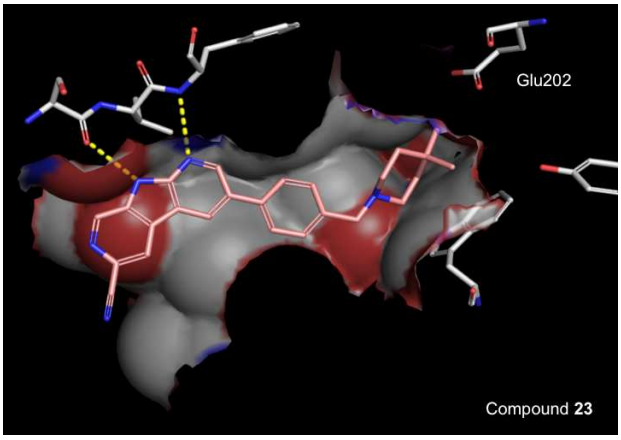

Trp86
Compound 19

(b)


Trp86
Compound 20


Trp86
Compound 21

(c)


Glu202
Compound 23

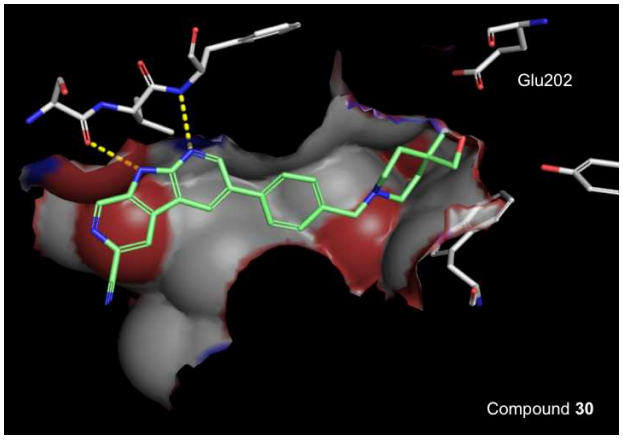
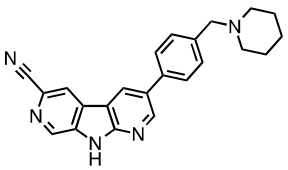

Glu202
Compound 30

Figure 3. Pairwise comparisons of binding modes predicted by docking to AChE can be used to rationalize differences in AChE inhibitory activity: (a) compound **1** (18 nM) vs. compound **19** (333 nM); (b) compound **20** (135 nM) vs. compound **21** (4.10 μM); (c) compound **23** (11 nM) vs. compound **30** (2.42 μM). Binding surface colors are atom-based (carbon grey, oxygen red, nitrogen blue).

Consideration of other pairs of similar analogs with contrasting potencies proved enlightening. Compound **20** docked efficiently, placing the *trans*-2,6-dimethylpiperidine moiety in a lipophilic environment and permitting a low energy chair conformation (Figure 3b). In contrast, isomer **21** is unable to achieve a low-energy conformation and remain within the bounds of the pocket. Compound **21** requires either a higher energy conformation or deformation of the protein, and suffers a 30-fold drop in affinity versus **20**.

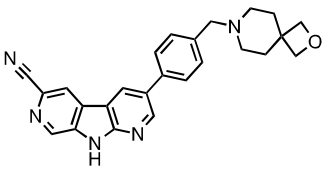
Compound **23** proved to be an exceptionally potent inhibitor of AChE, and is predicted by docking to bind efficiently and fill the hydrophobic space (Figure 3c). Oxetane **30** docks in a very similar conformation, but now suffers from the placement of oxygen in close proximity to the carboxylate (Glu202) and a subsequent destabilization of this mode. Experimentally a 220-fold reduction in activity is observed. These docking results allowed us to rationalize relative AChE binding potencies and qualitatively predict effects of basic amine substitution.

Having established SAR around the 1,7-diazacarbazole core and identified a number of analogs with an improved profile with respect to AChE binding, we utilized a screening cascade to select candidates for evaluation in our mouse pharmacodynamic (PD) model. Selection was made primarily using the results of isobologram analysis[22] to assess synergy with chemotherapeutics *in vitro*, mouse oral pharmacokinetics and kinase profiling. PD results provided a further selection criterion for evaluation in a mouse tumor xenograft model. Using these criteria, compounds **19** and **30** were considered to have the best overall profile and were selected for dosing in this efficacy model (Figure 4).



Compound **19**

ChK1 IC_{50}	1.0 nM
pHH3 EC_{50}	53 nM
Isobologram EC_{10fs}	10 nM
HT-29 GI_{50}	8.7 μM
AChE IC_{50}	0.33 μM



Compound **30**

ChK1 IC_{50}	2.5 nM
pHH3 EC_{50}	73 nM
Isobologram EC_{10fs}	16 nM
HT-29 GI_{50}	>2.5 μM
AChE IC_{50}	2.4 μM

Figure 4. *In vitro* activities of efficacy candidates **19** and **30**.

Isobologram analysis allows the demonstration of synergy in inducing cytotoxicity in HT-29 cells upon treatment with gemcitabine in combination with a ChK1 inhibitor.[8b] Dose ranging with both gemcitabine and ChK1 inhibitor allows generation of a two-dimensional dose response surface and determination of the concentration of sensitizer (ChK1 inhibitor) required to reduce by ten-fold the IC_{50} of gemcitabine-induced cytotoxicity (EC_{10fs}).[22] Both efficacy candidates **19** and **30** demonstrated excellent cellular chemopotentiation of gemcitabine, with EC_{10fs} of 10 nM and 16 nM respectively. In the absence of gemcitabine, compounds **19** and **30** demonstrated single agent cytotoxicity (GI_{50}) of 8.7 μM and >2.5 μM respectively. These data indicate a 870-fold, and greater than 156-fold margin for chemopotentiation over single agent cytotoxicity, and indicate that combination treatment is synergistic rather than additive.

Selectivity for ChK1 relative to other kinases was assessed in an extensive kinase profiling panel.[18] Compounds **19** and **30** were assayed at 100 nM concentration, representing 100-fold and 40-fold excess respectively over the ChK1 biochemical IC_{50}. Compound **19** exhibited greater than 80% inhibition of 15 of the 246 kinases assayed, and 50–80% inhibition of a further 17 kinases. Compound **30** exhibited greater than 80% inhibition of 21 of the 258 kinases assayed,

and 50–80% inhibition of a further 24 kinases. Cyclin-dependent kinases, important in regulating the cell cycle, were not significantly inhibited. These results indicate the selected compounds have good kinase selectivity profiles, with **19** modestly superior to **30**.

Table 4. Cross-species pharmacokinetic parameters for efficacy candidates **19** & **30**

Compound	Species	IV (solution, pH 4.5)			PO (suspension, pH 6.5)				PPB%
		Dose mg/kg	Cl mL/min/kg	Vss L/kg	Dose mg/kg	AUC hr.uM	C_{max} uM	F %	
19	Mouse	1	55.8	6.57	5	2.68*	0.386	86.5	95.0
	Rat	1	24.7	6.35	5	6.19	0.520	67.4	95.3
	Dog	1	118	23.4	2	0.101	0.021	13.1	86.8
	Cyno	1	45.8	25.7	2	0.869	0.044	43.8	88.3
30	Mouse	1	36.8	7.33	5	1.33*	0.191	48.5	96.2
	Rat	1	27.5	7.55	5	8.63	1.10	116	97.5
	Dog	1	10.9	9.63	2	3.51	0.200	46.7	94.8
	Cyno	1	30.8	17.1	2	0.91	0.086	34.0	95.1

* Due to the significant proportional increase when extrapolated after 9h, AUCs for mouse are to the 9h time point.

Consideration of our treatment hypothesis and dosing strategy—a single, appropriately timed dose following chemotherapeutic treatment—required that a ChK1 inhibitor achieve a tumor concentration above a threshold defined by cellular potency for a relatively short period. Cross-species pharmacokinetic parameters for compounds **19** and **30** are given in Table 4. Mouse PK indicated both compounds provided sufficient oral exposure to warrant dosing in our *in vivo* models. For compound **19**, rodents demonstrated moderate clearance with good oral bioavailability. However dog and cynomologous monkey demonstrated higher clearance (greater than liver blood flow) and high volumes of distribution. Compound **30** demonstrated similarly good oral exposures with improved consistency across species. Importantly, no sign of muscle

fasciculations was detected with either compound in any species, or upon dose escalation in rodents, consistent with the increased margins versus inhibition of AChE.

In our pharmacodynamics model, mice bearing HT-29 xenografts were treated with gemcitabine (60 mg/kg) followed after 24 h by a single oral dose of ChK1 inhibitor. Activation of checkpoint control in response to gemcitabine, and subsequent abrogation of G2/M checkpoint arrest in response to ChK1 inhibition, is evidenced by the level of activation of the histone H2AX. Phosphorylation of H2AX (to form γH2AX) occurs in response to DNA double-strand breaks (DSB) resulting from mitotic entry with unrepaired DNA damage.[23] The level of phosphorylation was monitored by Western blot analysis of tumor tissues harvested 16 h after ChK1 inhibitor administration. Compound **19** dosed orally at 10–40 mg/kg (Figure 5a) and compound **30** dosed orally at 12.5–50 mg/kg (Figure 5b) demonstrated an increase in γH2AX in a dose-dependent manner. In contrast, control arms indicated no increase in DSB upon treatment with either gemcitabine or ChK1 inhibitor alone.

Compounds **19** and **30** were next evaluated in a multi-dose mouse efficacy study. NCR nude mice[24] bearing HT-29 xenografts were treated with gemcitabine, followed after 24 h by ChK1 inhibitor dosed orally, a time interval that has been shown to be optimal in this model.[25] For compound **19**, mice received five treatment cycles at five day intervals (Figure 5c). For compound **30**, mice received four treatment cycles at four day intervals (Figure 5d). Efficacy was determined by monitoring tumor volume. Both compounds demonstrated improved reduction of tumor growth at all doses compared to gemcitabine alone, and in a dose-dependent manner. At the 50 mg/kg dose level, compound **30** demonstrated complete inhibition of tumor growth (111% inhibition, p value 0.0002). Body weights were unaffected in all treatment groups, indicating the test compounds did not reduce the tolerability relative to gemcitabine alone.[26]

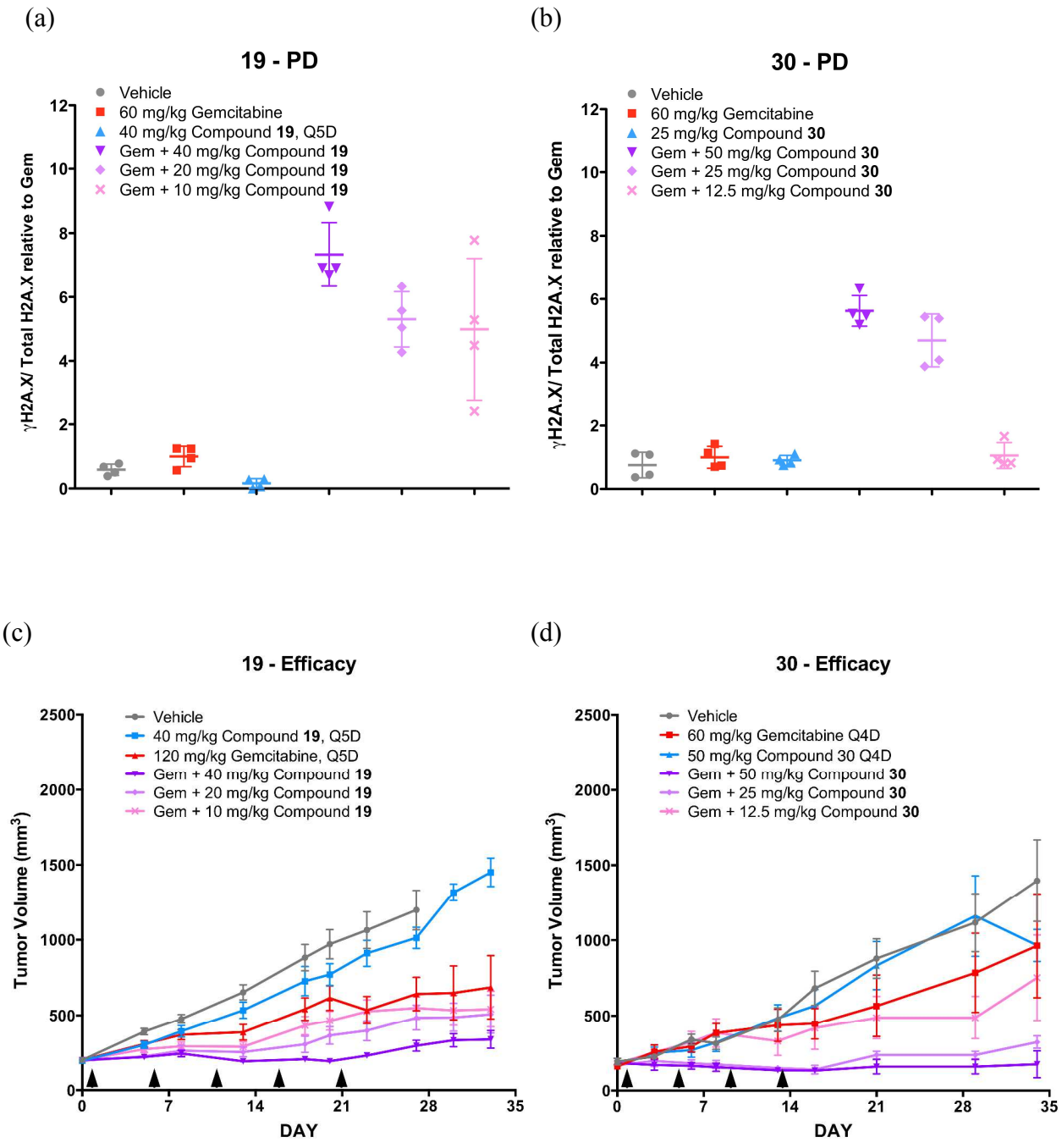
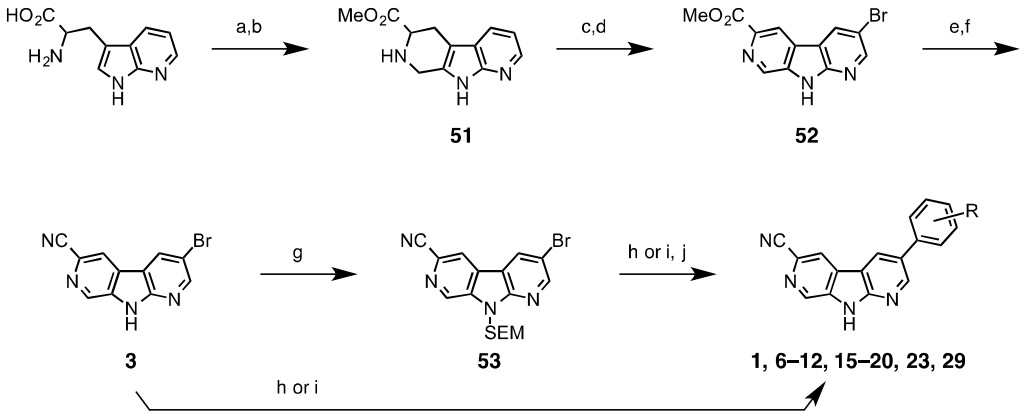


Figure 5. *In vivo* activity in NCR nude mice bearing HT29 p53^{mut} xenografts of compounds **19** and **30** dosed orally in MCT suspension 24 h following gemcitabine. Pharmacodynamics: increase in proportional γH2AX 16 h after treatment with (a) compound **19** or (b) compound **30**. Efficacy: dose dependent-reduction in tumor growth following (c) five cycles of gemcitabine

(120 mg/kg) in combination with compound **19**, or (d) four cycles of gemcitabine (60 mg/kg) in combination with compound **30**. Gemcitabine doses are indicated by arrows; the higher dose of gemcitabine and frequency of treatment used with compound **19** limits the value of direct comparison. Tumor growth inhibition and statistical analyses are provided in the supporting information.

Chemistry

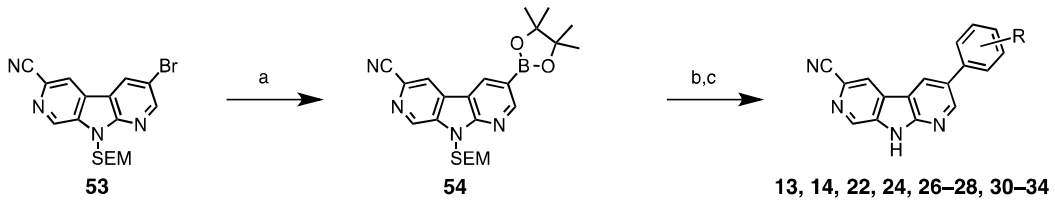
The synthesis of the key 3-bromo-6-cyano-1,7-diazacarbazole intermediate (**3**) was achieved in six steps from commercially available 7-azatryptophan (Scheme 1). Esterification and Pictet-Spengler reaction with formalin afforded an intermediate tetrahydro-1,7-diazacarbazole (**51**), and aromatization with selenium dioxide followed by regioselective bromination yielded methyl 9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxylate (**52**).[6] Conversion to the 6-carboxamide derivative was achieved by treatment with ammonia, and dehydration afforded intermediate **3**. Protection as the SEM derivative provided compound **53**, and either of intermediates **3** or **53** could serve as the biaryl coupling precursor. Suzuki-Miyaura couplings with various boronic acid or boronate esters and subsequent deprotection afforded the corresponding 3-aryl-6-cyano-1,7-diazacarbazoles (**1**, **6**–**10**, **12**, **18**–**20**, **23** and **29**) (Scheme 1). Similarly, Stille couplings with the appropriate aryl stannane and subsequent deprotection afforded compounds **11** and **15**–**17**.



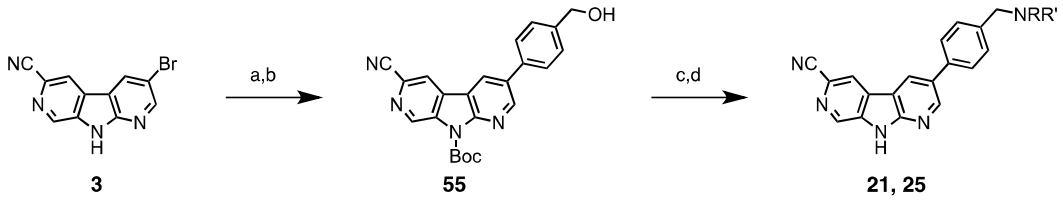
Scheme 1. Synthesis of 6-cyano-1,7-diazacarbazoles from 7-azatryptophan. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C–reflux; (b) $CH_2O_{(aq)}$, Py, 100 °C; (c) SeO_2, Py, dioxane, 100 °C; (d) Br_2, NaOAc, AcOH, 100 °C; (e) NH_3, MeOH, 140 °C, autoclave; (f) TFAA, NEt_3, THF, 0 °C–rt; (g) SEMCl, NaH, DMF; (h) Boronic acid or boronate ester, $Pd(dppf)Cl_2$, Cs_2CO_3(aq), dioxane, reflux; (i) Aryl stannane, $Pd(PPh_3)_4$, LiCl, dioxane, μW; (j) TBAF, THF, 50 °C.

Compound **53** could be converted to the corresponding boronate pinacol ester **54** (Scheme 2). Suzuki-Miyaura couplings of **54** with aryl halides, and subsequent removal of the SEM protecting group, provided an alternate route to 3-aryl derivatives (**13**, **14**, **22**, **24, 26**–**28** and **30**–**34)**.

Suzuki-Miyaura coupling of **3** with 4-(bromomethyl)phenylboronic acid at elevated temperature resulted in concomitant bromide hydrolysis, and subsequent Boc-protection afforded benzylic alcohol intermediate **55** (Scheme 3). Conversion to the mesylate and treatment with the appropriate secondary amines resulted in mesylate displacement and deprotection to provide benzylic amines **21** and **25**.



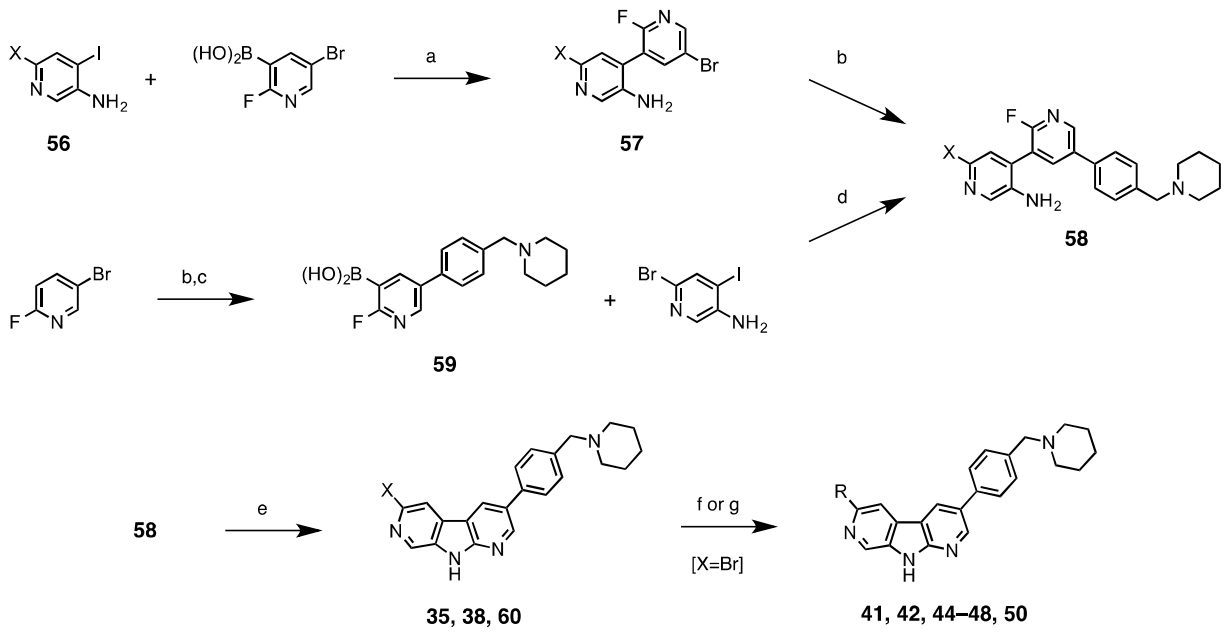
Scheme 2. 3-Aryl diazacarbazoles via reversed Suzuki-Miyaura coupling route. Reagents and conditions: (a) Bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, dioxane, DMSO, 120 °C; (b) Aryl bromide, $Pd(PPh_3)Cl_2$, Cs_2CO_3 (aq), DME-IMS-water, μW, 140 °C; (c) TBAF, THF, 55 °C.



Scheme 3. (4-Aminomethyl)phenyl derivatives via mesylate displacement route. Reagents and conditions: (a) 4-(bromomethyl)phenylboronic acid, $Pd(dppf)Cl_2$, KF(aq), MeCN, μW, 175 °C; (b) Boc_2O, MeCN, 50 °C; (c) MsCl, NEt_3, DCM, 0 °C–rt; (d) HNR^1R^2, NEt_3, MeCN, 50 °C.

Compound **2** was prepared by reduction of **53** with zinc and ammonium formate, and SEM deprotection. Compound **4** was prepared from boronate ester **54** by oxidation with *N*-methylmorpholine *N*-oxide and deprotection. Compound **5** was prepared from **53** by palladium-catalyzed arylation of *tert*-butyl carbamate, alkylation of the intermediate with sodium hydride and ethyl iodide, and double deprotection.

Synthesis of the 1,7-diazacarbaole core with various 6-substituents could be achieved in three steps from 6-substituted 3-amino-4-iodopyridines **56** (Scheme 4). Suzuki-Miyaura coupling of **56** with 5-bromo-2-fluoropyridine-3-boronic acid affords the corresponding bipyridyls **57**, and subsequent coupling with the appropriate aryl boronate provides **58** (X=H, OMe). Alternatively, boronic acid **59** can be prepared in two steps from 5-bromo-2-fluoropyridine. Suzuki-Miyaura coupling of **59** with 6-bromo-3-amino-4-iodopyridine provided a more convergent route to **58** (X=Br). Base-mediated cyclization of the bipyridyl intermediates **58** yielded the 1,7-diazacarbazoles (**35**, X=H; **38**, X=OMe; **60**, X=Br). Compounds **41**, **42**, **44**–**47** and **50** were synthesized from intermediate **60** (X=Br) by Suzuki-Miyaura couplings with the appropriate heteroaryl boronic acids. Compound **48** was synthesized from **60** by Stille coupling with 2-(tributylstannyl)pyrazine. Compound **49** was prepared by Stille coupling of 4-(tributylstannyl)pyridazine with **58** (X=Br) and subsequent base-mediated cyclization.



Scheme 4. Generalized biaryl synthesis of the 1,7-diazacarbazole core. Reagents and conditions: (a) Pd(dppf)Cl_2, KF(aq), MeCN, 95 °C; (b) 4-(Piperidin-1-ylmethyl)phenylboronic acid hydrobromide, Pd(PPh_3)Cl_2, KF(aq), MeCN, μW, 100 °C; (c) LDA, B(O^iPr)$_3$, THF, –10 °C; (d) Pd(dppf)Cl_2, KF(aq), MeCN, 90 °C; (e) NaHMDS, THF, rt; (f) Pd(dppf)Cl_2, Na_2CO_3(aq), MeCN, μW; (g) Aryl stannane, Pd(PPh_3)$_4$, LiCl, dioxane, μW.

6-Carboxamides **36** and **37** were synthesized from the corresponding 6-carboxylic acid methyl ester by thermal reaction with ammonia and ethylamine respectively. Reduction of the same ester with lithium aluminum hydride yielded compound **39**. Conversion of the methyl ester to 2,4,5-oxadiazole **43** was accomplished via the acid hydrazide, by treatment with hydrazine and subsequent condensation with ethyl formate. 6-Hydroxy compound **40** was prepared from the 6-methoxy analog **38** by demethylation with hydrogen bromide.

Conclusions

Lead compound **1** represented the prototype 1,7-diazacarbazole, a novel scaffold derived through structure-based design. Through a series of investigations we found the scaffold to be an exceptionally ligand efficient platform for building inhibitors of ChK1. We established the requirement for a basic amine to achieve the optimal ChK1 biochemical and cellular potency, and observed a broad tolerance of basic amine substitutions. By screening against AChE we were able to delineate potent ChK1 inhibition from AChE activity, and select compounds that maintained the excellent ChK1 potency of **1** with an improved margin.

Screening in the AChE assay demonstrated a much lesser tolerance of different amine substitutions, but with no clear trend. We turned to computational modeling of the AChE binding pocket, and were able to explain the observed high potency of compound **1**. Further, we could rationalize our empirical observations of AChE SAR through structure-based analyses. The AChE docking model proved to be a valuable tool for understanding existing SAR and guiding design. It is noted that the interactions predicted in this model, hinge-like hydrogen bonding and hydrophobic interactions in the H2 region, show great similarity to those utilized by many kinase inhibitors being investigated. This suggests that AChE inhibition might be a common undesired activity in other kinase inhibitor series, particularly those bearing a solvent-exposed basic amine.

Compounds were selected for evaluation in an *in vivo* pharmacodynamics model based primarily on cellular potency, kinase selectivity and mouse oral PK data. Compounds **19** and **30** were considered to have the best overall profiles, and demonstrated excellent efficacy in the mouse tumor xenograft model. These compounds were well tolerated *in vivo* with no evidence of muscle fasciculations. Compounds **19** (GNE-900)[7] and **30** (GNE-145)[7] have achieved our goal of identifying potent, selective and efficacious inhibitors of ChK1 with reduced acetylcholine

esterase inhibition, and improved safety, relative to lead compound **1**. Compound **19** has proved useful as an *in vivo* tool compound for investigating ChK1 biology.[8b,25]

Experimental Section

ChK1 AlphaScreen biochemical assay: *In vitro* inhibition of ChK1 kinase activity was assessed using homogenous AlphaScreen technology (Perkin Elmer) to monitor phosphorylation of a peptide substrate.[11] Active recombinant Checkpoint Kinase 1 (ChK1) was obtained from Invitrogen (Invitrogen cat #P3040). The substrate for ChK1 activity was biotinylated AKT substrate peptide (Cell Signaling Technology product #1065). The kinase reaction was carried out in 25 mM HEPES pH 7.5, 10 mM magnesium chloride, 5 mM α-glycerophosphate, 0.1 mg/ml Triton X-100, 100 μM sodium orthovanadate [Na_3VO_4], 2 mM dithiothreitol [DTT] 10 μM ATP and 0.15 nM ChK1 enzyme at 25˚C for 30 minutes. Control compound **1** exhibited an IC_{50} of 1.5 nM ± 0.382 nM (n=10). All other IC_{50} values are the geometric mean derived from a minimum of 2 independent assays, with a maximum of 2-fold variation observed between the two assays. Ligand efficiencies[16] were calculated as follows: ChK1 LE = 1.4*log(IC_{50})/heavy atom count.

Checkpoint abrogation cellular assay: HT-29 cells (ATCC) were pre-treated with 20 nM SN-38, washed, and then incubated with ChK1 inhibitor and 300 nM nocodazole for 24 hours. Following treatment, the cells were lysed and phospho-histone H3 (pHH3) content in the lysates was determined using Meso Scale phospho-histone H3 kit (product # K110EWA) following the instructions provided by the supplier (Meso Scale Discovery). Control compound **1** exhibited an IC_{50} of 97.7 nM ± 36.7 nM (n=6). All other reported IC_{50} values are the geometric mean derived from a minimum of 2 independent assays with a maximum of 2.5 fold variation observed between the two assays.

Isobologram assay: HT-29 cells (ATCC) were treated with varying concentrations of Gemcitabine Hydrochloride (LKT Laboratories #G1745) either alone or in combination with

ChK1 inhibitor for 72 hours. Cell viability was determined using Cell Titer-Glo Luminescent Cell Viability Assay (Promega #G7572). GI_{50} values were plotted in an isobologram plot to determine the effective concentration of ChK1 inhibitor that potentiates the potency of gemcitabine 10-fold (EC_{10fs}). Synergistic effects were determined using the combination index (CI) or the Bliss independence analysis methods.[22]

Acetylcholine esterase inhibition assay: A homogenous Horseradish peroxidase-coupled fluorescence assay (Life Technologies) was used to test for inhibition of AChE activity following exposure to ChK1 inhibitors.[15] Human AChE was obtained from Sigma and tested at 0.006 U/ml with 200 μM Amplex Red, 0.05 U/ml Horseradish peroxidase, 0.06 U/ml Choline oxidase, 0.25 μM Acetylcholine chloride (all Life Technologies) in the presence of test compound in proprietary assay buffer (Life Technologies). The enzyme assay was carried out at 25 °C for 30 min. Fluorescence signal was measured using a Flexstation/SpectraMax (Molecular Devices).

Kinase selectivity profiling: Screening of compounds **19** and **30** was performed using SelectScreen® Kinase Profiling Services (Invitrogen-Life Technologies, Madison, USA). Single-point inhibition data generated at 100 nM concentration for compounds **19** and **30**, for 246 and 258 kinases respectfully, are provided in the supporting material.

Pharmacodynamic studies: Randomized groups of HT-29 tumor-bearing mice (n=4) were pretreated with 60 mg/kg Gemcitabine or saline 24 hours before receiving the indicated oral doses of ChK1 inhibitor, or MCT vehicle. At 16 hours post–ChK1i dose, terminal samples were collected. Tumors were excised and flash-frozen for pharmacodynamic analysis.

Frozen tumors were crushed into powder on dry ice using a BioPulverizer (BioSpec Products) and extracted with ice-cold AB+ buffer. AB+ extraction buffer, designed for maximum recovery

of histones, is composed of 20 mmol/L Tris pH 7.5, 50 mmol/L NaCl, 0.5% SDS, 0.5% deoxycholate, and 0.5% Triton X-100 (w/v) and is supplemented with 1 mmol/L EDTA, 1 mmol/L dithiothreitol, 1 mmol/L NaF, 0.2 mmol/L sodium vanadate, 5 mmol/L □-glycerophosphate, protease inhibitors (Sigma-Aldrich), 1 mmol/L phenylmethysufonyl fluoride, and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich). After thorough disruption by sonication, lysates were clarified by centrifugation at 4 °C. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce), and brought to a standard concentration of 5 mg/mL.

Tumor lysates were loaded with 25 μg/lane and run on 4–12% NuPage Novex Bis-Tris gels (Life Technologies). Proteins were then transferred to nitrocellulose membranes using the iBlot system: Program 3 for 10 minutes (Life Technologies). For antigen detection, Western blot analyses were probed with primary antibodies: total H2AX (Upstate/Millipore) and pS139 H2AX (Cell Signaling Technology) at a 1:1000 ratio in LI-COR blocking buffer. Secondary detection antibody was goat-anti-rabbit immunoglobulin G (IgG; H&L) IR-Dye800 (Rockland Immunochemicals, Inc.) used at 1:10,000. The Western blot analyses were visualized with LI-COR IR imaging technology and band intensities were quantitated using Odyssey software (LI-COR Biosciences). For each lane, a ratio of γH2A.X:total H2A.X intensity was calculated. The ratio was then expressed as a fold-change from the average ratio of the 4 samples in the Gemcitabine only group. The Western blot images used in the generation of Figure 5a, for compound **19** and compound **30** are provided in the supporting material.

Efficacy studies: NCr nude mice (Taconic), ages 8 to 12 weeks, were inoculated with 5 x10^6 HT-29 cells in a 50:50 suspension of HEPES-buffered saline solution:Matrigel (BD Biosciences). When the tumor volumes reached between 125 and 250 mm^3, mice were

distributed into volume-matched cohorts. Gemcitabine (Gemzar; Eli Lilly) was formulated in saline and dosed intraperitoneally (i.p.). Compounds **19** and **30** were formulated as suspensions in 0.5% methylcellulose/ 0.2% Tween 80 (MCT), and administered by oral gavage. Tumor volume was measured in two dimensions (length and width) using Ultra Cal-IV calipers (model 54-10-111; Fred V. Fowler Company; Newton, MA). Body weights were collected two to three times per week, and body condition monitored daily; figures are provided in the supporting material. Mice with tumor volumes greater than 2000 mm^3 or with losses in body weight greater than 20% from their initial body weight were promptly euthanized, per IACUC guidelines.

Mean tumor volume and SEM values (n = 5, or n = 10) were calculated using GraphPad Prism software, version 6, at end of treatment. Two-way analysis of variance (ANOVA) and p-values were determined using the Tukey test with GraphPad Prism software. %TGI was calculated using linear mixed-effect modeling (LME). This method captures the maximum information from the study by using within animal correlations (repeated measurements of tumor volumes from individual animals over time) and addresses complications from unequal variances and animal censoring issues (e.g., modest dropouts due to non-treatment-related death of animals before study end). Curve fitting was applied to log 2-transformed individual tumor volume data using R package nlme, version 3.1–97 in R as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to Gemcitabine alone, such that: %TGI = 100 x 1 – $(AUC_{treatment}/day)$ / $(AUC_{Gemcitabine}/day)$.

Chemistry

Reagents and solvents purchased from commercial sources were used as obtained. Moisture or oxygen sensitive reactions were conducted under a nitrogen atmosphere using standard air-sensitive handing techniques. ^1H NMR spectra were recorded at ambient temperature and

chemical shifts are expressed in ppm relative to tetramethylsilane. The following abbreviations have been used in listing NMR data: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. The compounds described were determined to be sufficiently pure (>95%) by analytical HPLC analysis and LCMS analysis following final purification. Further details of chemistry procedures are given in the supplementary material.

Methyl 2-amino-3-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)propanoate bis hydrochloride salt. 7-Azatryptophan hydrate (100 g, 0.45 mol) was suspended in methanol (1 L) in a 3 L, 3-necked, round bottom flask fitted with a thermometer, pressure-equalizing dropping funnel and a mechanical stirrer. The mixture was cooled in ice and thionyl chloride (267 g, 2.24 mol) was added dropwise over *ca.* 30 mins. During the addition the reaction mixture became a thick paste. After the addition was complete, the ice bath was replaced by a heating block and the reaction mixture was heated to reflux and stirred for *ca.* 4 h. After cooling to room temperature the mixture was evaporated under vacuum and the residue triturated with methanol-diethyl ether (1:1) (*ca.* 1 L) and filtered to give the title compound (129 g, 99%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (s, 1H), 8.61 (br s, 2H), 8.54 (dd. J = 8.0 Hz, 1.06 Hz, 1H), 8.40 (dd, J = 8.4 Hz, 1.3 Hz, 1H), 7.61 (s, 1H), 7.41(m, 1H), 4.32 (m, 1H), 3.7 (s, 3H), 3.4 (d, J = 6.4 Hz, 2H). LCMS (ESI): *m/z*=220 $[M+H]^+$.

Methyl 6,7,8,9-tetrahydro-5*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxylate (**51**). Methyl 2-amino-3-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)propanoate bis hydrochloride salt (125 g, 0.43 mol) was suspended in pyridine (1 L) and treated with formaldehyde solution (37% in water) (38.5 mL, 0.47 mol). The resulting mixture was heated to 100 °C and stirred for 1 h. After cooling to room temperature and then in ice, the mixture was filtered and the solid washed with cold pyridine followed by dischloromethane and dried to give the title compound (85.7 g, 87%) as a

white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.7 (s, 1H), 10.2 (br s, 1H), 8.21 (m, 1H), 7.94 (m, 1H), 7.08 (m, 1H), 4.6 (m, 1H), 4.4 (m, 2H), 3.8 (s, 3H), 3.2 (m, 2H). LCMS (ESI): *m/z*=232 $[M+H]^+$.

Methyl 9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxylate. Methyl 6,7,8,9-tetrahydro-5*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxylate (85 g, 0.37 mol) was suspended in 1,4-dioxan (1 L) and treated with pyridine (150 mL) followed by selenium dioxide (61 g, 0.55 mol). The mixture was heated at 100 °C overnight with mechanical stirring. The resulting grey slurry was cooled to room temperature and filtered. The solid was washed with dioxan and dried as much as possible on the filter by pulling air through the cake. The resulting cake was heated to 100 °C in DMF (1.5 L) which left a heavy black solid in suspension. The black suspension (selenium residues) was removed by filtration and washed with a little hot DMF. The filtrate was treated with an equal volume (*ca.* 1.5 L) of water and the slurry cooled to room temperature and filtered. The solid was washed sequentially with water, methanol and diethyl ether and dried to give the title compound (58 g, 69%) as a pink solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.6 (br s, 1H), 8.99 (m, 2H), 8.57 (dd, J = 7.8 Hz, 1.7 Hz, 1H), 8.66 (dd, J = 4.8 Hz, 1.5 Hz, 1H), 7.39 (dd, J = 7.8 Hz, 4.8 Hz, 1H), 3.92 (s, 3H). LCMS (ESI): *m/z*=228 $[M+H]^+$.

Methyl 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxylate (**52**). Methyl 9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxylate (58 g, 0.26 mol) was suspended in glacial acetic acid (2 L) and treated with sodium acetate (66 g, 0.79 mol). The slurry was treated with bromine (122 g, 0.76 mol) over *ca.* 10 mins and the resulting mixture heated to 100 °C with mechanical stirring. The slurry became extremely thick during heating but stirring was continued for 90 mins. After cooling to ambient temperature the slurry was filtered and washed with acetic acid and dried as much as possible on the filter by pulling air through the cake. The cake was

collected and suspended in water (*ca.* 2 L) and the resulting slurry treated with aqueous sodium thiosulfate solution. Filtration resulted in a cake which was saturated with water, which was collected and dried at 70 °C under vacuum to provide the title compound (75.3 g, 96%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (s, 1H), 9.19 (d, *J* = 2.3 Hz, 1H), 9.04 (d, *J* = 1.0 Hz, 1H), 9.00 (d, *J* = 1.0 Hz, 1H), 8.73 (d, *J* = 2.3 Hz, 1H), 3.92 (s, 3H). LCMS (ESI): *m/z*=306/308 $[M+H]^+$.

3-Bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxamide. Methyl 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxylate (75 g, 0.25 mol) was suspended in a solution of 7M ammonia in methanol (ca. 800 mL) and the resulting slurry sealed in an autoclave and heated at 140 °C overnight. After cooling to room temperature the resulting slurry was evaporated to approximately half volume under vacuum and filtered. The solid was washed with methanol and dried to give the title compound (55.4 g, 78%) as a grey-green solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (dd, J = 6.6Hz, 2.3Hz, 2H), 9.01–8.90 (m, 3H), 8.69 (dd, J = 4.2 Hz, 2.3Hz, 2H), 8.09 (s, 1H), 7.53 (s, 1H). LCMS (ESI): *m/z*=291/293 $[M+H]^+$.

3-Bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**3**). 3-Bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxamide (55 g, 0.19 mol) was suspended in tetrahydrofuran (2 L) and treated with triethylamine (191 g, 1.9 mol). The mixture was cooled in ice and treated carefully with trifluoroacetic anhydride (198 g, 0.95 mol). The ice bath was removed and the mixture stirred at room temperature for 4 h. The resulting solution was poured onto 2M hydrochloric acid and the mixture filtered to remove a small amount of fine material which was hampering separation. The filtrate was separated and the aqueous phase further extracted with tetrahydrofuran. The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated. The residue was triturated with methanol to give 41 g (79%) of crude product. This

was recrystallized from glacial acetic acid (ca. 1 L) with a hot charcoal treatment to give the pure title compound (28.4 g, 55%) as a cream solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (s, 1H), 9.08–9.04 (m, 2H), 8.91 (d, J = 1.1 Hz, 1H), 8.79 (d, J = 2.3 Hz, 1H). LCMS (ESI): *m/z*=271/273 $[M+H]^+$.

3-Bromo-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**53**). To a suspension of 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (300 mg, 1.1 mmol) in DMF (2.5 mL), under an inert atmosphere, was added sodium hydride (65 mg, 1.3 mmol) and the reaction mixture was allowed to stir at ambient temperature for 30 min. After this time, the reaction mixture was cooled to 0 °C and 2-(trimethylsilyl)ethoxymethyl chloride (0.25 mL, 1.3 mmol) was added dropwise and then the resultant suspension was allowed to warm to room temperature. Water (0.5 mL) was added to the resultant suspension to quench the reaction, then the solvents were removed *in vacuo* and the residue was purified by flash chromatography (silica, 12 g column, Isco, 0-15% ethyl acetate in cyclohexane) to afford the title compound as an off-white crystalline solid (266 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (s, 1H), 9.08 (d, *J* = 1.1 Hz, 1H), 9.00 (d, *J* = 2.2 Hz, 1H), 8.95 (d, *J* = 1.1 Hz, 1H), 8.87 (d, *J* = 2.2 Hz, 1H), 4.37 (s, 2H), 2.87 (s, 6H). LCMS (ESI): *m/z*=403/405 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**19**). A suspension of 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**3**) (4.70 g, 17.2 mmol), 4-(piperidin-1-ylmethyl)phenylboronic acid (5.65 g, 25.8 mmol) and 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1.26 g, 10 mol%) in a mixture of aqueous cesium carbonate (18 mL, 2.0 M solution) and dioxane (55 mL) was evacuated and purged three times with nitrogen, and the mixture heated at reflux for 8 h under a nitrogen atmosphere. The reaction mixture was cooled to ambient temperature and filtered through

Celite®, with additional washings with a mixture of methanol-dichloromethane (1:1, 50 mL), and concentrated *in vacuo*. Flash chromatography of the residue (silica, 10% methanol-dichloromethane) afforded the title compound as a cream colored solid (2.27 g, 36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09-9.01 (m, 3H), 8.95 (d, *J* = 1.0 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 3.50 (s, 2H), 2.37 (m, 4H), 1.55–1.48 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=368 $[M+H]^+$.

Compounds **1**, **6**–**10**, **12**, **18**, **20**, **23** and **29** were prepared similarly to **19**:

3-(4-(4-Methylpiperazin-1-yl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**1**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.84 (s, 1H), 9.03–8.93 (m, 4H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.10 (d, *J* = 8.7 Hz, 2H), 3.22 (m, 4H), 2.54–2.46 (m, 4H), 2.24 (s, 3H). LCMS (ESI): *m/z*=369 $[M+H]^+$.

3-(4-Morpholinophenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**6**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H), 9.04–9.02 (m, 2H), 8.98 (d, *J* = 2.3 Hz, 1H), 8.94 (d, *J* = 1.0 Hz, 1H), 7.72 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 3.81 (t, *J* = 4.6 Hz, 4H), 3.24 (t, *J* = 4.6 Hz, 4H). LCMS (ESI): *m/z*=356 $[M+H]^+$.

3-(2-Hydroxyphenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**7**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 9.85 (s, 1H), 9.09 (s, 1H), 9.02 (s, 1H), 8.96 (s, 1H), 8.91 (s, 1H), 7.47–7.45 (m, 1H), 7.33–7.28 (m, 1H), 7.09–7.00 (m, 2H). LCMS (ESI): *m/z*=287 $[M+H]^+$.

N-(3-(6-Cyano-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridin-3-yl)phenyl)acetamide (**8**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 10.11 (s, 1H), 9.04–8.98 (m, 3H), 8.92 (s, 1H), 8.02 (s, 1H), 7.59–7.56 (m, 1H), 7.48–7.43 (m, 2H), 2.07 (s, 3H). LCMS (ESI): *m/z*=328 $[M+H]^+$.

3-(4-(Hydroxymethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**9**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 9.15 (s, 1H), 9.11–9.08 (m, 2H), 9.02 (s, 1H),

7.86–7.84 (d, *J* = 8.4 Hz, 2H), 7.57–7.55 (d, *J* = 8.4 Hz, 2H), 5.35–5.20 (s, 1H), 4.65 (s, 2H). LCMS (ESI): *m/z*=301 $[M+H]^+$.

3-(3-(Hydroxymethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**10**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 9.28 (m, 4H), 7.75 (s, 1H), 7.68–7.66 (m ,1H), 7.51–7.48 (m, 1H), 7.38–7.36 (m ,1H), 5.50 (br, 1H), 4.61 (s, 2H). LCMS (ESI): *m/z*=301 $[M+H]^+$.

3-(Thiophen-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**12**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 9.23–9.21 (m, 3H), 9.19–9.15 (m, 1H), 7.81–7.80 (m, 2H), 7.39–7.37 (m, 1H). LCMS (ESI): *m/z*=277 $[M+H]^+$.

3-(3-(4-Methylpiperazin-1-yl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**18**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09–8.98 (m, 3H), 8.95 (s, 1H), 7.42-7.30 (m, 2H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 3.33 (s, 4H), 2.50 (s, 4H), 2.25 (s, 3H). LCMS (ESI): *m/z*=368 $[M+H]^+$.

1-(4-Bromobenzyl)-*cis*-2,6-dimethylpiperidine. A mixture of 1-bromo-4-bromomethyl-benzene (500 mg, 2.0 mmol), *cis*-2,6-dimethylpiperidine (0.30 mL, 2.2 mmol) and potassium carbonate (332 mg, 2.4 mmol) in acetonitrile (20 mL) was heated under reflux for 2 h. The reaction mixture was allowed to cool to ambient temperature, the solid removed by filtration and the filtrate evaporated *in vacuo*. The resultant residue was partitioned between ethyl acetate (100 mL) and water (25 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to give the title compound as a pale brown oil (464 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 7.0 Hz, 2H), 3.71 (s, 2H), 2.56–2.36 (m, 2H), 1.68–1.61 (m, 1H), 1.61–1.47 (m, 2H), 1.33–1.24 (m, 3H), 1.01 (d, *J* = 6.2 Hz, 6H). LCMS (ESI): *m/z*=282/284 $[M+H]^+$.

(2*S*,6*R*)-2,6-Dimethyl-1-[4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)benzyl]piperidine. A degassed mixture of (2*S*,6*R*)-1-(4-bromobenzyl)-2,6-dimethylpiperidine (1.70 g, 6.0 mmol), *bis*(pinacolato)diborane (1.83 g, 7.2 mmol), 1,1′-[*bis*(diphenylphosphino) ferrocene]dichloro-palladium(II) (245 mg, 0.3 mmol) and potassium acetate (1.76 mg, 18.0 mmol) in dioxane (31 mL) and DMSO (4 mL) was heated under microwave irradiation at 150 °C for 30 minutes. The cooled reaction mixture was diluted with ethyl acetate (100 mL) then filtered and the filtrate was washed with water (100 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford the title compound as a beige solid (1.54 g, 78%) that was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 7.6 Hz, 2H), 3.88 (s, 2H), 2.58–2.25 (m, 2H), 1.51–1.70 (m, 4H), 1.43–1.21 (m, 14H), 1.10 (d, *J* = 6.2 Hz, 6H).

3-(4-(((2*R*,6*S*)-2,6-Dimethylpiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**20**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (d, *J* = 2.3 Hz, 1H), 9.04 (d, *J* = 1.1 Hz, 1H), 9.00 (d, *J* = 2.3 Hz, 1H), 8.93 (d, *J* = 1.1 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 3.78 (s, 2H), 2.55–2.42 (m, 2H), 1.66–1.52 (m, 3H), 1.37–1.20 (m, 3H), 1.00 (d, *J* = 6.2 Hz, 6H). LCMS (ESI): *m/z*=396 $[M+H]^+$.

1-(4-Bromobenzyl)-4,4-dimethylpiperidine. A mixture of 4-bromobenzyl bromide (500 mg, 2.0 mmol) and 4,4-dimethylpiperidine (249 mg, 2.2 mmol) and potassium carbonate (331 mg, 2.4 mmol) in acetonitrile (20 mL) was heated under reflux for 2 h. The reaction mixture was then cooled to ambient temperature and the reaction mixture concentrated under reduced pressure. The resultant oil was loaded onto an SCX-2 cartridge (10 g) and eluted with 2N ammonia in methanol to afford the title compound (323 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, *J*

= 8.3 Hz, 2H), 7.19 (d, *J* = 8.2 Hz, 2H), 3.44 (s, 2H), 2.35 (t, *J* = 5.4 Hz, 4H), 1.37 (t, *J* = 5.6 Hz, 4H), 0.90 (s, 6H).

4,4-Dimethyl-1-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl]-piperidine. A degassed mixture of 1-(4-bromobenzyl)-4,4-dimethylpiperidine (320 mg, 1.1 mmol), *bis*(pinacolato)diborane (346 mg, 1.4 mmol), 1,1′-[*bis*(diphenylphosphino)ferrocene] dichloro-palladium(II) (46.4 mg, 0.06 mmol) and potassium acetate (334 mg, 3.4 mmol) in dioxane (5.8 mL) and DMSO (0.6 mL) was heated under microwave irradiation at 150 °C for 30 minutes. The cooled reaction mixture was diluted with ethyl acetate (50 mL), filtered, and the filtrate washed with water (75 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford the title compound as a brown oil (264 mg, 71%) that was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.7 Hz, 2H), 3.63 (s, 2H), 2.47 (t, *J* = 5.3 Hz, 4H), 1.43 (t, *J* = 5.6 Hz, 4H), 1.38–1.31 (m, 12H), 0.91 (s, 6H).

3-(4-((4,4-Dimethylpiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**23**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.91 (d, *J* = 2.2 Hz, 1H), 8.72 (d, *J* = 2.2 Hz, 1H), 8.52 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 3.65 (s, 2H), 2.56–2.48 (m, 4H), 1.47 (t, *J* = 5.6 Hz, 4H), 0.96 (s, 6H). LCMS (ESI): *m/z*=396 $[M+H]^+$.

3-(4-(Morpholinomethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**29**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09–9.01 (m, 3H), 8.94 (d, *J* = 1.1 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.49 (d, *J* = 7.9 Hz, 2H), 3.60 (m, 4H), 3.54 (s, 2H), 2.40 (m, 4H). LCMS (ESI): *m/z*=370 $[M+H]^+$.

3-(Pyridin-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**15**). A degassed solution of 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**3**) (150 mg, 0.549 mmol), 2-(tributylstannyl)pyridine (607 mg, 1.65 mmol), tetrakis(triphenylphosphine)palladium(0) (32 mg, 5.0 mol%), lithium chloride (69.9 mg, 1.65 mmol) and *N*,*N*-diisopropylethylamine (239 µL, 1.37 mmol) in DMF (1.0 mL) was heated to 165 °C in a sealed vessel for 1.5 h and allowed to cool. Saturated aqueous potassium fluoride was added, and the resulting solids removed by filtration and washed with 4:1 DCM-methanol and water. The filtrate was separated, the aqueous phase washed with 4:1 DCM-methanol and the combined organic phases concentrated *in vacuo*. The crude residue was purified by chromatography (Biotage amino column, DCM–0.07 N ammonia in methanol) to afford a pale orange solid. This material was repurified by reverse-phase HPLC (C18, acetonitrile–0.1% aqueous ammonium hydroxide) to afford the title compound as a white crystalline solid (8.9 m g, 6.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 9.49 (d, *J* = 2.2 Hz, 1H), 9.43 (d, *J* = 2.2 Hz, 1H), 9.04 (dd, *J* = 11.6, 0.8 Hz, 2H), 8.75 (d, *J* = 4.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.98 (td, *J* = 7.8, 1.8 Hz, 1H), 7.43 (dd, *J* = 6.7, 4.9 Hz, 1H). LCMS (ESI): *m/z*=272 $[M+H]^+$.

Compounds **11**, **16** and **17** were prepared similarly to **15**:

3-(Furan-3-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**11**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85 (s, 1H), 8.98–8.94 (m, 3H), 8.81 (s, 1H), 8.27 (s, 1H), 7.81–7.80 (m, 1H), 7.05–7.04 (m, 1H). LCMS (ESI): *m/z*=261 $[M+H]^+$.

3-(Pyridin-3-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**16**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 9.16 (d, *J* = 2.4 Hz, 1H), 9.07–9.03 (m, 3H), 8.89 (d, *J* = 0.8 Hz, 1H), 8.65–8.64 (m, 1H), 8.31–8.29 (m, 1H), 7.64–7.63 (m, 1H). LCMS (ESI): *m/z*=272 $[M+H]^+$.

3-(Pyridin-4-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**17**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (s, 1H), 9.53 (d, *J* = 2.4 Hz, 1H), 9.35 (d, *J* = 2.0 Hz, 1H), 9.09 (d, *J* = 1.2 Hz, 1H), 9.01 (d, *J* = 6.8 Hz, 2H), 8.92 (d, *J* = 0.8 Hz, 1H), 8.50 (d, *J* = 6.8 Hz, 2H). LCMS (ESI): *m/z*=272 $[M+H]^+$.

3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**54**). A mixture of 3-bromo-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (1.41 g, 3.5 mmol), bis(pinacolato)diboron (980 mg, 3.85 mmol), potassium acetate (1.0 g, 10.5 mmol) and 1,1′-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (140 mg, 0.175 mmol) in dioxane (18 mL) and DMSO (2 mL) was heated at 120 °C, in a sealed vial and under argon, for 18 h. The reaction mixture was allowed to cool to ambient temperature then quenched by the addition of saturated aqueous sodium bicarbonate solution (200 mL) and extracted into ethyl acetate (200 mL). The organic layer was dried over sodium sulfate, filtered and evaporated *in vacuo* and the resultant residue was purified by flash chromatography (silica, 80 g column, Isco, 0-50% ethyl acetate in cyclohexane) to afford the title compound as a white crystalline solid (1.32 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (d, J = 1.0Hz, 1H), 9.05 (d, J = 1.6Hz, 1H), 8.86 (d, J = 1.6Hz, 1H), 8.37 (d, J = 1.0Hz, 1H), 6.03 (s, 2H), 3.61-3.54 (m, 2H), 1.41 (s, 12H), 0.94-0.87 (m, 2H), -0.10 (s, 9H). LCMS (ESI): *m/z*=451 $[M+H]^+$.

7-(4-Bromobenzyl)-2-oxa-7-azaspiro[3.5]nonane. A mixture of 4-bromobenzyl bromide (0.26 g, 1.04 mmol), triethylamine (0.21 mL, 2.07 mmol) and 2-oxa-7-azaspiro[3.5]nonane (0.50g, 2.07 mmol) in THF (20 mL) was heated under reflux for 5 h. The reaction mixture was cooled to ambient temperature, the solid removed by filtration and the filtrate was concentrated under reduced pressure. The resultant residue was loaded onto an SCX-2 cartridge (10 g) and

eluted with 2N ammonia in MeOH to afford the title compound (0.30 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.41 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 4.40 (s, 4H), 3.45–3.40 (m, 2H), 2.38–2.28 (s, 2H), 1.92–1.86 (s, 4H). LCMS (ESI): *m/z*=296/298 $[M+H]^+$.

3-(4-((2-Oxa-7-azaspiro[3.5]nonan-7-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]-dipyridine-6-carbonitrile (**30**). A suspension of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**54**) (200 mg, 0.5 mmol), 7-(4-bromobenzyl)-2-oxa-7-azaspiro[3.5]nonane (0.15 g, 0.495 mmol), bis(triphenylphosphine)dichloropalladium(II) (26 mg, 5 mol%) and cesium carbonate (0.15 g, 0.45 mmol) in a mixture of DME (5 mL), IMS (2 mL) and water (1.3 mL) was heated under microwave irradiation at 140 °C for 30 minutes. The cooled reaction mixture was diluted with water and extracted three times with ethyl acetate (20 mL). The organic phase was evaporated to dryness and then the crude residue was dissolved in a solution of TBAF in THF (3.15 mL, 1.0 M solution) and the reaction mixture was stirred at 55 °C for 12 h. After cooling the reaction mixture to ambient temperature, the reaction was quenched by the addition of saturated sodium carbonate solution and the organic components were extracted three times with ethyl acetate (20 mL). The organic layers were combined, dried over magnesium sulfate and then evaporated to dryness. The residual oil was purified by flash chromatography (silica: 10% MeOH: DCM) to afford the title compound as a cream colored solid (67 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$ plus CD_3OD) δ 9.02 (d, J = 1.0Hz, 1H), 8.90 (d, J = 2.2Hz, 1H), 8.69 (d, J = 2.2Hz, 1H), 8.49 (d, J = 1.0Hz, 1H), 7.67-7.62 (m, 2H), 7.48 (d, J = 8.0Hz, 2H), 4.45 (s, 4H), 3.56 (s, 2H), 2.48-2.31 (m, 4H), 1.97-1.87 (m, 4H). LCMS (ESI): *m/z*=410 $[M+H]^+$.

Compounds **13**, **14**, **22**, **24, 26**–**28** and **31**–**34** were prepared similarly to **30**:

3-(1-Methyl-1*H*-pyrazol-3-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**13**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.88 (br s, 1H), 9.14 (s, 2H), 9.02 (s, 1H), 8.98 (s, 1H), 7.82 (s, 1H), 6.84 (s, 1H), 3.94 (s, 3H). LCMS (ESI): *m/z*=275 $[M+H]^+$.

3-(1*H*-Imidazol-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**14**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, 1H), 12.74 (s, 1H), 9.26 (m, 2H), 9.05 (d, *J* = 1.1 Hz, 1H), 8.96 (d, *J* = 1.1 Hz, 1H), 7.34 (s, 1H), 7.12 (s, 1H). LCMS (ESI): *m/z*=261 $[M+H]^+$.

1-(4-Bromobenzyl)-3,3-dimethylpiperidine. A mixture of 4-bromobenzyl bromide (4.89 g, 19.5 mmol), 3,3-dimethylpiperidine (2.65 g, 23.5 mmol), potassium carbonate (8.09 g, 58.6 mmol) and sodium iodide (0.147g, 0.1 mmol) in THF (100 mL) was stirred at ambient temperature for 18 h, and then evaporated to dryness. The residue was treated with water (100 mL) and extracted with ethyl acetate (4 x 50 mL). The combined organic phase was washed with brine, dried over sodium sulfate and concentrated *in vacuo* to afford the title compound (5.39 g, 19.1 mmol, 98%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 3.36 (s, 2H), 2.30 (br s, 2H), 1.97 (br s, 2H), 1.64–1.54 (m, 2H), 1.29–1.18 (m, 2H), 0.91 (s, 6H). LCMS (ESI): *m/z*=282/284 $[M+H]^+$.

3-(4-((3,3-Dimethylpiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**22**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.69 (s, 1H), 9.12 (d, *J* = 1.1 Hz, 1H), 9.00 (d, *J* = 2.1 Hz, 1H), 8.68 (d, *J* = 2.1 Hz, 1H), 8.46 (d, *J* = 1.0 Hz, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 3.53 (s, 2H), 2.43–2.35 (m, 2H), 2.09–2.05 (m, 2H), 1.68–1.57 (m, 2H), 1.26 (t, *J* = 6.8 Hz, 2H), 0.97 (s, 6H). LCMS (ESI): *m/z*=396 $[M+H]^+$.

1-(2-(4-Bromophenyl)propan-2-yl)piperidine. In a three-necked round bottom flask fitted with a condenser and nitrogen stream, was placed a small quantity of ground glass and magnesium turnings (190 mg, 7.40 mmol). The mixture was stirred for 30 minutes under vacuum

then placed under nitrogen and iodine added (one small crystal), followed by the rapid addition of 1,4-dibromobenzene (2.43 g, 10.3 mmol) in 15 mL of anhydrous diethyl ether. The reaction mixture was then heated under reflux for 5 minutes. After this time, a solution of 1-(1-cyano-1-methyl-ethyl)-piperidine (1.0 g, 6.60 mmol) in anhydrous THF was added dropwise resulting in the formation of a white precipitate. The reaction mixture was heated under reflux for 1.5 h, before cooling to ambient temperature. The resultant mixture was treated with saturated aqueous potassium carbonate solution and extracted with DCM. The organic extracts were dried over magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by column chromatography (silica, 12 g column, Isco, 0–40% ethyl acetate in cyclohexane) to afford the title compound (200 mg, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.39 (m, 4H), 2.37 (t, *J* = 4.97 Hz, 4H), 1.48–1.46 (m, 6H), 1.28 (s, 6H). LCMS (ESI): *m/z*=282/284 $[M+H]^+$.

3-(4-(2-(Piperidin-1-yl)propan-2-yl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**24**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (d, *J* = 2.3 Hz, 1H), 9.04 (d, *J* = 1.1 Hz, 1H), 9.01 (d, *J* = 2.3 Hz, 1H), 8.94 (d, *J* = 1.1 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 3.62 (s, 2H), 3.19 (t, *J* = 6.9 Hz, 4H), 2.02 (p, *J* = 7.0 Hz, 2H). LCMS (ESI): *m/z*=396 $[M+H]^+$.

(*R*)-1-(4-Bromobenzyl)piperidin-3-ol. A mixture of 4-bromobenzyl bromide (1.0 g, 4.0 mmol), triethylamine (0.84 mL, 6.0 mmol) and (*R*)-3-methyl-piperidin-3-ol (1.11 g, 8.0 mmol) in THF (20 mL) was stirred at ambient temperature for 16 h. The solvent was evaporated and the resultant residue diluted with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was dried over sodium sulfate, filtered and evaporated. The material was purified by flash chromatography (silica, Biotage 50 g column,

50–100% ethyl acetate in cyclohexane) to afford the title compound (0.75 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.40 (m, 2H), 7.21–7.14 (m, 2,H), 3.85–3.76 (m, 1H), 3.45 (s, 2H), 2.45 (s, 3H), 2.29–2.16 (m, 1H), 1.86–1.70 (m, 1H) 1.67–1.45 (m, 3H); OH not observed. LCMS (ESI): *m/z*=270 $[M+H]^+$.

(*R*)-3-(4-((3-Hydroxypiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**26**). ^{1}H NMR (400 MHz, $CDCl_3$ & CD_3OD) δ 9.02–9.00 (m, 1H), 8.90–8.87 (m, 1H), 8.70–8.66 (m, 1H), 8.50–8.48 (m, 1H), 7.66–7.61 (m, 2H), 7.50–7.45 (m, 2H), 3.84–3.75 (m, 1H), 3.61 (s, 2H), 2.79–2.70 (m, 1H), 2.59–2.50 (m, 1H), 2.37–2.22 (s, 2H), 1.85–1.75 (m, 2H), 1.64–1.52 (m, 1H), 1.48–1.37 (m, 1H). LCMS (ESI): *m/z*=384 $[M+H]^+$.

3-(4-((4-Fluoropiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**27**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.08 (d, *J* = 2.3 Hz, 1H), 9.05 (d, *J* = 1.1 Hz, 1H), 9.02 (d, *J* = 2.3 Hz, 1H), 8.94 (d, *J* = 1.0 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 4.81–4.60 (m, 1H), 3.55 (s, 2H), 2.62–2.49 (m, 2H), 2.39–2.28 (m, 2H), 1.96–1.80 (m, 2H), 1.80–1.67 (s, 2H). LCMS (ESI): *m/z*=386 $[M+H]^+$.

1-(4-Bromobenzyl)piperidine-4-carbonitrile. A mixture of 4-bromobenzyl bromide (1.0 g, 4.0 mmol), triethylamine (0.84 mL, 6.0 mmol) and piperidine-4-carbonitrile (880 mg, 8.0 mmol) in THF (20 mL) was stirred at ambient temperature for 18 h. The reaction mixture was evaporated *in vacuo* and the residue partitioned between saturated sodium bicarbonate solution (100 mL) and dichloromethane (100 mL). The organic phase was separated, dried over sodium sulfate, filtered and evaporated *in vacuo*. The resultant residue was purified by flash chromatography (silica, Biotage 50 g column, 0–100% ethyl acetate in cyclohexane) to afford the title compound (1.10 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.41 (m, 2H); 7.21–7.15

(m, 2H); 3.44 (s, 2H); 2.70–2.57 (m, 3H); 2.38–2.22 (m, 2H); 1.99–1.74 (m, 4H). LCMS (ESI): *m/z*=280 $[M+H]^+$.

3-(4-((4-Cyanopiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**28**). ^{1}H NMR (400 MHz, $CDCl_3$ & CD_3OD) δ 9.02 (d, *J* = 1.1 Hz, 1H), 8.89 (d, *J* = 2.2 Hz, 1H), 8.68 (d, *J* = 2.2 Hz, 1H), 8.48 (d, *J* = 1.1 Hz, 1H), 7.67–7.61 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 3.61 (s, 2H), 2.77–2.66 (m, 3H), 2.49–2.36 (s, 2H), 2.03–1.86 (m, 4H). LCMS (ESI): *m/z*=393 $[M+H]^+$.

3-(6-(Piperidin-1-ylmethyl)pyridin-3-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**31**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 9.16 (dd, *J* = 5.4, 2.3 Hz, 1H), 9.09–9.07 (m, 2H), 8.95–8.93 (m, 2H), 8.21 (dd, *J* = 8.1, 2.5 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 3.64 (s, 2H), 2.44 (s, 4H), 1.56 (p, *J* = 5.5 Hz, 4H), 1.47–1.41 (m, 2H). LCMS (ESI): *m/z*=369 $[M+H]^+$.

2-Bromo-4-(piperidin-1-ylmethyl)thiazole. Methanesulfonyl chloride (0.48 mL, 6.2 mmol) was added dropwise to a cooled (0 °C) solution of (2-bromo-thiazol-4-yl)-methanol (1.00 g, 5.2 mmol) and triethylamine (1.60 mL, 11.4 mmol) in DCM (50 mL). The reaction mixture was allowed to warm to ambient temperature and stirred for 2 h. The mixture was diluted with DCM (50 mL) and washed with water (40 mL). The organic phase was separated, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford a yellow oil. The oil was dissolved in acetonitrile (30 mL), piperidine (0.84 mL, 11.4 mmol) and potassium carbonate (1.57 g, 11.4 mmol) were added and the reaction mixture was heated at 50 °C for 90 minutes. The reaction mixture was allowed to cool to ambient temperature and the solid removed by filtration. The filtrate was evaporated *in vacuo* and the resultant residue purified by flash column chromatography (silica, 12 g column, Isco, 0–75% ethyl acetate in cyclohexane) to afford the

title compound as a yellow oil (0.76 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (s, 1H), 3.61 (d, *J* = 0.9 Hz, 2H), 3.19 (t, *J* = 5.4 Hz, 1H), 2.45 (t, *J* = 5.1 Hz, 4H), 1.69–1.62 (m, 4H), 1.48–1.41 (m, 2H). LCMS (ESI): *m/z*=261/263 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)thiazol-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**32**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (d, *J* = 2.1 Hz, 1H), 9.05 (d, *J* = 2.1 Hz, 1H), 9.02 (d, *J* = 1.1 Hz, 1H), 8.50 (d, *J* = 1.1 Hz, 1H), 3.79 (s, 2H), 2.63–2.55 (m, 4H), 1.71–1.62 (m, 4H), 1.54–1.46 (m, 2H). LCMS (ESI): *m/z*=375 $[M+H]^+$.

3-(2-Ethyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**33**). ^{1}H NMR (400 MHz, $CDCl_3$ & CD_3OD) δ 9.01 (d, *J* = 1.0 Hz, 1H), 8.87 (d, *J* = 2.2 Hz, 1H), 8.66 (d, *J* = 2.2 Hz, 1H), 8.48 (d, *J* = 1.0 Hz, 1H), 7.47–7.42 (m, 2H), 7.22 (d, *J* = 7.8 Hz, 1H), 3.78 (s, 2H), 3.13–3.02 (m, 2H), 2.94–2.83 (m, 2H), 2.76–2.65 (m, 2H), 1.26 (t, *J* = 7.2 Hz, 3H). LCMS (ESI): *m/z*=354 $[M+H]^+$.

2-Bromo-5-ethyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine. To a suspension of 2-bromo-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (1.86 g, 8.50 mmol) in THF (31 mL) was added acetic acid (17 mL) then the reaction temperature was reduced to 0 °C and sodium borohydride (2.55 g, 42.5 mmol) was added in portions. After the addition was complete, the reaction mixture was heated at 60 °C for 1 h then the reaction mixture was cooled to ambient temperature and quenched by the addition of water. The resultant solution was partitioned between ethyl acetate (75 mL) and 1N sodium hydroxide solution (50 mL) and the layers separated. The organic phase was collected then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to give the title compound (1.87 g, 89%). ^{1}H NMR (300 MHz, CD_3OD) δ 6.78 (s, 1H), 3.50 (s, 2H), 2.81 (s, 4H), 2.62 (q, *J* = 7.2 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H).

3-(5-ethyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**34**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H), 10.68 (s, 1H), 9.05 (d, *J* = 1.0 Hz, 1H), 9.00 (s, 2H), 8.99 (s, 1H), 7.42 (s, 1H), 4.51 (d, *J* = 15.0 Hz, 1H), 4.27–4.16 (m, 1H), 3.85–3.73 (m, 1H), 3.40–3.14 (m, 5H), 1.36 (t, *J* = 7.2 Hz, 3H). LCMS (ESI): *m/z*=360 $[M+H]^+$.

***tert*-Butyl 6-cyano-3-(4-(hydroxymethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-9-carboxylate** (**55**). A mixture of 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbonitrile (**3**) (1.00 g, 3.70 mmol), 4-(bromomethyl)phenylboronic acid (1.18 g, 5.50 mmol) and 1,1′-[*bis*(diphenyl phosphino)ferrocene]dichloropalladium(II) (152 mg, 0.19 mmol) in acetonitrile (40 mL) and 2N aqueous potassium fluoride solution (20 mL) was divided equally across four 25 mL microwave vials. Each vial was degassed with nitrogen for 10 minutes and heated under microwave irradiation at 175 °C for 90 minutes. The cooled mixtures were combined, diluted with water (100 mL), the resulting solid collected by filtration, washed with water (50 mL) and diethyl ether (40 mL) and allowed to air dry. The solid was suspended in acetonitrile (250 mL), di-*tert*-butyldicarbonate (2.00 g, 9.17 mmol) was added and the mixture stirred at 5 °C for 1.5 h. The mixture was allowed to cool to ambient temperature, filtered to remove the solids and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography (silica, 80 g column, Isco, 0-70% ethyl acetate in DCM) to afford the title compound as a white solid (385 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 (d, *J* = 1.0 Hz, 1H), 9.08 (d, *J* = 2.3 Hz, 1H), 8.56 (d, *J* = 2.3 Hz, 1H), 8.38 (d, *J* = 1.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 4.81 (d, *J* = 5.9 Hz, 2H), 1.82 (s, 9H), 1.75 (t, *J* = 5.9 Hz, 1H). LCMS (ESI): *m/z*=301 $[M+H–Boc]^+$.

3-(4-((4-Hydroxypiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carbonitrile (**25**). Methanesulfonyl chloride (60 μL, 0.70 mmol) was added to a solution of *tert*-butyl 6-cyano-3-(4-(hydroxymethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-9-carboxylate (**55**) (224 mg, 0.60 mmol) and triethylamine (110 μL, 0.80 mmol) in DCM (20 mL). The mixture was allowed to warm to ambient temperature and stirred for 2 h. The mixture was diluted with DCM (200 mL) and washed with water (100 mL). The separated organic phase was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford the crude mesylate intermediate as yellow oil (374 mg, 99%). A portion of the crude mesylate (100 mg, 0.209 mmol) in acetonitrile (5 mL) at ambient temperature was treated with triethylamine (140 μL, 1.02 mmol) and 4-hydroxypiperidine (30 mg, 0.3 mmol). The mixture was heated to 50 °C for 1 h, allowed to cool to ambient temperature and the resulting precipitate collected by filtration and dried *in vacuo*. The residue was purified by flash chromatography (silica, Biotage 50 g column, 0-20% methanol in DCM) to afford the title compound as a pale solid (22 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.90 (d, *J* = 2.2 Hz, 1H), 8.71 (d, *J* = 2.2 Hz, 1H), 8.52 (m, 1H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.50 (d, *J* = 7.9 Hz, 2H), 3.72–3.63 (m, 1H), 3.63 (s, 2, H), 2.90–2.83 (m, 2H), 2.30–2.20 (m, 2H), 1.95–1.86 (m, 2H), 1.68–1.57 (m, 2H). LCMS (ESI): *m/z*=384 $[M+H]^+$.

Compound **21** was prepared similarly to **25**:

3-(4-(((3*R*,5*S*)-3,5-Dimethylpiperidin-1-yl)methyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]-dipyridine-6-carbonitrile (**21**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (dd, *J* = 1.1, 0.5 Hz, 1H), 8.91 (d, *J* = 2.2 Hz, 1H), 8.71 (d, *J* = 2.2 Hz, 1H), 8.51 (dd, *J* = 1.1, 0.5 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 3.60 (s, 2H), 2.91–2.88 (m, 1H), 2.90–2.84 (m, 1H), 1.79–1.68 (m, 4H), 1.55 (t, *J* = 11 Hz, 2H), 0.86 (d, *J* = 6.4 Hz, 6H). LCMS (ESI): *m/z*=396 $[M+H]^+$.

9-((2-(Trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile. A mixture of 3-bromo-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**53**) (5.6 g, 14 mmol), ammonium formate (8.8 g, 139 mmol) and zinc (9.1 g, 139 mmol) in THF (85 mL) was heated at 75 °C for 10 h. The reaction mixture was allowed to cool to room temperature, filtered through a pad of celite, and washed with DCM (200 mL). The filtrate was concentrated *in vacuo* and purified by flash chromatography (silica, 120 g, Isco, 5–45% ethyl acetate in heptane) to afford the title compound as a white solid (3.6 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.73 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.46 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.39 (s, 1H), 7.39 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.01 (s, 2H), 3.60 (t, *J* = 8.0 Hz, 2H), 0.93 (t, *J* = 8.0 Hz, 2H), –0.09 (s, 9H). LCMS (ESI): *m/z*=325 $[M+H]^+$.

9*H*-Pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**2**). To a solution of 1 M TBAF in THF (10 mL) at room temperature was added 9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (200 mg, 0.62 mmol) and the reaction mixture was then heated to 50 °C for 4.5 h. The reaction mixture was cooled to ambient temperature, diluted with saturated sodium hydrogen carbonate solution (15 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to give a white solid. The resultant white solid was triturated with acetonitrile (4 mL), collected by filtration and left to air dry to afford the title compound as a white solid (108 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (d, *J* = 1.1 Hz, 1H), 8.93 (d, *J* = 1.1 Hz, 1H), 8.76 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.71 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.44 (dd, *J* = 7.9, 4.8 Hz, 1H). LCMS (ESI): *m/z*=195 $[M+H]^+$.

3-Hydroxy-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile. To a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9-((2-

(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile **(54)** (245 mg, 0.54 mmol) in THF (2.0 mL), under an inert atmosphere, was added *N*-methylmorpholine *N*-oxide (191 mg, 1.63 mmol) and the reaction mixture was heated under reflux for 90 minutes. The reaction mixture was allowed to cool to ambient temperature and the solvent removed *in vacuo*. The residue was purified by flash chromatography (silica, 12 g column, Isco 0–50% ethyl acetate in cyclohexane) to afford the title compound as a white solid (180 mg, 97%). ^{1}H NMR (300 MHz, CD_3OD) δ 9.10 (d, *J* = 1.0 Hz, 1H), 8.64 (d, *J* = 1.3 Hz, 1H), 8.35 (d, *J* = 2.7 Hz, 1H), 8.04 (d, *J* = 2.7 Hz, 1H), 5.99 (s, 2H), 3.59 (t, *J* = 8.0 Hz, 2H), 0.86 (t, *J* = 8.0 Hz, 2H), –0.14 (s, 9H). LCMS (ESI): *m/z*=341 $[M+H]^+$.

3-Hydroxy-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**4**). A solution of 3-hydroxy-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (90.9 mg, 0.267 mmol) in 1,4-dioxane (0.5 mL) was treated with 48% aqueous hydrobromic acid (0.5 mL) and heated at 75 °C for 10 min. The cooled reaction mixture was made basic to pH 12 with 6N aqueous sodium hydroxide solution and adjusted to pH 7–9 with dropwise addition of 1N hydrochloric acid. The solid was collected via centrifugation, the aqueous supernate discarded, and the solid dissolved in 1–2 mL of DMSO and purified by preparative HPLC (0–30% acetonitrile–0.1% aqueous formic acid) to afford the title compound as an off-white solid (19 mg, 33%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 9.94 (s, 1H), 8.94 (s, 1H), 8.87 (s, 1H), 8.32 (d, *J* = 2.7 Hz, 1H), 8.08 (d, *J* = 2.7 Hz, 1H). LCMS (ESI): *m/z*=211 $[M+H]^+$.

***tert*-Butyl (6-cyano-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']-dipyridin-3-yl)carbamate**. A mixture of 3-bromo-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridine-6-carbonitrile (**53**) (1.53 g, 3.79 mmol), *tert*-butyl carbamate (888 mg, 7.58 mmol), and cesium carbonate (2.47 g, 7.58 mmol) were suspended in 1,4-dioxane (30

mL) degassed and purged with nitrogen three times. 4,5-*bis*(Diphenylphosphino)-9,9-dimethylxanthene (219 mg, 0.379 mmol) and *tris*(dibenzylideneacetone)dipalladium(0) (173 mg, 0.189 mmol) were added and the reaction mixture heated at 90 °C for 25 h. The cooled reaction mixture was pre-absorbed onto silica gel and purified by flash chromatography on silica (silica, 120 g column, Biotage, 10–60% ethyl acetate in heptane) to afford the title compound as a white-yellow solid (1.3 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 9.26 (s, 1H), 9.02 (s, 1H), 8.95 (s, 1H), 8.67 (d, J = 2.4, 1H), 5.98 (s, 2H), 3.55 (t, J = 7.9, 2H), 1.52 (s, 9H), 0.81 (t, J = 7.9, 2H), –0.12 – –0.23 (m, 9H). LCMS (ESI): m/z=440 $[M+H]^+$.

***tert*-Butyl (6-cyano-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']-dipyridin-3-yl)(ethyl)carbamate**. To a solution of *tert*-Butyl (6-cyano-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b:*5,4-*c*']-dipyridin-3-yl)carbamate (108 mg, 0.25 mmol) in THF (2.0 mL) was added sodium hydride (14.2 mg, 0.59 mmol). After the bubbling had ceased, iodoethane (47.3 μL, 0.592 mmol) was added and the reaction mixture heated at 55 °C overnight. The cooled reaction mixture was diluted with water and ethyl acetate, the layers separated, and the aqueous phase extracted into ethyl acetate. The combined organic phase was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resultant residue was dissolved in ethyl acetate and absorbed onto silica gel for purification by flash chromatography (silica, 12 g column, Biotage, 1–50% ethyl acetate in heptane) to afford the title compound as a viscous yellow oil (96 mg, 83%) that was used without further purification.

3-(Ethylamino)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carbonitrile (**5**). A solution of *tert*-butyl (6-cyano-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b:*5,4-*c*']-dipyridin-3-yl)-carbamate (161 mg, 0.345 mmol) in 1,4-dioxane (500 μL) was treated with 48% aqueous hydrobromic acid (500 μL) and heated at 75 °C for 10 min. The cooled reaction mixture was

made basic to pH 12 with 6N aqueous sodium hydroxide solution and then adjusted to pH 7–9 with dropwise addition of 1N hydrochloric acid. The solid was collected *via* centrifugation, the aqueous supernate discarded, and the solid dissolved in 1–2 mL of DMSO and purified by preparative (5–85% acetonitrile–0.1% aqueous ammonium hydroxide) to afford the title compound as a yellow solid (22 mg, 26%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (d, J = 1.0 Hz, 1H), 8.78 (d, J = 0.9 Hz, 1H), 8.22 (d, J = 2.7 Hz, 1H), 7.78 (d, J = 2.7 Hz, 1H), 5.76 (t, J = 5.5 Hz, 1H), 3.20–3.07 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H). LCMS (ESI): *m/z*=211 $[M+H]^+$.

5-Bromo-2-fluoro-[3,4'-bipyridin]-3'-amine (**57**, X=H). A mixture of 4-iodo-pyridin-3-ylamine (1.027 g, 4.67 mmol), 2-fluoro-5-bromopyridine-3-boronic acid (2.05 g, 9.33 mmol), and [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (1:1) (191 mg, 0.233 mmol) in acetonitrile (12 mL) and 1N aqueous potassium fluoride solution (12 mL) was heated at 95 °C for 3 h. The reaction mixture was allowed to cool, treated with additional portions of the boronic acid (0.5 eq) and of catalyst (5.0 mol%), and heated under reflux overnight under a nitrogen atmosphere. The mixture was allowed to cool, diluted with DCM and water, and filtered to remove the solids. The filtrate layers were separated and the aqueous phase was extracted into DCM, and the combined organic phase concentrated *in vacuo*. The resultant residue was redissolved in 20% methanol–DCM and absorbed onto silica gel for purification by flash chromatography (silica, 100g column, Biotage, 1–20% methanol–DCM) to afford the title compound as a brown solid (861 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 1.1 Hz, 1H), 8.18 (dd, J = 8.3, 2.5 Hz, 1H) 8.11 (s, 1H), 7.81 (d, J = 4.8 Hz, 1H), 7.01 (d, J = 4.8 Hz, 1H), 5.37 (s, 2H). LCMS (ESI): *m/z*=268/270 $[M+H]^+$.

2-Fluoro-5-(4-(piperidin-1-ylmethyl)phenyl)-[3,4'-bipyridin]-3'-amine (**58**, X=H). A degassed mixture of 5-bromo-2-fluoro-[3,4']bipyridinyl-3'-ylamine (861 mg, 3.21 mmol), 4-

piperidin-1-ylmethyl-phenyl boronic acid (816 mg, 3.72 mmol) and *bis*(triphenylphosphine)palladium(II) chloride (169 mg, 0.241 mmol) in acetonitrile (14 mL) and 1N aqueous potassium fluoride solution (8.0 mL) was heated under microwave irradiation at 100 °C for 25 minutes. The cooled reaction mixture was diluted with water and 20% methanol–DCM, the layers separated, and the aqueous phase extracted into 20% methanol–DCM. The combined organic phase was concentrated *in vacuo,* and the resultant residue dissolved in DCM/methanol, absorbed onto celite, and purified by flash chromatography (silica, 100 g column, Biotage, 0–20% methanol–DCM) to afford the title compound as a dark orange foam (753 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.20 (d, J = 7.1 Hz, 1H), 8.13 (s, 1H), 7.84 (d, J = 4.8 Hz, 1H), 7.74 (m, 2H), 7.43 (m, 2H), 7.07 (d, J = 4.9 Hz, 1H), 5.31 (s, 2H), 3.29 (s, 2H), 2.35 (m, 4H), 1.51 (m, 4H), 1.40 (m, 2H). LCMS (ESI): *m/z*=363 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine (**35**). A 1N solution of bis(trimethylsilyl)amide in THF (2.78 mL, 2.80 mmol) was added dropwise to a solution of 5-(4-piperidin-1-ylmethyl-phenyl)-2-fluoro-[3,4']bipyridinyl-3'-ylamine (101 mg, 0.278 mmol) in THF (5.0 mL) at ambient temperature. The mixture was stirred for 1 h at ambient temperature and then treated with water. The resultant brown solution was partitioned between DCM and water, the layers separated, and the aqueous phase extracted into 20% methanol–DCM. The combined organic phase was concentrated *in vacuo*. The resultant residue was absorbed onto silica gel, and purified by flash chromatography (silica, 1–20% methanol–DCM) to provide a light orange-yellow solid (39.9 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.24 (s, 1H), 8.99 (d, J = 1.8 Hz, 1H), 8.93 (s, 1H), 8.92 (d, J = 1.8 Hz, 1H), 8.45 (d, J = 5.2 Hz, 1H), 8.22 (d, J = 5.2 Hz, 1H), 7.77 (d, J = 7.5 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 3.49 (s, 2H), 2.36 (m, 4H), 1.52 (m 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=343 $[M+H]^+$.

***tert*-Butyl (6-methoxypyridin-3-yl)carbamate**. A mixture of 6-methoxypyridin-3-amine (14 g, 0.11 mol) and di-*tert*-butyldicarbonate (32 g, 0.15 mol) in 1,4-dioxane (100 mL) was heated at 75 °C for 16 h. The cooled reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (150 mL). The organic phase was separated, dried over sodium sulfate, filtered and evaporated *in vacuo* to afford a residue that was purified by flash chromatography (silica, 120 g column, Isco, 0–40% ethyl acetate in hexanes) to afford the title compound as a pink solid (20 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 2.8 Hz, 1H), 7.80 (br s, 1H), 6.70 (d, J = 8.9 Hz, 1H), 6.39 (s, 1H), 3.90 (s, 3H), 1.51 (s, 9H). LCMS (ESI): *m/z*=225 $[M+H]^+$.

***tert*-Butyl (4-iodo-6-methoxypyridin-3-yl)carbamate**. *n*-Butyllithium (2.5 M in hexanes, 100 mL, 240 mmol) was added dropwise over 1 h to a cooled (–78 °C) mixture of *tert*-butyl-6-methoxypyridin-3-ylcarbamate (16 g, 71 mmol) and *N,N,N',N'*-tetramethylethylenediamine (34 mL, 221 mmol) in diethyl ether (100 mL). The reaction was stirred at –78 °C for 30 minutes, then warmed to -20 °C and left stirring for 3 h. The reaction mixture was transferred *via* cannula over fifteen minutes to a cold (–78 °C) solution of 1-chloro-2-iodoethane (48 g, 243 mmol) in diethyl ether (50 mL). On complete addition, the reaction mixture was allowed to warm to room temperature and left stirring at this temperature for 16 h. The reaction was quenched with saturated aqueous ammonium chloride (30 mL) and water (200 mL) then extracted with ethyl acetate (2 x 100 mL). The combined organic layer was washed with saturated sodium sulfite (50 mL), 1N hydrochloric acid (100 mL), water (100 mL), saturated sodium bicarbonate solution (100 mL) and brine (50 mL), dried over sodium sulfate, and evaporated to give a residue which was purified by flash chromatography (silica, 120 g column, Isco, 0–40% ethyl acetate in hexanes) to afford the title compound as a white crystalline solid (18 g, 72%). ^{1}H NMR (400

MHz, $CDCl_3$) δ 8.45 (br s, 1H), 7.22 (s, 1H), 6.28 (br s, 1H), 3.89 (s, 3H), 1.52 (s, 9H). LCMS (ESI): *m/z*=351 $[M+H]^+$.

4-Iodo-6-methoxypyridin-3-amine. A solution of (6-methoxy-4-iodo-pyridin-3-yl)carbamic acid *tert*-butyl ester (18 g, 51 mmol) in DCM (50 mL) and TFA (50 mL) was stirred at ambient temperature for 1 h and then evaporated *in vacuo*. The resultant residue was treated with saturated aqueous sodium hydrogen carbonate solution (25 mL), diluted with water (100 mL) and extracted twice into ethyl acetate (100 mL). The organic layer was separated, dried over sodium sulfate, filtered and evaporated *in vacuo* to afford the title compound as a brown foam (10 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.14 (s, 1H), 3.84 (s, 3H), 3.72 (s, 2H). LCMS (ESI): *m/z*=251 $[M+H]^+$.

5-Bromo-2-fluoro-6'-methoxy-[3,4'-bipyridin]-3'-amine (**57**, X=OMe). A mixture of 6-methoxy-4-iodopyridin-3-amine (9.5 g, 38 mmol), 5-bromo-2-fluoropyridin-3-ylboronic acid (16.7 g, 76 mmol), and 1,1′-[*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (1.5 g, 2 mmol) in 1N aqueous potassium fluoride (95 mL) and acetonitrile (200 mL) was heated at 95 °C for 16 h. The cooled reaction mixture was diluted with water (100 mL) and extracted with DCM (3 x 100 mL). The combined organic layer was dried over sodium sulfate, filtered, and evaporated to afford a residue which was purified by flash chromatography (silica, 120 g column, Isco, 0–40% ethyl acetate in hexanes) to afford the title compound as a yellow/orange solid (8.0g, 71%), used directly in the following step. LCMS (ESI): *m/z*=225 $[M+H]^+$.

2-Fluoro-6'-methoxy-5-(4-(piperidin-1-ylmethyl)phenyl)-[3,4'-bipyridin]-3'-amine (**58**, X=OMe). A mixture of 6'-methoxy-2-fluoro-[3,4']bipyridinyl-3'-ylamine (1.0 g, 3.4 mmol), 4-(piperidin-1-ylmethyl)phenylboronic acid hydrobromide (1.7 g, 5.7 mmol), and *bis*(triphenylphosphine)palladium(II) dichloride(0.18 g, 0.25 mmol) in 1N aqueous potassium

fluoride (8.4 mL) and acetonitrile (12 mL) was heated under microwave irradiation at 100 °C for 25 minutes. The cooled reaction was diluted with water (100 mL) and extracted with DCM (3 x 50 mL). The combined organic layer was, dried over sodium sulfate, filtered, and purified by flash chromatography (silica, 25 g column, Biotage, 0–10% methanol–DCM containing 1% 2 M ammonia in methanol) to afford the title compound as a yellow-orange solid (1.0g, 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.74 (s, 3H), 7.42 (s, 2H), 6.65 (s, 1H), 4.71 (s, 2H), 3.77 (s, 3H), 3.47 (s, 2H), 2.34 (s, 4H), 1.51 (s, 4H), 1.40 (s, 2H). LCMS (ESI): *m/z*=298/300 $[M+H]^+$.

6-Methoxy-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine (**38**). To a solution of 6'-methoxy-2-fluoro-[3,4']bipyridinyl-3'-ylamine (43 mg, 0.11 mmol) in anhydrous tetrahydrofuran (1.8 mL) was added sodium *bis*(trimethylsilyl)amide (1N solution in THF, 0.33 mL, 0.332 mmol) under a flow of nitrogen. The reaction was left to stir at room temperature for 1 h then quenched with acetic acid (1 mL). The reaction mixture was evaporated *in vacuo* to afford a residue that was purified by preparative HPLC (0–30% acetonitrile–0.1% aqueous formic acid) to afford the title compound as a beige solid (10 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.92 (d, J = 2.1 Hz, 1H), 8.85 (d, J = 2.3 Hz, 1H), 8.47 (s, 1H), 7.73 (d, J = 8.2 Hz, 2H), 7.64 (s, 1H), 7.42 (d, J = 8.2 Hz, 2H), 3.92 (s, 3H), 3.48 (s, 2H), 2.36 (s, 4H), 1.51 (m, 4H), 1.40 (m, 2H). LCMS (ESI): *m/z*=373 $[M+H]^+$.

6-Bromo-4-iodonicotinic acid. *n*-Butyllithium (2.5 M in hexanes, 297 mL, 0.743 mol) was added over 1 h to a cooled (–25 °C) solution of 2,2,6,6,-tetramethylpiperidine (131 mL, 0.77 mol) in tetrahydrofuran (1 L). The mixture was allowed to stir for 16 h at –25 °C and then cooled to –55 °C before addition of solid 6-bromonicotinic acid (50.0 g, 0.25 mmol). The mixture was allowed to warm to –20 °C and stirred for 2 h. The reaction mixture was cooled to –70 °C then

poured onto a pre-cooled (–70 °C) solution of iodine (188.5 g, 0.74 mol) in tetrahydrofuran (500 mL). The mixture was then poured into the original reaction vessel and the contents allowed to warm to ambient temperature and stirred for 1 h. The solvent was evaporated and the resultant residue dissolved in water (500 mL) and washed with dichloromethane (3 x 300 mL). The aqueous phase was separated and the pH adjusted to 2 by the addition of concentrated hydrochloric acid. Aqueous sodium metabisulfite solution (20% w/w, 30 mL) was added and the solid which deposited was collected by filtration. The resultant solid was washed with water (75 mL) and pentane (75 mL) and dried at 75 °C under vacuum to furnish the title compound as a tan solid (53.1 g, 65%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.35 (s, 1H). LCMS (ESI): *m/z*=328/330 $[M+H]^+$.

***tert*-Butyl (6-bromo-4-iodopyridin-3-yl)carbamate**. A mixture of 6-bromo-4-iodo-nicotinic acid (18.3 g, 55.7 mmol), diphenylphosphoryl azide (18 mL, 83.6 mmol) and triethylamine (23.5 mL, 167.2 mmol) in *tert*-butanol (110 mL) and toluene (120 mL) was heated at 110 °C for 3 h. The mixture was allowed to cool to ambient temperature then evaporated under reduced pressure. The resultant oil was treated with water (150 mL) and extracted with ethyl acetate (2 x 300 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to give a black solid. The resultant black solid was triturated with methanol (75 mL), collected by filtration, then washed with diethyl ether (30 mL) and left to air dry to afford the title compound as a brown solid (7.5 g, 34%). The filtrate was evaporated and purified by flash chromatography on a pad of silica. The pad was washed with 20% ethyl acetate in cyclohexane. Collecting all fractions containing product followed by evaporation *in vacuo* and trituration with cyclohexane afforded further title compound (8.9 g, 40%) as a white solid (combined yield 16.4

g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.87 (s, 1H), 6.64 (s, 1H), 1.54 (s, 9H). LCMS (ESI): *m/z*=399/401 $[M+H]^+$.

6-Bromo-4-iodopyridin-3-ylamine. (6-Bromo-4-iodo-pyridin-3-yl)carbamic acid *tert*-butyl ester (13.6 g, 34.1 mmol) was dissolved in dichloromethane (150 mL) and trifluoroacetic acid (50 mL) was added. The resultant solution was stirred at ambient temperature for 2 h then evaporated under reduced pressure. The resultant residue was treated with a saturated solution of sodium hydrogen carbonate and the resultant solid was treated with water (50 mL) and extracted with ethyl acetate (2 x 200 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford the title compound as an off-white solid (10.0 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.73 (s, 1H), 4.14 (s, 2H). LCMS (ESI): *m/z*=299/301 $[M+H]^+$.

2-Fluoro-5-(4-(piperidin-1-ylmethyl)phenyl)pyridine. A degassed mixture of 5-bromo-2-fluoropyridine (578 μL, 5.60 mmol), 4-piperidin-1-ylmethyl-boronic acid (1.23 g, 5.6 mmol), 1,1′-[*bis*(diphenylphosphino)ferrocene] dichloropalladium(II) (205 mg, 5 mol%) in 1N potassium fluoride solution (4 mL) and acetonitrile (9 mL) was heated under microwave irradiation at 100 °C for 15 min. The resultant crude mixture was diluted with water (10 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was purified by flash chromatography (silica, 40 g column, Isco, 0–10% (2N ammonia in MeOH) in DCM) to afford the title compound (1.27 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 2.5 Hz, 1H), 7.96 (td, *J* = 8.5, 2.5 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 6.99 (dd, *J* = 8.5, 3.0 Hz, 1H), 3.52 (s, 2H), 2.40 (br. s, 4H), 1.59 (p, *J* = 6.0 Hz, 4H), 1.44 (t, *J* = 6.0 Hz, 2H). LCMS (ESI): *m/z*=271 $[M+H]^+$.

(2-Fluoro-5-(4-(piperidin-1-ylmethyl)phenyl)pyridin-3-yl)boronic acid (**59**). Lithium diisopropylamine (7.1 mL, 14.1 mmol) was added to a solution of 2-fluoro-5-(4-piperidin-1-ylmethylphenyl)-pyridine (1.27 g, 4.70 mmol) and triisopropyl borate (3.26 mL, 14.1 mmol) in anhydrous THF (12 mL) at –10°C. The resultant solution was stirred between –10 °C and 0 °C for 1 h, and then quenched by the addition of saturated aqueous ammonium chloride (10 mL). The reaction mixture was diluted with water (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was dried over magnesium sulfate and concentrated *in vacuo.* The crude oil was triturated with cyclohexane: DCM (3:1) to afford the title compound as a beige solid (677 mg, 46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.35–8.31 (m, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2 H), 3.54–3.42 (m, 2H), 2.44–2.24 (m, 4H), 1.57–1.45 (m, 4H), 1.44–1.36 (m, 2H), 1.20 (d, *J* = 6.5 Hz, 2H). LCMS (ESI): *m/z*=315 $[M+H]^+$.

6'-Bromo-2-fluoro-5-(4-(piperidin-1-ylmethyl)phenyl)-[3,4'-bipyridin]-3'-amine (**58**, X=Br). A mixture of 6-bromo-4-iodopyridin-3-amine (3.3 g, 8.0 mmol), 2-fluoro-3-boronic acid-5-(4-piperidin-1-ylmethylphenyl)pyridine (3.2 g, 10.0 mmol), and 1,1′-[*bis*(diphenylphosphino)-ferrocene]dichloropalladium(II) (360 mg, 0.44 mmol) in 1N aqueous potassium fluoride (21 mL) and acetonitrile (45 mL) was heated at 90 °C for 4 h. The cooled reaction was diluted with water (100 mL)and extracted with DCM (3 x 50 mL). The combined organic layer was dried over sodium sulfate, filtered, and evaporated to afford a residue which was purified by flash chromatography (silica, 25 g column, Biotage, 0–10% methanol–DCM containing 1% 2M ammonia in methanol) to afford the title compound as a yellow-orange solid (3.2 g, 91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.25 (d, *J* = 7.3 Hz, 1H), 7.90 (s, 1H), 7.74 (d, *J* = 6.5 Hz, 2H), 7.42 (d, *J* = 6.5 Hz), 7.35 (s, 1H), 5.57 (s, 2H), 3.47 (s, 2H), 2.34 (s, 4H), 1.51 (s, 4H), 1.40 (s, 2H).

6-Bromo-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine (**60**, X=Br). To a solution of 6'-bromo-2-fluoro-[3,4']bipyridinyl-3'-ylamine (1.5 g, 3.4 mmol) in anhydrous tetrahydrofuran (41 mL) was added sodium *bis*(trimethylsilyl)amide (1N solution in THF, 10.5 mL, 10 mmol) under a flow of nitrogen. The reaction mixture was left to stir at room temperature for 1 h then quenched by the addition of acetic acid (1 mL). The reaction mixture was evaporated *in vacuo* to afford a residue that was purified by flash chromatography (silica, 25 g column, Biotage, 0–10% methanol–DCM containing 1% 2N ammonia in methanol) to afford the title compound as an off-white solid (620 mg, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 9.03 (d, J = 2.5 Hz, 1H), 8.95 (d, J = 2.5 Hz, 1H), 8.71 (s, 1H), 8.52 (s, 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 3.51 (s, 2H), 2.38 (s, 4H), 1.52 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=422/424 $[M+H]^+$.

5-(3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridin-6-yl)oxazole (**41**). A mixture of 6-bromo-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine (**60**, X=Br) (150 mg, 0.36 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)oxazole (140 mg, 0.71 mmol), 1,1′-[*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (29 mg, 0.004 mmol) in saturated aqueous sodium carbonate (0.5 mL) and acetonitrile (5 mL) was heated under microwave irradiation at 130 °C for 30 minutes. The cooled reaction mixture was diluted with DCM (20 mL) and methanol (2 mL) and washed with water (15 mL). The organic phase was separated, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford a residue that was purified by preparative HPLC (20-60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford the title compound as an off-white solid (42 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 9.12 (d, J = 2.2 Hz, 1H), 8.96 (s, 1H), 8.94 (d, J = 2.2 Hz, 1H), 8.67

(s, 1H), 8.52 (s, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.66 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (s, 4H), 1.51 (m, 4H), 1.40 (m, 2H). LCMS (ESI): *m/z*=410 $[M+H]^+$.

4-(3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c'*]dipyridin-6-yl)isoxazole (**44**). A degassed mixture of 6-bromo-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine (**60**, X=Br) (130 mg, 0.31 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (140 mg, 0.72 mmol) and 1,1'-[*bis*(diphenylphosphino)ferrocene]-dichloropalladium(II) (19 mg, 7.5 mol%) in 1.0 M aqueous potassium acetate (0.62 mL) and acetonitrile (3.0 mL) was heated under microwave irradiation at 85 °C for 18 minutes. The cooled mixture was treated with DMSO and acetic acid to dissolve solids, and volatiles removed *in vacuo*. The residual aqueous solution was purified directly by preparative HPLC (15-60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford the pure title compound as a pale solid (10 mg, 8.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 9.47 (s, 1H), 9.18 (s, 1H), 8.95 (s, 1H), 8.94 (d, *J* = 2.3 Hz, 1H), 8.89 (d, *J* = 2.2 Hz, 1H), 8.68 (s, 1H), 7.76 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (s, 4H), 1.52 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=410 $[M+H]^+$.

Compounds **42**, **45**–**47** and **50** were prepared similarly to **41**:

5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole

A mixture of 5-bromothiazole (0.54 mL, 6.1 mmol), *bis*(pinacolato)diborane (1.56 g, 6.1 mmol), 1,1′-[*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (250 mg, 0.3 mmol), and potassium acetate (1.8 g, 18.3 mmol) in 1,4-dioxane (20 mL) was heated at 100 °C for 12 hours. The cooled mixture was diluted with DCM (50 mL), washed with water (15 mL), and the separated organic phase dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The residue was redissolved in DCM (10 mL) and filtered through a silica pad which was

washed with DCM (2 x 20 mL). The combined filtrate was concentrated *in vacuo* to afford the title compound as brown solid, used without further purification.

5-(3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridin-6-yl)thiazole (**42**). ^{1}H NMR (300 MHz, CD_3OD & CDCl3) δ 8.93 (d, *J* = 1.1 Hz, 1H), 8.92 (d, *J* = 0.7 Hz, 1H), 8.85 (d, *J* = 2.2 Hz, 1H), 8.82 (d, *J* = 2.2 Hz, 1H), 8.56 (d, *J* = 1.1 Hz, 1H), 8.43 (d, *J* = 0.7 Hz, 1H), 7.75–7.70 (m, 2H), 7.55–7.49 (m, 2H), 3.71 (s, 2H), 2.67–2.53 (m, 4H), 1.74–1.63 (m, 4H), 1.59–1.47 (m, 2H); one exchangeable not observed. LCMS (ESI): *m/z*=426 $[M+H]^+$.

6-(1-Methyl-1*H*-pyrazol-4-yl)-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine (**45**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (d, *J* = 1.1 Hz, 1H), 8.81 (d, *J* = 2.2 Hz, 1H), 8.77 (d, *J* = 2.2 Hz, 1H), 8.31 (d, *J* = 1.1 Hz, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 4.01 (s, 3H), 3.60 (s, 2H), 2.56–2.44 (m, 4H), 1.71–1.58 (m, 4H), 1.55–1.45 (m, 2H). LCMS (ESI): *m/z*=423 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-6-(pyridin-3-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine (**46**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 9.36 (d, *J* = 2.0 Hz, 1H), 9.05 (d, *J* = 2.3 Hz, 1H), 9.04 (s, 1H), 8.99 (s, 1H), 8.94 (d, *J* = 2.2 Hz, 1H), 8.60 (dd, *J* = 1.6, 3.2 Hz, 1H), 8.50 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.78 (d, *J* = 8.2 Hz, 2H), 7.55 (s, 1H), 7.46 (d, *J* = 8.1 Hz, 2H), 3.51 (s, 2H), 2.38 (s, 4H), 1.53 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=420 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-6-(pyridin-4-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine (**47**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 9.10 (s, 1H), 9.07 (d, *J* = 2.2 Hz, 1H), 9.06 (s, 1H), 8.96 (d, *J* = 2.2 Hz, 1H), 8.70 (d, *J* = 6.1 Hz, 2H), 8.15 (d, *J* = 6.1 Hz, 2H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (s, 4H), 1.58–1.47 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=420 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-6-(pyrimidin-5-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine (**50**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 9.52 (s, 2H), 9.21 (s, 1H), 9.08 (d, *J* = 1.7 Hz, 2H), 9.02 (d, *J* = 2.2 Hz, 1H), 8.96 (d, *J* = 2.2 Hz, 1H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (s, 4H), 1.53 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=421 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-6-(pyrazin-2-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine (**48**). A degassed mixture of 6-bromo-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine (**60**, X=Br) (60 mg, 0.14 mmol), 2-(tributylstannyl)pyrazine (74 mg, 0.20 mmol), tetrakis(triphenylphosphine)palladium(0) (8.4 mg, 5.2 mol%) and lithium chloride (4.9 mg, 0.12 mmol) in 1,4-dioxane (1.0 mL) was heated to 120 °C in a sealed vessel for 16 h. A second portion of 2-(tributylstannyl)pyrazine (105 mg, 0.28 mmol) was added and the mixture heated to 120 °C for a further 24 h, and allowed to cool. The mixture was diluted with dichloromethane (40 mL) and methanol (5 mL) and washed with saturated aqueous potassium fluoride (10 mL) and water (10 mL). The aqueous washes were extracted with 4:1 DCM-methanol, and the combined organic phases dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude residue was purified by chromatography (silica, 10 g, Biotage, 0–10% methanol–dichloromethane containing 1% 2M ammonia in methanol) and repurified by reverse-phase HPLC (C18, acetonitrile–0.1% aqueous ammonium hydroxide) to afford the title compound as a white solid (10 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 9.62 (d, *J* = 1.4 Hz, 1H), 9.28 (s, 1H), 9.19 (d, *J* = 2.3 Hz, 1H), 9.06 (s, 1H), 8.95 (d, *J* = 2.2 Hz, 1H), 8.78–8.72 (m, 1H), 8.66 (d, *J* = 2.5 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (s, 4H), 1.58–1.48 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=421 $[M+H]^+$.

2-Fluoro-5-(4-(piperidin-1-ylmethyl)phenyl)-6'-(pyridazin-4-yl)-[3,4'-bipyridin]-3'-amine. A degassed mixture of 6'-bromo-2-fluoro-5-(4-piperidin-1-ylmethyl-phenyl)-[3,4']bipyridinyl-3'-ylamine (**58**, X=Br) (200 mg, 0.45 mmol), 4-(tributylstannyl)pyridazine (334 mg, 0.91 mmol), *tetrakis*(triphenylphosphine)palladium(0) (39 mg, 0.034 mmol) and lithium chloride (192 mg, 4.5 mmol) in 1,4-dioxane (5 mL) was heated at 110 °C for 24 h. The cooled reaction mixture was diluted with dichloromethane (20 mL) and methanol (2 mL) and washed with water (15 mL). The organic phase was separated, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford a residue that was purified by flash chromatography (silica, 10 g, Biotage, 0–10% methanol–dichloromethane containing 1% 2M ammonia in methanol) to afford a brown residue that was taken to the next step without further purification.

3-(4-(Piperidin-1-ylmethyl)phenyl)-6-(pyridazin-4-yl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine (**49**). Sodium *bis*-(trimethylsilyl)amide (1N solution in THF, 1.4 mL, 1.4 mmol) was added to a solution of 6'-(pyridazin-4-yl)-2-fluoro-[3,4']bipyridinyl-3'-ylamine (120 mg, 0.27 mmol) in anhydrous tetrahydrofuran (5 mL) and the reaction was left to stir at room temperature for 5 h. The reaction was quenched by the addition of acetic acid (1 mL) and the solvent removed *in vacuo* to afford a residue that was purified by preparative HPLC (20–60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford the title compound as a pale orange solid (37 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 10.01 (s, 1H), 9.35 (d, *J* = 5.4 Hz, 1H), 9.25 (s, 1H), 9.12 (s, 1H), 9.05 (d, *J* = 2.1 Hz, 1H), 8.98 (d, *J* = 2.1 Hz, 1H), 8.34 (m, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 2H), 3.52 (s, 2H), 2.38 (s, 4H), 1.53 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=421 $[M+H]^+$.

Methyl 3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxylate. A suspension of methyl 3-bromo-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carboxylate

(**52**) (1.00 g, 3.27 mmol), (4-(piperidin-1-ylmethyl)phenyl)boronic acid (830 mg, 3.79 mmol) and 1,1′-[*bis*(diphenylphosphino)-ferrocene]dichloropalladium(II) dichloromethane complex (200 mg, 245 μmol) in 1 M aqueous potassium phosphate (2.0 mL) and acetonitrile (2.0 mL) was heated at under microwave irradiation 120 °C for 30 min. The cooled mixture was diluted with water (100 mL) and extracted three times with DCM (50 mL). The combined organic layer was dried over sodium sulfate, filtered, and evaporated to afford a residue which was purified by flash chromatography (silica, 25 g column, Biotage, 0–10% methanol–DCM containing 1% 2 M ammonia in methanol) to afford the title compound as a yellow-orange solid (3.2 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (br s, 1H), 9.20 (d, *J* = 2.3 Hz, 1H), 9.07 (s, 1H), 9.00 (s, 1H), 8.98 (d, *J* = 2.2 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 3.93 (s, 3H), 3.49 (s, 2H), 2.37 (t, *J* = 5.0 Hz, 4H), 1.52 (p, *J* = 5.5 Hz, 4H), 1.41 (dt, *J* = 11.3, 4.4 Hz, 2H).

3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*′]dipyridine-6-carboxamide (**36**). A solution of methyl 3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carboxylate (67.3 mg, 0.168 mmol) in 7 M ammonia in methanol (8.0 mL) was heated to 70 °C for 24 h in a sealed tube. The mixture was allowed to cool and concentrated *in vacuo*. The residue was dissolved in DMSO and purified by preparative HPLC (2–60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford a pale yellow fluffy solid (10.3 mg, 16%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.56 (s, 1H), 9.18 (d, *J* = 2.2 Hz, 1H), 9.00 (s, 1H), 8.96 (d, *J* = 2.1 Hz, 1H), 8.90 (s, 1H), 8.10 (s, 1H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.53 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 3.49 (s, 2H), 2.36 (m, 4H), 1.56–1.47 (m, 4H), 1.40 (m, 2H). LCMS (ESI): *m/z*=386 $[M+H]^+$.

***N*-Ethyl-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carboxamide** (**37**). A solution of methyl 3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-

b:5,4-*c*']dipyridine-6-carboxylate (152 mg, 0.380 mmol) in 2 M ethylamine in THF (8.0 mL) was heated to 80 °C for 4 days in a sealed tube. The mixture was allowed to cool and concentrated *in vacuo*. The residue was redissolved in DMSO and purified by preparative HPLC (2–60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford a dark brown solid (74.9 mg, 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.19 (d, *J* = 2.2 Hz, 1H), 8.99 (s, 1H), 8.97 (d, *J* = 2.2 Hz, 1H), 8.91 (s, 1H), 8.72 (m, 1H), 8.24 (s, 1H), 7.81 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 3.47–3.33 (m, 2H), 2.38 (m, 4H), 1.53 (m, 4H), 1.42 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 3H). LCMS (ESI): *m/z*=414 $[M+H]^+$.

(3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridin-6-yl)methanol (**39**). A 1.0 M solution of lithium aluminum hydride in THF (1.2 mL, 1.2 mmol) was slowly added to a suspension of methyl 3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carboxylate (48.0 mg, 0.120 mmol) in THF at 0 °C. The mixture was slowly warmed to ambient temperature. A further portion of 1.0 M solution of lithium aluminum hydride in THF (1.2 mL, 1.2 mmol) was added slowly at ambient temperature. After 1 h, the mixture was treated with aqueous ammonium chloride solution, and diluted with water and 50% methanol–DCM. The resulting mixture was treated with Rochelle's salt and allowed to stir vigorously for 2 h. The solids were removed by filtration, and the layers were separated. The aqueous phase was extracted with 20% methanol–DCM and the combined organic phase was concentrated *in vacuo*. The residue was purified by preparative HPLC (2–60% acetonitrile–0.1% aqueous ammonium hydroxide) to provide a light-yellow solid (7.1 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1H), 9.02 (m, 1H), 8.90 (m, 1H), 8.82 (m, 1H), 8.28 (m, 1H), 7.79 (m, 2H), 7.46 (m, 2H), 5.40 (t, *J* = 5.7 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 2H), 3.59–3.41 (m, 2H), 2.38 (m, 4H), 1.54 (m, 4H), 1.42 (m, 2H). LCMS (ESI): *m/z*=373 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-7,9-dihydro-6*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridin-6-one (**40**). To 6-Methoxy-3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine (200 mg, 0.5 mmol) was added a solution of hydrogen bromide in acetic acid (33%, 5 mL) and the reaction was heated at 100 °C for 16 h. The cooled reaction mixture was then evaporated *in vacuo* to afford a residue that was purified by preparative HPLC (0–30% acetonitrile–0.1% aqueous formic acid) to afford the title compound as a bright yellow solid (50 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (s, 1H), 8.91–8.74 (m, 2H), 8.28 (s, 1H), 7.72 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.29 (s, 1H), 6.68–6.44 (s, 1H), 3.48 (s, 2H), 2.36 (s, 4H), 1.51 (m, 4H), 1.40 (m, 2H). LCMS (ESI): *m/z*=359 $[M+H]^+$.

3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbohydrazide. A solution of methyl 3-(4-(piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c*']dipyridine-6-carboxylate (425 mg, 1.06 mmol) in ethanol (4.6 mL) was treated with hydrazine hydrate (1.29 mL, 26.5 mmol) at reflux for 1 h under a nitrogen atmosphere. The solution was allowed to cool to ambient temperature and the resulting precipitate was collected by filtration, washed with ethanol, and dried under vacuum to afford a yellow solid, used without purification (337 mg, 79%).

2-(3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridin-6-yl)-1,3,4-oxadiazole (**43**). A solution of 3-(4-(Piperidin-1-ylmethyl)phenyl)-9*H*-pyrrolo[2,3-*b*:5,4-*c′*]dipyridine-6-carbohydrazide (53.8 mg, 0.134 mmol) in DMF (0.81 mL) was treated with trimethoxymethane (1.21 mL, 11.0 mmol) and heated at 120 °C for 24 h. The mixture was cooled, treated with trimethoxymethane (1.0 mL) and a few drops of acetic acid, and heated under reflux for 48 h. The mixture was allowed to cool, treated with saturated sodium hydrogen carbonate, and the resulting white precipitate collected by filtration and washed with water. The

filtrate was extracted with DCM, 20% methanol–DCM, and the organic phase dried over anhydrous sodium sulfate, combined with the isolated solid and concentrated *in vacuo*. The residue was purified by preparative HPLC (2–60% acetonitrile–0.1% aqueous ammonium hydroxide) to afford a light-yellow flaky solid (6.0 mg, 27%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77–12.69 (s, 1H), 9.41 (s, 1H), 9.26 (d, *J* = 2.2 Hz, 1H), 9.18 (s, 1H), 9.08 (s, 1H), 9.00 (d, *J* = 2.2 Hz, 1H), 8.31 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 3.50 (s, 2H), 2.37 (m, 4H), 1.52 (m, 4H), 1.41 (m, 2H). LCMS (ESI): *m/z*=411 $[M+H]^+$.

Supporting Information

Details of the AChE *in silico* docking model are provided in the supplementary material. Also available are *in vivo* pharmacokinetic procedures. Western blot images generated in the pharmacodynamic studies, and used in the preparation of Figure 5a, as well as body weight data generated in the efficacy studies are also provided. Kinase selectivity profiling data for **19** and **30**, and crystallographic collection and refinement data are tabulated.

Accession Codes

Coordinates and structure factors for the ChK1 complexes with compounds **1**, **7**, **8** and **19** are deposited with the Protein Data Bank (PDB) with accession codes 4QYH, 4RVL, 4RVK and 4RVM respectively.

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Abbreviations

ANOVA: analysis of variance; Biotage: Biotage® SNAP pre-packed silica flash chromatography cartridge; DSB: double-strand breaks; IMS: industrial methylated spirits; Isco: Teledyne-Isco CombiFlash® purification system utilizing pre-packed silica flash chromatography cartridge; $psNEt_2$: polymer supported diethylamine (SiliCycle); SCX-2: strong cationic exchange resin; SEM: (2-(trimethylsilyl)ethoxy)methyl.

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(5) Data from www.clinicaltrials.gov. (a) LY2606368 is under evaluation for the treatment of breast or ovarian cancer associated with the BRCA1/2 mutation, non-high risk triple negative breast cancer, and high-grade serious ovarian cancer at low genetic risk (NCT02203513). LY2606368 is classified as a dual ChK1 and ChK2 inhibitor. (b) MK-8776 (SCH-900776) is under evaluation in combination with cytarabine for the treatment of relapsed relapsed acute myeloid leukemia (NCT01870596); (c) LY-2603618 has been evaluated in combination with pemetrexed and cisplatin for the treatment of non-small cell lung cancer (NCT01139775), in combination with pemetrexed for the treatment of advanced or metstatic non-small cell lung cancer (NCT00988858), also in combination with gemcitabine for the treatment of pancreatic cancer (NCT00839332).

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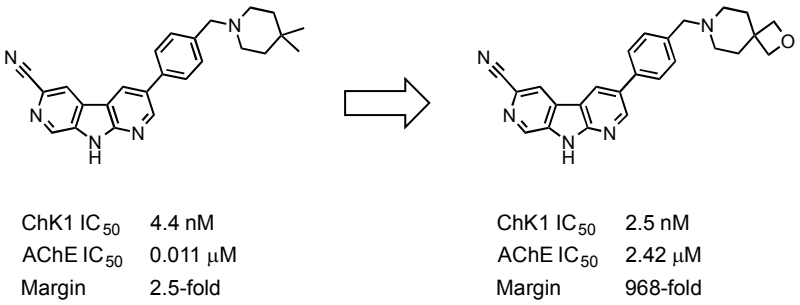
(26) Western blot images for the pharmacodynamics studies (Figures 5a, 5b), and body weight data and statistical analyses for the efficacy studies (Figures 5c, 5d) are available in the supporting material.

Table of Contents graphic:



ChK1 IC_{50}	4.4 nM
AChE IC_{50}	0.011 μM
Margin	2.5-fold

ChK1 IC_{50}	2.5 nM
AChE IC_{50}	2.42 μM
Margin	968-fold