Identification of Potent and Selective Amidobipyridyl Inhibitors of Protein Kinase D

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The synthesis and biological evaluation of potent and selective PKD inhibitors are described herein. The compounds described in the present study selectively inhibit PKD among other putative HDAC kinases. The PKD inhibitors of the present study blunt phosphorylation and subsequent nuclear export of HDAC4/5 in response to diverse agonists. These compounds further establish the central role of PKD as an HDAC4/5 kinase and enhance the current understanding of cardiac myocyte signal transduction. The in vivo efficacy of a representative example compound on heart morphology is reported herein.

Introduction

Acute and/or chronic stresses placed on the heart can result in compensations such as pathological cardiac hypertrophy.[1] Pathological cardiac hypertrophy is not, at present, delineated as either an adaptive or maladaptive stress response in the cardiac myocytes. However, pathologic cardiac hypertrophy is linked to increased morbidity and mortality.[2] Specifically, left ventricular hypertrophy is an independent risk factor for cardiac disease.[3] Given that heart failure[4] continues to be a leading cause of death, a greater understanding of the underlying mechanisms of cardiac hypertrophy and the identification of suitable agents to inhibit such processes is of significant interest.

The three isoforms of the protein kinase D (PKD[a]) family, PKD1, PKD2, and PKD3, have been shown to play a role in growth factor signaling and in stress-induced signaling.[5] PKD1 phosphorylates histone deacetylase 5 (HDAC5) in cardiac myocytes, induces the binding of 14−3−3 protein to the phosphoserine motif of HDAC5, and leads to nuclear export through a CRM1-dependent mechanism. The result of HDAC5 export is increased transcriptional activity of prohypertrophic genes, such as myocyte enhancer factor 2 (MEF2). MEF2 gene transcription in the myocyte, in turn, alters myocyte growth, contraction, calcium handling, and metabolism.[5,6] Thus, inhibitors of PKD may prove beneficial in slowing or altering the progressing of pathological hypertrophy.

Growing interest in the development of small molecule inhibitors of PKD stems from their potential in disease therapy and as valuable tools to further dissect of the role of PKD in the disease. In addition to its role in heart failure, PKD has also been found to be a key mediator of signals controlling DNA synthesis, cell growth, and proliferation.[7,8] Pharmacological inhibition of PKD has also been shown to inhibit prostate cancer cell growth and migration.[8] Published reports of PKD inhibitors[8,9] range from allosteric modulators to partially selective type I kinase inhibitors. In most cases, the potential to understand PKD-mediated pathways is limited by a lack of selectivity for PKD.[10]

High throughput screening (HTS) and subsequent optimization efforts identified 2,6-naphthyridine **1**[11] as a potent PKD inhibitor (Figure 1). Compound **1** also povided potent inhibition of HDAC5 nuclear export (PKD1 IC_{50} = 0.6 nM, HDACexp IC_{50} = 32 nM) in cardiac myocytes and is 1000-fold selective for PKD versus the upstream activator kinases (PKCs). However, **1** lacked selectivity against other putative HDAC kinases to clearly define the role of PKD in cardiac myocyte stress response. For example, **1** partially inhibits a set of putative HDAC kinases. Thus, more selective PKD inhibitors were sought.

Attempts to produce an X-ray crystal structure of compound **1** bound to PKD1 were unsuccessful; however, it is known that **1** is an ATP competitive type I inhibitor of PKD1, and thus, it is possible to propose a binding mode for **1** as depicted in Figure 1a. Structure−activity relationship (SAR) analysis suggests that the key interaction of **1** with PKD1 is a hydrogen bond pair of the alkylaminopyridine to hinge region residue Leu662. Additional interactions of note are a hydrogen bond of the A-ring nitrogen with the catalytic Lys612 and a salt bridge of the distal piperazine nitrogen with Glu710. Early SAR showed that replacement of the A-ring nitrogen in **1** by a carbon atom afforded compounds with 10- to 20-fold loss in potency.[11] This finding is in accord with proposed binding mode of **1** wherein the A-ring nitrogen makes a hydrogen bond contact with the catalytic Lys612. Given this understanding of the binding mode of **1**, analogues that mimic

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[a] Abbreviations: PKD, protein kinase D; PKC, protein kinase C; TAB, thoracic aortic banded; DSS, Dahl salt-sensitive; HDAC, histone deacetylase; MEF2, myocyte enhancer factor 2; GFP, green fluorescence protein; LV, left ventricle; TL, tibia length; IVRT, isovolumic relaxation time; MAP, mean arterial pressure; PMA, phorbol 12-myristate 13-acetate; PE, phenylephrine; PGF2α, prostaglandin F 2α; ET-1, endothelin-1; LPA, lipopolysaccharide A; PMBCs, peripheral blood mononuclear cells; TFA, trifluoroacetic acid.
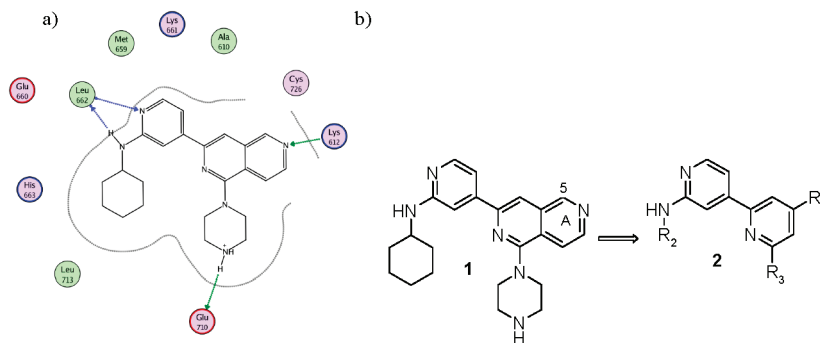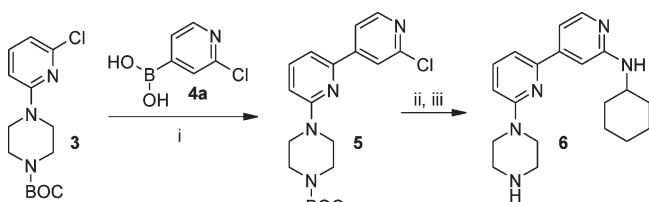
Figure 1. (a) Proposed binding mode of 2,6-naphthyridine **1** to the PKD1 active site. (b) Truncation of A ring of 2,6-naphthyridine PKD inhibitors (**1**) to provide bipyridyl compounds of type **2**.

Scheme 1[a]

[a] Reagents and conditions: (i) **4a**, Pd(Ph$_3$P)$_4$, aq Na$_2$CO$_3$, CH$_3$CN, 90 °C; (ii) cHexNH$_2$, Pd(t-Bu$_3$P)$_2$, t-BuONa, 1,4-dioxane, 130 °C; (iii) TFA, DCM, room temp.

the binding to the Lys612 while maintaining the hinge interaction were sought to improve upon **1**. One such series of compounds arose from truncation of the A-ring of **1** to provide bipyridyls such as **2** that afford the opportunity to introduce alternative hydrogen bond acceptor groups at R$_1$. Herein, we describe the design and optimization of this bipyridyl series of PKD inhibitors.

Chemistry

Compounds wherein R$_1$ = H were prepared by a three-step process (Scheme 1). Suzuki coupling of commercially available chloropyridine **3** to boronic acid **4a** provided bipyridyl **5**. Subsequent palladium catalyzed amination using Pd(t-Bu$_3$P)$_2$[12] provided truncated analogue **6**.

To generate analogues with R$_1$ ≠ H (Scheme 2), treatment of citrazinic acid **7** with phosphorus oxybromide followed by quenching the intermediate acid bromide with methanol provided the requisite 2,4,6-trisubstituted pyridine **8**. Nucleophilic substitution of bromide **8** by amine **9** followed by Suzuki coupling with pyridyl-4-boronic acids **4a** and **4b** provided bipyridyls **10a** and **10b**, respectively. Conversion of the R$_1$ ester of **10a** and **10b** to an amide was accomplished by either of two methods: (1) treatment with *tert*-butylamine under Weinreb amidation[13] conditions to generate the corresponding amide **11a**; (2) treatment with methanolic ammonia to give the primary amide **11b** directly. Chloropyridine **11a** underwent palladium catalyzed amination by analogy to Scheme 1. Removal of the BOC and *tert*-butyl groups upon treatment with TFA at elevated temperature provided the desired products **12b,c,d,f,g,h**. Alternatively, suitable amines, such as cyclohexylamine, displaced the fluoride of **11b**, which, following deprotection, provided target compounds **12a** and **12g**. One of the limitations in the conversion of **11b** to **12a** was competing transamidation at R$_1$ to give an undesired cyclohexylamide. The route to compounds **12** from **11a**, employing

the *tert*-butylamide, proved to be more robust, avoided the byproduct formation and allowed for greater diversity at R$_2$.

To enhance diversity at R$_1$, primary amides **12a,b,e** were converted to nitriles **12i−k** by treatment with trifluoroacetic anhydride (Scheme 2). For additional diversity at R$_1$, intermediates with R$_1$ = −OTf and −Br were generated (Scheme 3). By S$_N$Ar of 2,6-dibromo-4-nitropyridine **13** with BOC-piperazine, followed by Suzuki coupling with **4a** or **4b**, compound **14a** or **14b** were accessed by analogy to Schemes 1 and 2 above. Subsequent direct or palladium catalyzed coupling of an amine, such as cyclohexylamine, with **14a** or **14b**, respectively, afforded 2-aminopyridines **15a−c**, which were protected with a BOC group to give intermediates **16a−c**. It is noteworthy to highlight the conversion of **16a−c** to **17a−c** by treatment with potassium hydroxide in which the nitro group served as a masked hydroxyl group (Scheme 3). Utilization of a nitro group as a leaving group in S$_N$Ar reactions is known but is less commonly utilized than halogens. In addition, hydrolyses of 4-nitropyridines have been demonstrated but typically with less functionalized compounds.[14] Conversion of the resulting 4-hydroxypyridine to triflate **18b** and **c** was accomplished upon treatment with 2-(N,N-bis-(trifluoromethylsulfonyl)amino)pyridine and triethylamine. Alternatively, bromide **18a** was prepared by reaction with phosphorus oxytribromide. Under these reaction conditions, the BOC groups were cleaved, thus requiring reintroduction of the BOC group by treatment with BOC anhydride and triethylamine. The bromide **18a** and triflates **18b** and **18c** underwent Suzuki coupling to provide pyrazoles **19a−d**.

Compounds with R$_2$ = cyclohexyl showed good in vitro properties and profiles, so additional exploration of R$_1$ and R$_3$ was carried out leaving the R$_2$ group constant. To that end, stannane **21** (Scheme 4) was prepared by selective displacement of the fluoride of 4-iodo-2-fluoropyridine **20** with cyclohexylamine, followed by palladium mediated stannylation with hexamethylditin. With stannane **21** in hand, Stille coupling with 2-chloropyridine **22** afforded bipyridyl **23**, which was converted to targets by facile heating in dioxane with the appropriate amine (HNR$_4$R$_5$). Installation of the amide to give compounds **24a−i** followed as outlined above. Likewise, 2,6-dichloro-4-substituted pyridine **25a** (R$_1$ = CF$_3$) or **25b** (R$_1$ = CHF$_2$) were treated with amine **9** to give intermediates **26a** and **26b**. Coupling of **26a** and **26b** by sequences outlined above provided analogues **24j** and **24k**.

Given the promising in vitro data for amides like **12a** and the potential for in vivo hydrolysis of the primary amide, it was reasonable to explore the impact of cyclizing such compounds back onto the pyridine core as in **30** and **31** (Scheme 5). Intermediate **27**[15] underwent light-mediated benzylic bromination followed

Scheme 2[a]
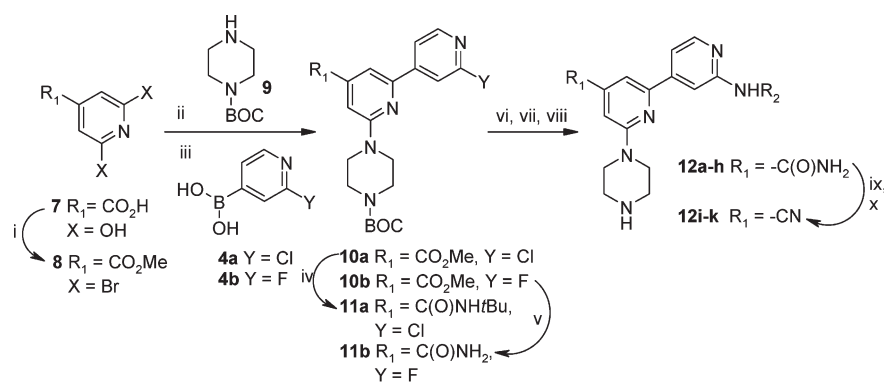
[a] Reagents and conditions: (i) POBr$_3$, 130 °C, then MeOH; (ii) **9**, Et$_3$N, 1,4-dioxane, 110 °C; (iii) **4a** or **4b**, Pd(dppf)Cl$_2$·CH$_2$Cl$_2$, aq Na$_2$CO$_3$, DME, 80 °C; (iv) AlMe$_3$, *t*-BuNH$_2$, PhMe, 110 °C; (v) 7 M NH$_3$/MeOH, 90 °C; (vi) **11b**, *c*-hexylamine, 130 °C or NaHMDS, R$_2$NH$_2$, THF, 80 °C; (vii) **11a**, R$_2$NH$_2$, Pd(*t*Bu$_3$P)$_2$, *t*-BuONa, 1,4-dioxane, 110 °C; (viii) TFA/DCM room temp; or TFA 120 °C, microwave for R$_1$ = CONH-*t*-Bu; (ix) TFAA, Et$_3$N, DCM, room temp; (x) NaBH$_4$, MeOH, room temp.

Scheme 3[a]
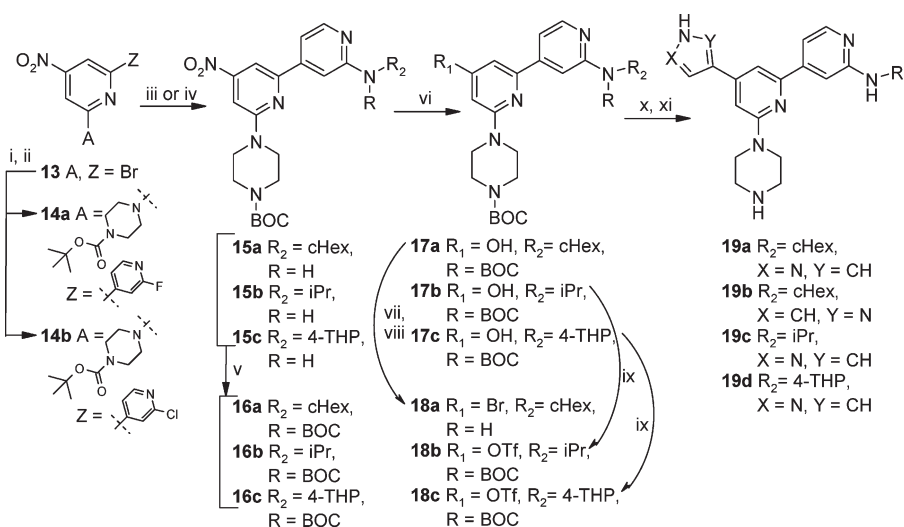
[a] Reagents and conditions: (i) **9**, Et$_3$N, 1,4-dioxane, 110 °C; (ii) **4a** or **4b**, Pd(dppf)Cl$_2$·CH$_2$Cl$_2$, aq Na$_2$CO$_3$, DME, 90 °C; (iii) R$_2$NH$_2$, 110 °C; (iv) R$_2$NH$_2$, Pd(*t*-Bu$_3$P)$_2$, *t*-BuONa, 110 °C, 1,4-dioxane; (v) BOC$_2$O, DMAP, CH$_3$CN/DCM reflux; (vi) KOH, DMSO, room temp; (vii) POBr$_3$, 130 °C; (viii) BOC$_2$O, Et$_3$N, DCM, room temp; (ix) 2-(*N*,*N*-bis(trifluoromethylsulfonyl)amino)pyridine, Et$_3$N, DCM 0 °C to room temp; (x) R$_1$B(OH)$_2$, Pd(dppf)Cl$_2$·CH$_2$Cl$_2$, aq Na$_2$CO$_3$, DME, 130 °C, microwave; (xi) TFA/DCM, room temp.

by aminolysis, affording the desired lactam in one pot. Subsequent displacement of one chlorine gave a separable mixture of regioisomers **28** and **29**. Stille coupling of **22** with either **28** or **29**, followed by deprotection of the piperazine with TFA, provided target compounds **30** and **31**.

Compound Evaluation

In vitro, compounds were evaluated for their ability to inhibit the target PKD1, as well as PKD isoforms 2 and 3 and representative PKC isoforms PKCα and PKCδ. All compounds in the present study were found to inhibit the entire PKD family (1, 2, and 3) with similar potency. The HDAC nuclear export assay, a cellular readout of PKD activity, monitored the ability of the inhibitors to prevent prostaglandin F2α-stimulated nuclear export of GFP-HDAC5 in the cardiac myocyte.

In vivo, selected compounds were evaluated for their ability to block cardiac hypertrophy with daily administration for 14 days in a rat model of disease. The two rat models utilized in the present study were the TAB rat, a surgical model of pressure-overload-induced cardiac hypertrophy, recalling that PKD1 knockout was

shown to blunt cardiac hypertrophy in the TAB mouse. A second model, the Dahl salt-sensitive (DSS) rat, is a genetic model of high-salt-induced hypertension and cardiac hypertrophy. In all rat studies, the effect of compound treatment on blood pressure was monitored to control for the anticipated benefit of antihypertensives to cardiac hypertrophy in both models.

Results

The simple truncated analogue **6** proved to be a potent PKD inhibitor (PKD1 IC$_{50}$ = 43 nM) with cellular activity (HDACexp IC$_{50}$ = 516 nM), albeit to a lesser extent than **1** (PKD1 IC$_{50}$ = 1 nM, HDACexp IC$_{50}$ = 32 nM). However, the biochemical and cellular efficacy of **6** was encouraging for further exploration. Indeed, as hypothesized above, introducing a moiety at R$_1$ capable of making a hydrogen bond contact with Lys612 such as in **12a** (Figure 2) afforded a compound of equal potency (PKD1 IC$_{50}$ = 1 nM, HDACexp IC$_{50}$ = 77 nM) to the naphthyridine comparator **1**. Therefore, it was demonstrated that a primary R$_1$ amide is capable of serving as an effective replacement for the A-ring of **1**.

Scheme 4[a]
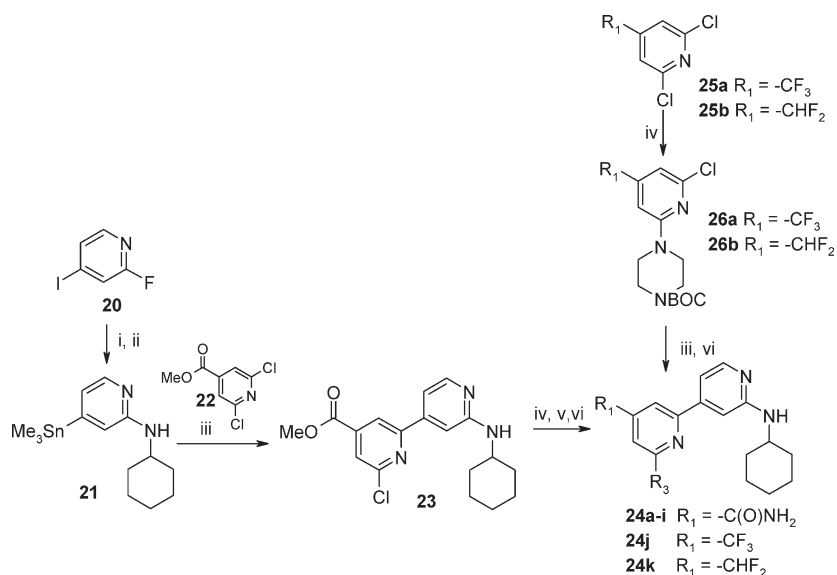
[a] Reagents and conditions: (i) cHexNH₂, 120 °C; (ii) Me₃SnSnMe₃, Pd(Ph₃P)₄, PhMe, 100 °C; (iii) **22**, CsF, Pd(t-Bu₃P)₂, 1,4-dioxane, 100 °C; (iv) amine, Et₃N, 1,4-dioxane, 90 °C; (v) 7 M NH₃/MeOH, 90 °C; (vi) TFA/DCM, room temp.

Scheme 5[a]
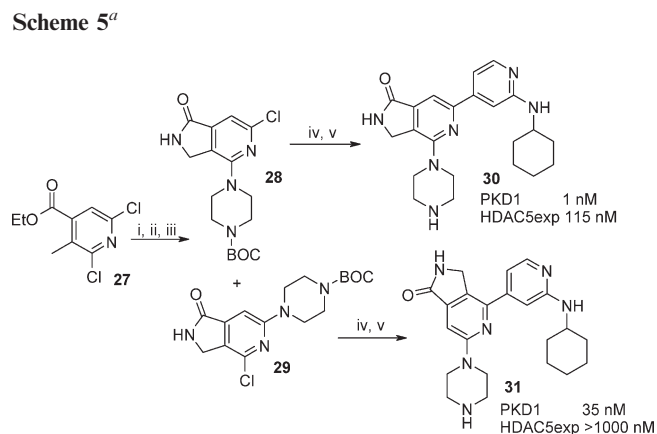
[a] Reagents and conditions: (i) NBS, benzoylperoxide, heat lamp, CCl₄, 60 °C; (ii) NH₄OH, THF, room temp; (iii) **9**, Et₃N, 1,4-dioxane, 120 °C; (iv) **21**, CsF, Pd(tBu₃P)₂, 1,4-dioxane, 100 °C; (v) TFA/DCM, room temp.

Exploration of the R₂ position led to the general observation that, while a variety of substituents provided potent inhibition of PKD1, simple branched alkyls provided optimal cellular activity. For example, R₂ = cyclohexyl (**12a**; PKD1 IC_{50} = 1 nM, HDACexp IC_{50} = 77 nM), isopropyl (**12d**; PKD1 IC_{50} = 1 nM, HDACexp IC_{50} = 438 nM), and cyclopentyl (**12g**; PKD1 IC_{50} = 6 nM, HDACexp IC_{50} = 202 nM) offered comparable biochemical activity but superior cellular activity to R₂ = ethyl (**12h**; PKD1 IC_{50} = 4 nM, HDACexp IC_{50} > 1000 nM). The difference is most significant in the cellular HDAC5 export assay. One surprising finding is that while **12c** is a potent inhibitor of PKD1 in the enzymatic assay, it failed to inhibit nuclear export of HDAC5 below 1000 nM. Similar potency to **12a** was attained with aryl and heteroaryl groups such as **12b** (PKD1 IC_{50} = 1 nM, HDACexp IC_{50} = 24 nM), **12e** (PKD1 IC_{50} = 1 nM, HDACexp IC_{50} = 674 nM), and **12f** (PKD1 IC_{50} = 1 nM, HDACexp IC_{50} = 478 nM), yet only the simple phenyl analogue **12b** provided analogous cellular activity to that seen when R₂ = cyclohexyl.

The R₁ nitrile analogues (**12i–k**; PKD1 IC_{50} = 5–18 nM) proved to be less potent than the parent amides in the enzymatic
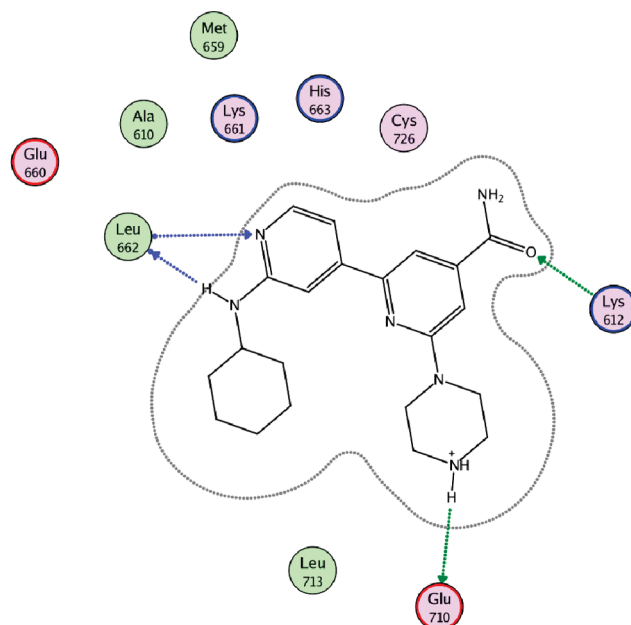

Figure 2. Proposed binding mode of bipyridyl **12a** to the PKD1 active site.

assay. Intriguingly, **12i** (PKD1 IC_{50} = 18 nM, HDACexp IC_{50} = 66 nM) provided similar efficacy in the HDAC5 export assay to that of **12a** even with ∼20-fold lower potency in the PKD1 assay (Table 1). This could be due to inherent differences in the cellular permeability of the nitrile (Caco-2 cells, P(B-A)/P(A-B) = 0.44) compared to the amide (Caco-2 cells, P(B-A)/P(A-B) = 7.89). A similar trend in activity was seen with each of the nitriles and the corresponding parent amides.

A variety of heterocycles were explored as potential hydrogen bond donor/acceptor replacements for the primary amide in **12a**. Pyrazole isomers **19a–d** provided the most promising examples (Table 1). It is instructive to note the ability of pyrazole **19a** to closely mimic **12a**. Pyrazole isomer **19a** was more potent than the alternative isomer **19b**, which may indicate a preference for

Table 1. PKD Inhibition (IC$_{50}$, nM) and HDAC5 Export (EC$_{50}$, nM) Activity for Compounds[a]

Cmpd	R$_1$	R$_2$	R$_3$	PKD1	PKD2 (3)	HDACexp
12a	-C(O)NH$_2$	*c*Hex		1	9 (1)	77
12b	-C(O)NH$_2$	Ph		1	1 (1)	24
12c	-C(O)NH$_2$			1	9 (1)	>1000
12d	-C(O)NH$_2$	*i*Pr		1	8 (1)	438
12e	-C(O)NH$_2$			1	7 (1)	674
12f	-C(O)NH$_2$	2-ClPh-		1	8 (1)	478
12g	-C(O)NH$_2$	*c*Pent		6	59 (5)	202
12h	-C(O)NH$_2$	Et		4	31 (3)	>1000
12i	CN	*c*Hex		18	130 (16)	66
12j	-CN	Ph		5	35 (4)	378
12k	-CN			8	68 (7)	428
19a		*c*Hex		< 1	1 (< 1)	17
19b		*c*Hex		4	62 (5)	704
19c		*i*Pr		< 1	2 (< 1)	92
19d				< 1	2 (< 1)	234
24a	-C(O)NH$_2$	*c*Hex		2	18 (2)	>1000
24b	-C(O)NH$_2$	*c*Hex		68	540 (64)	>1000
24c	-C(O)NH$_2$	*c*Hex		6	60 (6)	679
24d	-C(O)NH$_2$	*c*Hex		8	86 (6)	831
24e	-C(O)NH$_2$	*c*Hex		14	168 (12)	>1000

Table 1. Continued

Cmpd	R₁	R₂	R₃	PKD1	PKD2 (3)	HDACexp
24f	-C(O)NH$_2$	*c*Hex	[structure]	25	238 (18)	752
24g	-C(O)NH$_2$	*c*Hex	[structure]	46	212 (46)	>1000
24h	-C(O)NH$_2$	*c*Hex	[structure]	55	427 (64)	>1000
24i	-C(O)NH$_2$	*c*Hex	[structure]	>1000	-	-
24j	-CF$_3$	*c*Hex	[structure]	20	101 (13)	342
24k	-CHF$_2$	*c*Hex	[structure]	17	76 (13)	568

[a] Values are the mean of at least two experiments.

Table 2. Pharmacokinetic Parameters Selected PKD Inhibitors[a]

parameter	12a	12a[b]	1	12j	24j	30
AUC$_{0-8h}$, po (μM·h)	BQL[c]	25.8 ± 3.7	0.19 ± 0.18	1.1 ± 0.2	0.73 ± 0.07	0.1 ± 0.05
CL ((mL/min)/kg)	97 ± 26		32 ± 12	31		86
Vd$_{ss}$ (L/kg)	22 ± 6		12 ± 1	7.8		35
$t_{1/2}$ (h)	3.8 ± 1.7		5.2 ± 1.9	3.1		6.2
C_{max} (nM)	BQL[c]	8568 ± 375	0.12 ± 0.03	231 ± 49	251 ± 50	31 ± 25
F (%)	NC[d]		4	18 ± 3	31 ± 3	5 ± 3

[a] Sprague−Dawley rat PK. Animals were dosed either iv (2 mg/kg, $n = 2$) or po (5 mg/kg, $n = 3$) with compound in 10% NMP, 10% Cremophor, 80% pH 4.63 buffer vehicle. PK parameters are derived from iv dosing and are reported as the mean ± SD. [b] Dahl-S rat: subcutaneous; 50 mg/kg; 10% 1 N HCl, 90% Captisol in pH buffer (10%); AUC is 0−24 h. [c] Below quantitation limits. [d] Not calculated, since oral exposure was BQL.

orientation of the H-bond donor/acceptor relative to both **1** and **12a**. Likewise, pyrazole **19d** was among the most efficacious PKD inhibitors evaluated to date in the HDAC5 export cellular assay, while its comparator amide analogue **12c** provided no measurable efficacy below one micromolar.

Alteration of R$_3$ beyond the parent piperazine proved to be less fruitful. In general, with basic amine-containing R$_3$ groups, such as in **24a−h**, moderate levels of PKD inhibition (IC$_{50}$ < 100 nM) were achieved. However, in most cases, loss of potency in the cell accompanied these subtle structural changes. Again, this may be a consequence of changes in permeability in the cellular assay. Compounds **12a** with **24a**, which have similar activity in the enzymatic assays, showed wide differences in cellular activities (EC$_{50}$ for **24a** is > 1000 nM, while for **12a** it is 77 nM). Removal of the basic amine, such as **24i**, produced compounds typically lacking activity in the enzymatic assay.

Following low-dose iv and po administration of compound **12a** to rats, a set of pharmacokinetic parameters were determined. Compound **12a** had high clearance and no oral bioavailability in rat PK (Table 2). The low bioavailability is likely the result of poor permeability, as discussed above, since the compound has an aqueous solubility of 860 μM (at pH 6.8). Many of the modifications described above were aimed at replacing the amide moiety of **12a** with groups to improve the in vivo PK-ADME profile. Many of the compounds analyzed, such as **12b,c,e** showed similar behavior to **12a** in that they had high clearance and limited or no oral bioavailability. The most promising changes were conversion of the amide to a nitrile as exemplified by **12j**, which provided 18%

oral bioavailability and attenuated clearance. Additionally, it was found that when R$_1$ = trifluoromethyl, as in **24j**, reasonable exposure was attained when dosed orally. Compounds **30** and **31** were designed specifically to ask whether or not the lactam would provide improved oral exposure relative to the primary amide **12a**. Indeed, **30**, provided some oral availability, albeit still at only 5%. In the exploration of alternative dosing, it was found, however, that **12a** could be dose subcutaneously (sc) (Table 2, Figure 4) to provide suitable exposures for evaluation of the compounds in vivo in several models of cardiac hypertrophy.

Key to further establishing the central role of PKD in cardiac myocyte growth signaling was the identification of an inhibitor that was more selective than **1**. To that end, it was fortuitous that **12a** demonstrated improved selectivity, relative to **1**, for PKD among other putative HDAC 4/5 kinases, most notably CaMKII, MARK1, and MARK2 (Table 3).[16a] In a broader kinase panel, **12a** was also found to be more selective than **1**.[16b] If the proposed binding modes depicted in Figures 1a and 2 accurately predict respective binding of the inhibitors to PKD1, then it is clear from the similarity in structure that the increased selectivity of **12a** over **1** must be a result of unfavorable interactions of the R$_1$ amide of **12a** with the kinases in question. At present, in the absence of X-ray crystallographic information this remains a working hypothesis. By comparison of the proposed binding modes for **1** and **12a**, it would be reasonable to hypothesize that substitution at the C-5 of the naphthyridine should improve the kinase selectivity of **1**. In addition to improved kinase selectivity, **12a** also shows minimal activity against a panel of receptors
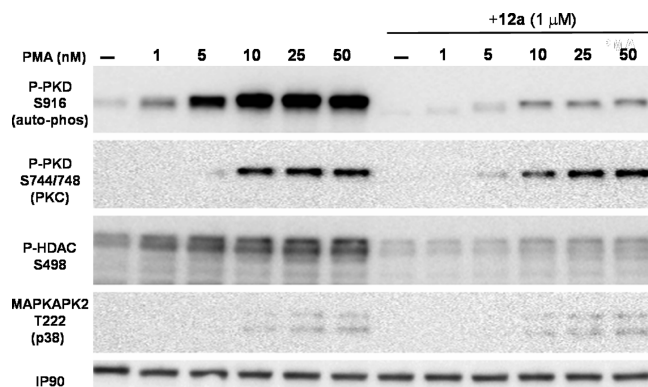
Figure 3. Compound **12a** (1 μM) blunts autophosphorylation of PKD at Ser916 and phosphorylation of the PKD substrate HDAC at Ser498 while not reducing PKC mediated phosphorylation of PKD at Ser744/748 in ex vivo stimulation of peripheral blood mononuclear cells (PBMCs) stimulated with PMA.
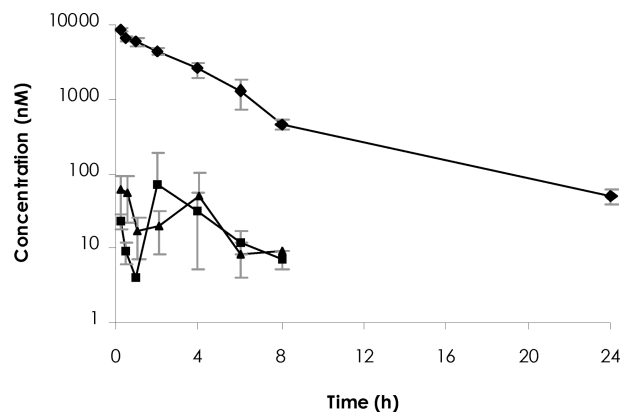

Figure 4. Dahl salt-sensitive rat plasma exposure following 50 mg/kg sc and 100 mg/kg (solution and suspension) oral administration of **12a**: (◆) 50 mg/kg sc solution; (▲) 100 mg/kg po suspension; (■) 100 mg/kg po solution.

Table 3. PKD Inhibitor **12a** Is Selective against Other Kinases

entry	kinase	% inhibition at 1 μM [a]	
		12a	**1**
1	hCaMKIδ	6	11
2	rCaMKIIα	0	36
3	hCaMKIIβ	3	36
4	hCaMKIIδ	26	80
5	hCaMKIV	1	1
6	hMARK1	24	82
7	hMARK2	27	83
8	hGRK5	2	5
9	hPKCδ	22	51
10	hPKCϵ	−3	34[b]

[a] Mean of n = 2 measurements. Inhibition assays conducted by Invitrogen Selectscreen. [b] Mean of $n \geq 2$ experiments; a more complete comparison is provided in the Supporting Information.

and importantly is selective for PKD over α1AR (IC$_{50}$ = 3000 nM) and β1AR (IC$_{50}$ = 8300 nM).

Compound **12a**, reported elsewhere as BPKDi,[17] was shown to inhibit endogenous PKD1 signaling. Specifically, treatment of neonatal rat ventricular myocytes (NRVMs) with **12a** (1 μM) blunted autophosphorylation of PKD1 (Ser916) but not PKC mediated phosphorylation of Ser744/748 in response to PMA, ET-1, PGF2α, and PE. It was also reported that **12a** (1 μM) blocks agonist-dependent phosphorylation of both GFP tagged and endogenous HDAC4 and HDAC5 in neonatal rat ventricular myocytes (NRVMs). Treatment of NRVMs (infected with GFP-tagged HDAC5) with 1 μM **12a** also blocked agonist-driven nuclear export of HDAC5.

In an ex vivo assay, **12a** was also shown to blunt autophosphorylation of PKD (Ser916) and downstream PKD mediated phosphorylation of HDAC (Ser498) in PBMCs in response to increasing concentrations of PMA (Figure 3). Importantly, this blockade of signaling was accomplished without effecting the phosphorylation of the PKC sites (Ser744/748) on PKD. However, it is important to note that **12a** is more effective in blocking the phosphorylation of HDAC than the phosphorylation of PKD1 at Ser916 in response to PMA stimulation.

In order to evaluate the effect of **12a** in a model of cardiac hypertrophy, it was necessary to dose the compound sc, given its limited oral bioavailability. A dramatic change in plasma exposure with **12a** is realized on changing from po to sc dosing (Figure 4). While sc dosing at 50 mg/kg in the DSS rat provides good exposure (AUC$_{0-8h}$ = 25.8 μM·h), the highest

dose that could be used daily without an effect on blood pressure was 5 (mg/kg)/day.

It is known that DSS rats given a high-salt diet exhibit cardiac hypertrophy and thus offer a model of disease. Blood pressure and cardiac morphology results were obtained following dosing of **12a** (0.5, 1.5, or 5 (mg/kg)/day sc) in the DSS rat for 14 days. No effect on mean blood pressure is observed at the highest dose, 2 h after the final dose (Figure 5a). Additionally, the compound did not affect cardiac hypertrophy (as measured by the ratio of left ventricle mass to tibia length) at any of the three doses (Figure 5b). Ex vivo analysis of PBMCs isolated from the DSS rat study revealed that PKD autophosphorylation (Figure 6a) was also unaffected. By contrast, **12a** at the highest dose (5 (mg/kg)/day sc) significantly reduced HDAC phosphorylation (Figure 6b), indicating that blockade of pathway was achieved. The concentration of **12a** (LV 12 ± 2 μM, plasma 0.23 ± 0.8 μM) on day 14 (5 mg/kg sc) in the DSS rat was well above the level required to inhibit PKD1 and HDAC5 nuclear export in vitro (PKD1 IC$_{50}$ = 1 nM, HDACexp IC$_{50}$ = 77 nM, Table 1).

In the second model of cardiac hypertrophy, the thoracic aortic banded rat (TAB), **12a** (5 (mg/kg)/day sc) likewise did not affect cardiac hypertrophy as measured by LV/TL (Figure 7). The PBMC biomarker readouts from the TAB rat study with **12a** mimicked the findings from the DSS rat. Therefore, while no effect on PKD autophosphorylation (Figure 8a) was observed, **12a** significantly reduced HDAC phosphorylation (Figure 8b) in PBMCs isolated from the TAB rat.

Conclusions

In the preceding paper (DOI: 10.1021/jm100075z),[11] naphthyridine **1** demonstrated reduction of cardiac hypertrophy in the DSS rat model. However, interpretation of the data is complicated by suboptimal kinase and receptor selectivity. The aim of the present study, to identify a compound with improved kinase and receptor selectivity and sufficient exposure to study PKD inhibition in vivo, was realized with compound **12a**. Amidobipyridyl inhibitor **12a** has the desired properties to complement the findings of comparator naphthyridine **1**. Like **1**, potent PKD inhibitor **12a** clearly demonstrated the ability to control PKD autophosphorylation and HDAC5 phosphorylation in cells. In addition, **12a** controls nuclear export without disrupting upstream PKC signaling.[16]

While **12a** did not demonstrate suitable PK to enable oral dosing, the compound was evaluated in models of cardiac hypertrophy by employing sc dosing. The maximal dose of
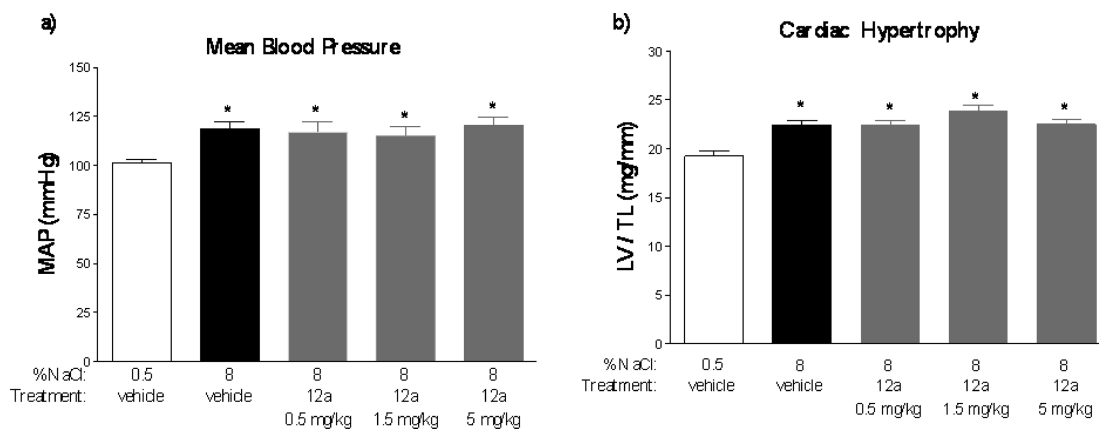
Figure 5. Effect of compound **12a** at 0.5, 1.5, 5 (mg/kg)/day sc for 14 days in the DSS rat on (a) mean blood pressure and (b) cardiac hypertrophy (left ventricular mass/tibia length) on day 14. Compound **12a** did not increase the mean blood pressure observed with high (8%) salt diet. Compound **12a** did not reduce cardiac hypertrophy (LV/TL) relative to controls: (∗) $p < 0.05$ vs 0.05% NaCl plus vehicle.
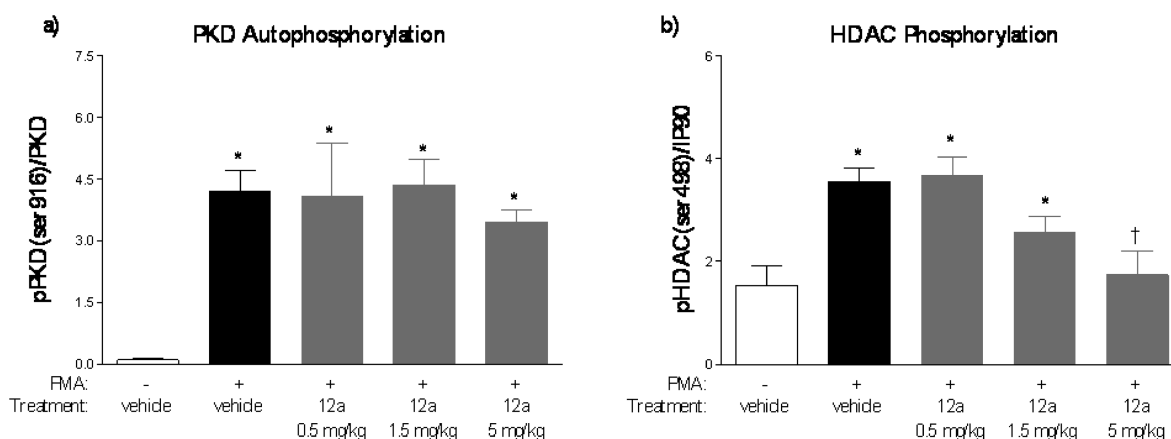

Figure 6. Biomarker readout in the DSS rat in a surrogate cell type on day 14. In peripheral blood mononuclear cells (PBMCs), PMA-stimulated, (a) autophosphorylation of PKD at Ser916 is not blunted by compound **12a** and (b) phosphorylation of the PKD substrate HDAC at Ser498 is blunted by **12a** given 5 mg/kg sc: (∗) $p < 0.05$ vs. vehicle; (#) $p < 0.05$ vs vehicle plus PMA.
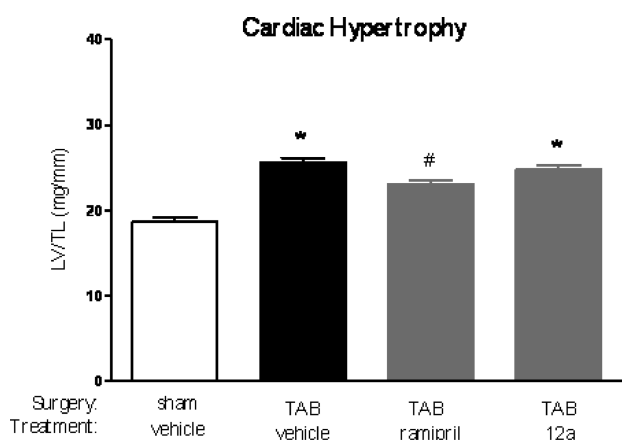

Figure 7. Effect of compound **12a** at 5 (mg/kg)/day sc in the thoracic aortic banded (TAB) rat on cardiac hypertrophy (left ventricular mass/tibia length): (∗) $p < 0.05$ vs vehicle; (#) $p < 0.05$ vs vehicle plus TAB.

12a that could be employed, in the absence of effects on blood pressure, was 5 (mg/kg)/day sc. In both models, **12a** given daily at 5 mg/kg sc produced no significant reduction of cardiac hypertrophy relative to vehicle controls. In the DSS rat, both plasma and cardiac (LV) exposures were significantly greater than the PKD1 and HDACexp IC_{50} values. Taken in combination with the PBMC biomarker data, the exposures (LV $12 \pm 2 \ \mu$M, plasma $0.23 \pm 0.8 \ \mu$M) are consistent with the inhibition of HDAC5 phosphorylation in the heart. By analogy to naphthyridine **1**, discussed in the preceding paper (DOI: 10.1021/jm100075z), bipyridyl **12a** produced a marked reduction in HDAC phosphorylation in the PBMCs. Compounds **1** (PKD1 $IC_{50} = 0.6$ nM; HDACexp $IC_{50} = 32$ nM) and **12a** (PKD1 $IC_{50} = 1$ nM; HDACexp $IC_{50} = 77$ nM) are equivalent with respect to in vitro potencies, and both achieve cardiac exposure 100- to 1000-fold in excess of the cellular IC_{50} values. Yet only the highest dose of **1** (50 (mg/kg)/day po, cardiac exposure $60 \ \mu$M) controls both PKD autophosphorylation and cardiac hypertrophy. The higher cardiac exposure and lower kinase selectivity of **1** versus **12a** may be contributing factors to the reduction of PKD autophosphorylation (Ser916) and cardiac hypertrophy. Nonetheless, both **1** and **12a** blunt the target phosphorylation event, which is the phosphorylation of the PKD substrate HDAC5.

A major finding in this study is that the PKD selective inhibitor **12a** failed to reduce cardiac hypertrophy in two distinct animal models (DSS and aortic banded rats). This is seemingly in contradiction to previously published work demonstrating that genetic loss of PKD1 protects from pressure-overload-induced cardiac hypertrophy. There are several possibilities to explain these results. First, it is possible that inhibition of PKD1 kinase activity is not equivalent to
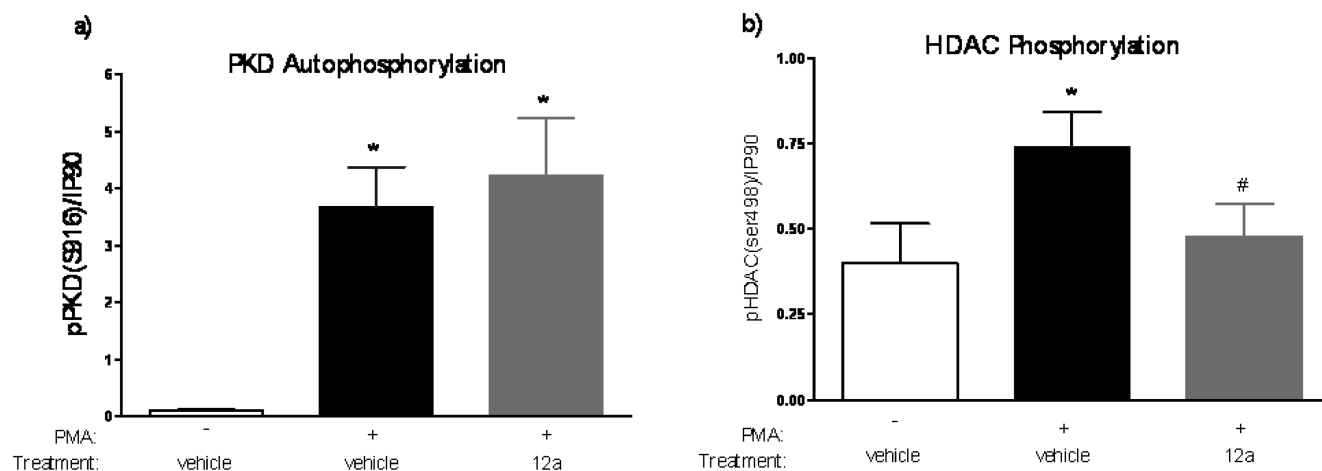
Figure 8. Ex vivo. In peripheral blood mononuclear cells (PBMCs), PMA-stimulated, (a) autophosphorylation of PKD at Ser916 is not blunted by compound **12a** and (b) phosphorylation of the PKD substrate HDAC at Ser498 is reduced: (∗) $p < 0.05$ vs vehicle; (#) $p < 0.05$ vs vehicle plus PMA.

total loss of the protein as in the PKD1 knockout mouse. The PKD1 protein may have other functions such as to serve as a scaffold or to possess a signaling function that contributes to its prohypertrophic activity. In addition, because of technical limitations, we were unable to detect pHDAC5 in the heart. Although **12a** was able to reduce pHDAC5 in PBMCs, we cannot be certain that the same is true in the heart in spite of the high cardiac exposure of the compound. Finally, it is possible that there is a difference in the animal models tested. The models used here are both in the rat, while the PKD1 knockout is obviously in the mouse. There may be a fundamental difference in the role of PKD1 in the rat versus the mouse in the control of cardiac cell growth. Nonetheless, **12a** represents a potent and selective PKD inhibitor that has proven to be a valuable tool to further define the role of PKD1 in cardiac hypertrophy and failure.

Experimental Section

PKD1 Assay. The assay to measure protein kinase D1 (PKD1) activity was a time-resolved fluorescence resonance transfer (TR-FRET) assay using PerkinElmer's LANCE technology. In this case, a biotinylated syntide-2 peptide was used as the substrate in this reaction. Phosphorylation of the syntide-2 substrate was detected by a specific antibody that recognizes the phosphorylated peptide. A second fluorophore, APC, was conjugated to streptavidin that binds the biotinylated syntide-2 peptide. For detection, the europium fluorophore can be excited by 340 nM light which then emits at 615 nM. Therefore, when the europium labeled secondary antibody binds on the phosphorylated peptide, it was brought into close contact with the APC and excites this fluorophore. The APC emission was at 665 nM and the (665 nM)/(615 nM) ratio was a readout of PKD1 activity. This assay was performed with full length wild-type enzyme that was expressed and purified from Sf9 insect cells. The reaction buffer consists of 35 mM Tris-HCl, pH7.5, 5 mM $MgCl_2$, 0.02% Tween-20, 20 μM ATP, 1 mM DTT, and 0.2 μg/mL PKD1 enzyme. The enzyme reaction was initiated by the addition of 2 μM syntide-2 peptide substrate and the reaction carried out for 50 min at room temperature. The reaction was stopped by a stop/detection buffer consisting of 50 mM EDTA, 0.18 mg/mL rabbit polyclonal anti-phospho syntide-2 antibody, 0.5 nM europium labeled anti-rabbit IgG, and 10 nM streptavidin conjugated APC. After a 1 h incubation with the stop/detection buffer, the reaction was read on an Envision 2100 reader using a LANCE Eu/APC dual protocol. As described above, a (665 nM)/(615 nM) ratio was determined to measure

substrate phosphorylation and enzyme activity. Compounds were typically tested in an 11-point dose response fashion in triplicate for each concentration used. IC_{50} values were calculated using an activity base (IDBS) software program.

PKD2 Assay. The assay to measure protein kinase D2 (PKD2) activity was a time-resolved fluorescence resonance transfer (TR-FRET) assay using PerkinElmer's LANCE technology. In this case, a biotinylated syntide-2 peptide was used as the substrate in this reaction. Phosphorylation of the syntide-2 substrate was detected by a specific antibody that recognizes the phosphorylated peptide. A second fluorophore, APC, was conjugated to streptavidin that binds the biotinylated syntide-2 peptide. For detection, the europium fluorophore can be excited by 340 nM light which then emits at 615 nM. Therefore, when the europium labeled secondary antibody binds on the phosphorylated peptide, it was brought into close contact with the APC and excites this fluorophore. The APC emission was at 665 nM and the (665 nM)/(615 nM) ratio was a readout of PKD2 activity.

PKD3 Assay. The assay was performed with full length wild-type enzyme purchase from Invitrogen. The reaction buffer consists of 35 mM Tris-HCl, pH7.5, 5 mM $MgCl_2$, 0.02% Tween-20, 20 μM ATP, 1 mM DTT, and 0.2 μg/mL PKD2 enzyme. The enzyme reaction was initiated by the addition of 2 μM syntide-2 peptide substrate and the reaction carried out for 50 min at room temperature. The reaction was stopped by a stop/detection buffer consisting of 50 mM EDTA, 0.18 mg/mL rabbit polyclonal anti-phospho syntide-2 antibody, 0.5 nM europium labeled anti-rabbit IgG, and 10 nM streptavidin conjugated APC. After a 1 h incubation with the stop/detection buffer, the reaction was read on an Envision 2100 reader using a LANCE Eu/APC dual protocol. As described above, a (665 nM)/(615 nM) ratio was determined to measure substrate phosphorylation and enzyme activity. Compounds were typically tested in an 11-point dose response fashion in triplicate for each concentration used. IC_{50} values were calculated using an activity base (IDBS) software program.

The assay to measure protein kinase D3 (PKD3) activity was a time-resolved fluorescence resonance transfer (TR-FRET) assay using PerkinElmer's LANCE technology. In this case, a biotinylated syntide-2 peptide was used as the substrate in this reaction. Phosphorylation of the syntide-2 substrate was detected by a specific antibody that recognizes the phosphorylated peptide. A second fluorophore, APC, was conjugated to streptavidin that binds the biotinylated syntide-2 peptide. For detection, the europium fluorophore can be excited by 340 nM light which then emits at 615 nM. Therefore, when the europium labeled secondary antibody binds on the phosphorylated peptide, it was brought into close contact with the APC and excites

this fluorophore. The APC emission was at 665 nM, and the (665 nM)/(615 nM) ratio was a readout of PKD3 activity.

PKC Assays. The compounds of the invention were tested for their activity on different PKC isotypes according to the following method. Assay was performed in a white with clear bottom 384-well microtiter plate with nonbinding surface. The reaction mixture (25 μL) contains 1.5 μM of a tridecapeptide acceptor substrate that mimics the pseudo substrate sequence of PKCα with the Ala $\rightarrow$ Ser replacement, 10 μM ^{33}P-ATP, 10 mM Mg(NO$_3$)$_2$, 0.2 mM CaCl$_2$, PKC at a protein concentration varying from 25 to 400 ng/mL (depending on the isotype used), lipid vesicles (containing 30 mol % phosphatidylserine, 5 mol % DAG, and 65 mol % phosphatidylcholine) at a final lipid concentration of 0.5 mM, in 20 mM Tris-HCl buffer, pH 7.4, and 0.1% BSA. Incubation was performed for 60 min at room temperature. Reaction was stopped by adding 50 μL of stop mix (100 mM EDTA, 200 μM ATP, 0.1% Triton X-100, 0.375 mg/ well streptavidin-coated SPA beads in phosphate buffered saline without Ca, Mg. After 10 min of incubation at room tempera- ture, the suspension was spun down for 10 min at 300g. Incorporated radioactivity was measured in a Trilux counter for 1 min. IC$_{50}$ measurement was performed on a routine basis by incubating a serial dilution of inhibitor at concentrations ranging between 1 and 1000 nM. IC$_{50}$ values were calculated from the graph by curve fitting with XL fit software. Human recombinant PKCα was obtained from Oxford Biomedical Research and was used under the assay conditions as described above. Human recombinant PKCδ was obtained from Oxford Biomedical Research and was used under the assay conditions as described above.

HDAC Export Assay. Compounds were evaluated in the HDAC5 nuclear exposrt assay, a 384-well plate-based assay that enables HTS to identify small molecules that block agonist- dependent nuclear export of HDAC5. This assay employs the Cellomics high content imaging platform (Giuliano and Taylor, 1998) and adenovirus encoding green fluorescent protein (GFP) tagged HDAC5. Neonatal rat ventricular myocytes (NRVMs) were infected with GFP-HDAC5 encoding virus and plated on gelatin-coated 384-well dishes. Cells were exposed to compound and stimulated with a prostaglandin (PGF2α), which is a potent stimulus for HDAC5 nuclear export. Following 2 h of stimula- tion, cells were fixed and GFP-HDAC5 localization was quan- tified using the Cellomics system, which provides a readout of relative fluorescence intensity in the cytoplasmic versus nuclear compartment.

DSS Rat Model. Six to seven week-old male Dahl salt- sensitive (DSS) rats ($n = 50$) from Harlan Labs were fed base diet (0.49% NaCl) and allowed to acclimate for 1 week prior to being separated into five weight-matched groups. Rats were maintained on the grain based diet (0.49% NaCl) or switched to grain diet containing 8.0% NaCl for 2 consecutive weeks. Coincident with diet switch rats were administered (sc, 1.0 mL/kg) vehicle (10% 1 N HCl/90% 10% captisol in pH 4.6 buffer) or test compound **12a** (0.5, 1.5, or 5 mg/kg) in vehicle. At the completion of study, steady-state hemodynamics were de- termined. The rats were then sacrificed, and tissues were col- lected for morphological and biochemical analysis.

TAB Rat Model. Seven to eight week-old male Sprague– Dawley rats ($n = 30$) from Charles River Labs were allowed normal chow and water ad libitum and allowed to acclimate for 1 week. Rats were then instrumented with an aortic occlusion cuff or underwent sham surgery. Coincident with the surgical procedure, rats were administered (sc, 1.0 mL/kg) vehicle (acidified captisol) or **12a** (5 (mg/kg)/day sc) dissolved in vehicle for 2 weeks. On the final day of study, end-point cardiac per- formance and steady-state hemodynamics were determined by ultrasound and direct cardiac catheterization, respectively. Following completion of the measurement protocol, the rats were sacrificed, and tissues were collected for morphological and biochemical analysis.

Chemistry. General. NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. The following abbreviations are used to denote signal patterns: s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad. Flash chromatography was conducted using grade 60 230−400 mesh silica gel from Fisher Chemical (S825-1) or by utilizing the CombiFlash Companion from Teledyne Isco, Inc. and RediSep Rf disposable normal phase silica gel columns (4−300 g). Thin layer chromatography was performed using 2.5 cm × 7.5 cm glass-backed TLC silica gel 60 F$_{254}$ plates from EMD Chemicals, Inc. (15341-1) and visualized by UV light. HPLC purifications were performed on a Gilson preparative HPLC system controlled by Unipoint software using X-Bridge phenyl, C8, C18, or RP18 30 mm × 50 mm columns with 5 μm particle size. The purity of all compounds was ≥95%, unless otherwise noted. Low-resolution mass spectra were recorded using an Agilent 1100 series LCMS spectrometer.

4-(2′-Chloro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (5). A mixture of 4-(6-bromopyridin-2-yl)- piperazine-1-carboxylic acid *tert*-butyl ester (1.98 g, 5.78 mmol), 2-chloropyridine-4-boronic acid (1.0 g, 6.35 mmol), Pd(Ph$_3$P)$_4$ (0.330 g, 0.289 mmol), aqueous solution of Na$_2$CO$_3$ (5.7 mL, 2.0 M), and CH$_3$CN (10 mL) was sparged with argon for 10 min. The vessel was then sealed, and the contents were heated to 90 °C for 4 h. The mixture was then allowed to cool and concentrated under reduced pressure. The residue was taken up in CH$_2$Cl$_2$ and washed with H$_2$O. The aqueous layer was further extracted with CH$_2$Cl$_2$ (2 × 50 mL). The combined organic layers were then dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 20−30% EtOAc/ hexanes gradient) to give the title compound 4-(2′-chloro[2,4′]- bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester: MS (ESI) m/z 375.0, 376.9 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.44 (d, J = 5.3 Hz, 1 H), 7.93 (s, 1 H), 7.78 (dd, J = 5.1, 1.5 Hz, 1 H), 7.61 (dd, J = 8.5, 7.5 Hz, 1 H), 7.16 (d, J = 7.3 Hz, 1 H), 6.73 (d, J = 8.6 Hz, 1 H), 3.55−3.69 (m, 8 H), 1.50 (s, 9 H).

Cyclohexyl-(6-piperazin-1-yl[2,4′]bipyridinyl-2′-yl)amine (6). A mixture of 4-(2′-chloro[2,4′]bipyridinyl-6-yl)piperazine-1-car- boxylic acid *tert*-butyl ester (0.300 g, 0.801 mmol), Pd(*t*-Bu$_3$P)$_2$ (0.041 g, 0.080 mmol), NaO-*t*-Bu (0.115 g, 1.20 mmol), cyclo- hexylamine (0.18 mL, 1.60 mmol), and 1,4-dioxane (4 mL) was sparged with argon for 10 min. The vessel was then sealed, and the contents were heated to 130 °C for 8 h. The mixture was then allowed to cool followed by concentration. The residue was then separated via flash chromatography (SiO$_2$, EtOAc/hexanes gradient) to give 4-(2′-cyclohexylamino[2,4′]bipyridinyl-6-yl)- piperazine-1-carboxylic acid *tert*-butyl ester: MS (ESI) m/z 438.0 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.99 (d, J = 5.3 Hz, 1 H), 7.61−7.69 (m, 1 H), 7.19 (d, J = 7.6 Hz, 1 H), 7.15 (s, 1 H), 7.01 (d, J = 5.6 Hz, 1 H), 6.88 (d, J = 8.6 Hz, 1 H), 6.32−6.61 (m, 1 H), 3.68−3.78 (m, 1 H), 3.59 (d, J = 10.4 Hz, 4 H), 3.42−3.50 (m, 4 H), 1.94 (d, J = 15.9 Hz, 2 H), 1.73 (d, J = 19.5 Hz, 2 H), 1.60 (d, J = 20.5 Hz, 1 H), 1.43 (s, 9 H), 1.26−1.40 (m, 2 H), 1.11−1.26 (m, 3 H).

To a solution of 4-(2′-cyclohexylamino[2,4′]bipyridinyl-6-yl)- piperazine-1-carboxylic acid *tert*-butyl ester (0.220 g, 0.503 mmol) and CH$_2$Cl$_2$ (7 mL) was added TFA (5 mL). After being stirred for 1 h, the solution was concentrated. The residue was taken up in CH$_2$Cl$_2$ (50 mL) and washed with a saturated aqueous solution of Na$_2$CO$_3$. The aqueous layer was further extracted with CH$_2$Cl$_2$ (2 × 50 mL). The combined organic layers were then dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was then purified via semipreparative HPLC (10−90% CH$_3$CN/H$_2$O gradient with 0.1% NH$_4$OH) to give the title compound cyclohexyl-(6-piperazin-1-yl[2,4′]bipyridinyl-2′-yl)- amine: MS (ESI) m/z 338.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.99 (d, J = 5.3 Hz, 1 H), 7.61 (t, J = 8.0 Hz, 1 H), 7.12 (s, 1 H), 7.10−7.17 (m, 1 H), 6.98 (d, J = 5.6 Hz, 1 H),

6.82 (d, *J* = 8.6 Hz, 1 H), 6.43 (d, *J* = 7.8 Hz, 1 H), 3.66−3.80 (m, 1 H), 3.44−3.55 (m, 4 H), 3.31 (s, 1 H), 2.77−2.86 (m, 4 H), 1.87−1.98 (m, 2 H), 1.67−1.78 (m, 2 H), 1.55−1.65 (m, 1 H), 1.26−1.39 (m, 2 H), 1.12−1.25 (m, 3 H).

2,6-Dibromoisonicotinic Acid Methyl Ester (8). A mixture of citrazinic acid **7** (5.0 g, 32.2 mmol) and $POBr_3$ (27.5 g, 96.8 mmol) was heated at 130 °C. Upon completion of the reaction, the thick slurry was cooled to 0 °C and carefully quenched with MeOH (250 mL). The reaction mixture was concentrated in vacuo and then partitioned between dichloromethane and a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give a tan solid that was clean enough by NMR/LCMS for further use (7.5 g, 79%): (ESI) *m/z* 295.8 (M + 1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 8.10 (s, 2 H), 4.05 (s, 3 H).

6-(4-*tert*-Butoxycarbonylpiperazin-1-yl)-2′-chloro[2,4′]bipyridinyl-4-carboxylic Acid Methyl Ester (10a). 2,6-Dibromoisonicotinic acid methyl ester (5.0 g, 17.0 mmol), piperazine-1-carboxylic acid *tert*-butyl ester (3.2 g, 17.0 mmol), and Et_3N (3.5 mL, 25.5 mmol) were stirred in 1,4-dioxane (75 mL) at 110 °C in a 150 mL pressure vessel until the reaction was completed by LCMS. The reaction vessel was cooled to room temperature and concentrated in vacuo. The residue was taken up in acetonitrile/water (1:9). A tan solid precipitate was collected by filtration and dried to give the title compound . The purity was sufficient by NMR/LCMS to use for further transformations (5.4 g, 80%): MS (ESI) *m/z* 402.0 (M + 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.21 (s, 1 H), 7.03 (s, 1 H), 3.85 (s, 3 H), 3.50−3.55 (m, 4 H), 3.44−3.49 (m, 4 H), 1.41 (s, 9 H).

4-(6-Bromo-4-methoxycarbonylpyridin-2-yl)piperazine-1-carboxylic acid *tert*-butyl ester (1.5 g, 3.76 mmol) and 2-chloro-4-pyridine boronic acid (0.71 g, 4.51 mmol) were stirred in DME (25 mL). To this was added a 2.0 M solution of Na_2CO_3 (6.0 mL, 11.28 mmol) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (0.31 g, 0.37 mmol). This above suspension was heated to 80 °C for 4 h. The mixture was diluted with EtOAc (25 mL) and partitioned between organic and saturated $NaHCO_3$ (×2). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude residue was purifed via flash chromatography (SiO_2, EtOAc/heptanes gradient) to afford the compound as a pale-yellow solid (1.40 g, 87%): MS (ESI) *m/z* 433.2 (M + 1); ^{1}HNMR (400 MHz, CD_2Cl_2) δ ppm 8.37 (d, *J* = 4.5 Hz, 1 H), 7.92 (d, *J* = 1.5 Hz, 1 H), 7.79 (dd, *J* = 5.1, 1.5 Hz, 1 H), 7.60 (s, 1 H), 7.26 (s, 1 H), 3.86 (s, 3 H), 3.57−3.68 (m, 4 H), 3.41−3.53 (m, 4 H), 1.39 (s, 9 H).

6-(4-*tert*-Butoxycarbonylpiperazin-1-yl)-2′-fluoro[2,4′]bipyridinyl-4-carboxylic Acid Methyl Ester (10b). 10b was prepared as described for **10a**: MS (ESI) *m/z* 417.4 (M + 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.32 (d, *J* = 5.3 Hz, 1 H), 7.78−7.83 (m, 1 H), 7.70 (s, 1 H), 7.60 (s, 1 H), 7.35 (s, 1 H), 4.00 (s, 3 H), 3.70−3.76 (m, 4 H), 3.59−3.65 (m, 4 H).

2′-Cyclohexylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (12a). 6-(4-*tert*-Butoxycarbonylpiperazin-1-yl)-2′-fluoro[2,4′]bipyridinyl-4-carboxylic acid methyl ester (435.0 mg, 1.00 mmol) was dissolved in a 7.0 M NH_3/MeOH solution (25 mL) and heated at 90 °C in a sealed pressure vessel .Upon completion, the reaction was concentrated in vacuo and the residue obtained was used without further purification (398.0 mg, 95%): (ESI) *m/z* 402.1 (M + 1); ^{1}H NMR (400 MHz, CD_3CN) δ ppm 8.30 (d, *J* = 5.3 Hz, 1 H), 7.91−7.98 (m, 1 H), 7.71 (s, 1 H), 7.58 (d, *J* = 1.0 Hz, 1 H), 7.21 (d, *J* = 1.0 Hz, 1 H), 6.98 (s, 1 H), 6.23 (s, 1 H), 3.64−3.76 (m, 4 H), 3.48−3.59 (m, 4 H), 1.48 (s, 9 H).

4-(4-Carbamoyl-2′-fluoro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester (160.0 mg, 0.39 mmol) was dissolved in neat cyclohexylamine (8 mL) and heated at 130 °C in a sealed pressure vessul until the reaction was complete. The reaction was concentrated in vacuo and the residue purified via semi-preparative HPLC (5−50% CH_3CN/H_2O gradient with 0.1% NH_4OH) to give 4-(4-carbamoyl-2′-cyclohexylamino[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester (95.0 mg,

50%): (ESI) *m/z* 481.4 (M + 1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm 8.01 (d, *J* = 5.6 Hz, 1 H), 7.53 (s, 1 H), 7.18 (s, 1 H), 7.02 (dd, *J* = 5.3, 1.3 Hz, 1 H), 6.97 (s, 1 H), 4.60 (br s, 1 H), 3.55−3.65 (m, 4 H), 3.44−3.51 (m, 4 H), 1.91−2.05 (m, 2 H), 1.63−1.76 (m, 2 H), 1.53−1.63 (m, 2 H), 1.39 (s, 9 H), 1.10−1.25 (m, 4 H).

To a solution of 4-(4-carbamoyl-2′-cyclohexylamino[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester (96.0 mg, 0.20 mmol) and CH_2Cl_2 (5.00 mL) was added TFA (5.0 mL). After being stirred for 2 h, the solution was concentrated. The residue was taken up in CH_2Cl_2 (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with fresh CH_2Cl_2 (2 × 10 mL). The combined organic layers were then dried (Na_2SO_4), filtered, and concentrated. The residue was purified via semipreparative HPLC (10−90% CH_3CN/H_2O gradient with 0.1% NH_4OH) to give the title compound (35.0 mg, 46%): MS (ESI) *m/z* 381.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.17 (s, 1 H), 8.02 (d, *J* = 5.3 Hz, 1 H), 7.60 (s, 1 H), 7.52 (s, 1 H), 7.20 (s, 1 H), 7.16 (s, 1 H), 7.03 (dd, *J* = 5.4, 1.4 Hz, 1 H), 6.47 (d, *J* = 7.8 Hz, 1 H), 3.67−3.84 (m, 1 H), 3.48−3.63 (m, 4 H), 2.76−2.92 (m, 4 H), 1.93 (dd, *J* = 11.7, 2.4 Hz, 2 H), 1.67−1.80 (m, 2 H), 1.53−1.66 (m, 1 H), 1.10−1.41 (m, 5 H); HRMS (ESI) calcd *m/z* 381.2403 (M + 1), found *m/z* 381.2395 (M + 1). Anal. ($C_{21}H_{28}N_6O \cdot 1H_2O$) calcd, C 63.29, H 7.59, N 21.09; found, C 63.24, H 7.52, N 21.08.

2′-Cyclopentylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (12g). The title compound was prepared by a similar method to **12a**: MS (ESI) *m/z* 367.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.18 (s, 1 H), 8.04 (d, *J* = 5.6 Hz, 1 H), 7.61 (s, 1 H), 7.54 (s, 1 H), 7.22 (s, 1 H), 7.15 (s, 1 H), 7.05 (dd, *J* = 5.3, 1.5 Hz, 1 H), 6.58 (d, *J* = 6.8 Hz, 1 H), 4.09−4.24 (m, 1 H), 3.52−3.65 (m, 4 H), 2.80−2.98 (m, 4 H), 1.85−1.98 (m, 2 H), 1.63−1.78 (m, 2 H), 1.37−1.62 (m, 4 H).

2′-(1-Methyl-1*H*-pyrazol-3-ylamino)-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (12e). A solution of NaHMDS (0.5 mL, 0.48 mmol, 1.0 M THF) was added to a solution of 3-amino-1-methylpyrazole (0.024 g, 0.24 mmol) in THF (3 mL) at ambient temperature. Then 4-(4-carbamoyl-2′-fluoro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester (50 mg, 0.12 mmol) was added. The reaction mixture was sealed and heated to 80 °C for 3 h. The reaction was quenched with *i*-PrOH and concentrated in vacuo. The residue was purified by flash chromatography (2−10% MeOH/DCM) to afford 4-(4-carbamoyl-2′-(2-methyl-2*H*-pyrazol-3-yl)piperazine-1-carboxylic acid *tert*-butyl ester: MS (ESI) *m/z* 479.3 (M + 1).

4-(4-Carbamoyl-2′-(2-methyl-2*H*-pyrazol-3-yl)piperazine-1-carboxylic acid *tert*-butyl ester (175 mg, 0.37 mmol) and TFA (5 mL) were stirred in CH_2Cl_2 (5 mL) at 25 °C for 2 h. After being stirred for 2 h, the solution was concentrated. The residue was purified via semipreparative HPLC (10−90% CH_3CN/H_2O gradient with 0.1% NH_4OH) to give 2′-(1methyl-1*H*-pyrazol-3-ylamino)-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic acid amide: MS (ESI) *m/z* 379.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.94 (br s, 2 H), 8.20−8.37 (m, 2 H), 8.12 (s, 1 H), 7.80 (s, 1 H), 7.76 (s, 1 H), 7.71 (s, 1 H), 7.57 (d, *J* = 5.4 Hz, 1 H), 7.46 (s, 1 H), 6.24 (d, *J* = 2.1 Hz, 1 H), 3.88−3.98 (m, 4 H), 3.85 (s, 3 H), 3.28 (br s, 4 H).

6-Piperazin-1-yl-2′-(tetrahydropyran-4-ylamino)[2,4′]bipyridinyl-4-carboxylic Acid Amide. (12c). To a solution of toluene (60 mL) and trimethylaluminum (23.1 mL, 46.3 mmol) was added *tert*-butylamine (4.9 mL, 46.3 mmol). This solution was stirred at room temperature for 10 min before 6-(4-*tert*-butoxycarbonylpiperazin-1-yl)-2′-chloro[2,4′]bipyridinyl-4-carboxylic acid methyl ester (2.5 g, 5.78 mmol) was added portionwise. The resulting suspension was heated at 110 °C until LCMS indicated complete reaction. The mixture was cooled to ambient temperature and quenched carefully with MeOH. The gelatinous suspension was filtered and the filter cake washed well with MeOH. The filtrate was concentrated in vacuo and the residue purified via flash chromatography (SiO_2, EtOAc/heptanes gradient) to afford the 4-(4-*tert*-butylcarbamoyl-2′-chloro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester as a yellow solid (2.05 g, 75%): MS (ESI)

m/z 474.1 (M + 1); ^{1}H NMR (400 MHz, CD$_2$Cl$_2$) δ ppm 8.35 (d, *J* = 5.1 Hz, 1 H), 7.88 (s, 1 H), 7.76 (dd, *J* = 5.3, 1.5 Hz, 1 H), 7.21 (s, 1 H), 6.93 (s, 1 H), 5.95 (br s, 1 H), 3.55−3.65 (m, 4 H), 3.44−3.51 (m, 4 H), 1.39 (s, 18 H).

A mixture of 4-(4-*tert*-butylcarbamoyl-2′-chloro[2,4′]bipyridinyl-6-yl)-piperazine-1-carboxylic acid *tert*-butyl ester (225.0 mg, 0.47 mmol), Pd(*t*-Bu$_3$P)$_2$ (24.0 mg, 0.047 mmol), NaO-*t*-Bu (141.0 mg, 1.41 mmol), 4-aminotetrahydropyran (0.14 mL, 1.4l mmol), and 1,4-dioxane (5 mL) was sparged with argon for 10 min. The vessel was sealed, and the contents were heated to 130 °C for 2 h. The mixture was allowed to cool and concentrated under reduced pressure. The residue was purified via flash chromatography (SiO$_2$, EtOAc/hexanes gradient) to give 4-[4-carbamoyl-2′-(tetrahydropyran-4-ylamino)[2,4′]bipyridinyl-6-yl]piperazine-1-carboxylic acid tertbutyl ester (150 mg, 59%): MS (ESI) *m/z* 539.2 (M + 1); ^{1}H NMR (400 MHz, CD$_2$Cl$_2$) δ ppm 8.13 (d, *J* = 5.3 Hz, 1 H), 7.26 (s, 1 H), 7.13 (dd, *J* = 5.3, 1.5 Hz, 1 H), 7.07 (s, 1 H), 7.01 (s, 1 H), 6.07 (br s, 1 H), 4.76 (br s, 1 H), 3.93−4.16 (m, 1 H), 3.65−3.76 (m, 4 H), 3.58 (dd, *J* = 6.3, 4.0 Hz, 4 H), 1.50 (s, 18 H), 1.29 (d, *J* = 6.6 Hz, 6 H).

A mixture of 4-[4-carbamoyl-2′-(tetrahydropyran-4-ylamino)[2,4′]bipyridinyl-6-yl]piperazine-1-carboxylic acid *tert*-butyl ester (115.0 mg, 0.21 mmol) and TFA (8 mL) was stirred in a microwave at 120 °C for 2 h. After being stirred for 2 h, the solution was concentrated. The residue was purified via semi-preperative HPLC (10−90% CH$_3$CN/H$_2$O gradient with 0.1% NH$_4$OH) to give the title compound (95.0 mg, 75%): MS (ESI) *m/z* 383.1 (M + 1); ^{1}H NMR (400 MHz, MeOH-*d$_4$*) δ ppm 7.90 (d, *J* = 5.6 Hz, 1 H), 7.46 (s, 1 H), 7.16 (s, 1 H), 7.13 (s, 1 H), 7.07 (dd, *J* = 5.6, 1.5 Hz, 1 H), 3.80−3.95 (m, 3 H), 3.55−3.66 (m, 4 H), 3.41−3.53 (m, 2 H), 2.78−2.94 (m, 4 H), 1.82−1.98 (m, 2 H), 1.33−1.54 (m, 2 H).

2′-Ethylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (12h). The title compound was prepared by a similar method as described for **12c**: MS (ESI) *m/z* 327.1 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 8.18 (s, 1 H), 8.04 (d, *J* = 5.3 Hz, 1 H), 7.60 (s, 1 H), 7.53 (s, 1 H), 7.20 (s, 1 H), 7.13 (s, 1 H), 7.07 (dd, *J* = 5.4, 1.5 Hz, 1 H), 6.56 (t, *J* = 5.4 Hz, 1 H), 3.49−3.59 (m, 4 H), 3.24−3.36 (obs q, 2 H), 2.73−2.87 (m, 4 H), 2.38 (br s, 1 H), 1.15 (t, *J* = 7.1 Hz, 3 H).

4′-*tert*-Butylcarbamoyl-2″-isopropylamino-3,4,5,6-tetrahydro-2*H*-[4,2′;6′,4″]terpyridine-1-carboxylic Acid *tert*-Butyl Ester (12d). The title compound was prepared by a similar method as described for **12c**: MS (ESI) *m/z* 341.1 (M + 1); ^{1}H NMR (400 MHz, MeOH-*d$_4$*) δ ppm 7.89 (d, *J* = 6.1 Hz, 1 H), 7.45 (d, *J* = 1.0 Hz, 1 H), 7.09−7.15 (m, 2 H), 7.05 (dd, *J* = 5.7, 1.6 Hz, 1 H), 3.82−4.01 (m, 1 H), 3.51−3.65 (m, 4 H), 2.79−2.92 (m, 4 H), 1.15 (d, *J* = 6.6 Hz, 6 H).

2′-(2-Chlorophenylamino)-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (12f). The title compound was prepared by a similar method as described for **12c**: MS (ESI) *m/z* 409.1 (M + 1); ^{1}H NMR (400 MHz, MeOH-*d$_4$*) δ ppm 8.16 (d, *J* = 5.4 Hz, 1 H), 7.88 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.66 (s, 1 H), 7.59 (s, 1 H), 7.44 (d, *J* = 6.8 Hz, 2 H), 7.28 (dt, 1 H), 7.23 (s, 1 H), 7.05 (dt, *J* = 7.7, 1.5 Hz, 1 H), 3.61−3.73 (m, 4 H), 2.89−3.01 (m, 4 H).

2′-Phenylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic Acid Methyl Amide (12b). The title compound was prepared by a similar method as described for **12c**: (ESI) *m/z* 375.1 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 8.18 (d, *J* = 5.4 Hz, 1 H), 7.62 (d, *J* = 8.0 Hz, 2 H), 7.53 (d, *J* = 8.5 Hz, 2 H), 7.38 (dd, *J* = 5.6, 1.5 Hz, 1 H), 7.31 (app t, 2 H), 7.26 (s, 1 H), 7.00 (t, 1 H), 3.66−3.83 (m, 4 H), 2.95−3.10 (m, 4 H).

2′-Cyclohexylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carbonitrile (12i). 2′-Cyclohexylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carboxylic acid amide (1.00 g, 2.08 mmol) in CH$_2$Cl$_2$ (21 mL) and Et$_3$N (1.45 mL, 10.4 mmol) was stirred at 0 °C before adding trifluoroacetic acid anhydre (0.9 mL, 6.33 mmol). The solution was then allowed to warm to room temperature. After 2 h, the mixture was diluted with CH$_2$Cl$_2$ and extracted with a saturated aqueous NaHCO$_3$ solution. The separated organic

layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue (100 mg) was dissolved in MeOH (2.0 mL) and treated with NaBH$_4$ (14 mg, 0.36 mmol) at 0 °C. After 2 h, the mixture was evaporated and the residue was taken up in CH$_2$Cl$_2$/TFA (2:1) and stirred for 3 h at 0 °C. The acid was neutralized by washing with a saturated aqueous solution of NaHCO$_3$. The residue was purified by column chromatography (5−10% MeOH/CH$_2$Cl$_2$) to give the title compound: MS (ESI) *m/z* 363.3 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 8.03 (d, *J* = 5.3 Hz, 1 H), 7.45 (s, 1 H), 7.30 (s, 1 H), 7.15 (s, 1 H), 7.03 (dd, *J* = 5.4, 1.4 Hz, 1 H), 6.50 (d, *J* = 7.7 Hz, 1 H), 3.67−3.81 (m, 1 H), 3.51−3.65 (m, 4 H), 2.73−2.88 (m, 4 H), 1.85−1.99 (m, 2 H), 1.66−1.78 (m, 2 H), 1.54−1.66 (m, 1 H), 1.09−1.41 (m, 6 H).

2′-Phenylamino-6-piperazin-1-yl[2,4′]bipyridinyl-4-carbonitrile (12j). The title compound was prepared by a similar method as described for **12**: MS (ESI) *m/z* 357.2 (M + 1); ^{1}H NMR (400 MHz, MeOH-*d$_4$*) δ ppm 8.16 (d, *J* = 5.6 Hz, 1 H), 7.51−7.57 (m, 2 H), 7.48−7.52 (m, 1 H), 7.39 (s, 1 H), 7.24−7.35 (m, 3 H), 7.14 (s, 1 H), 6.91−7.03 (m, 1 H), 3.57−3.74 (m, 4 H), 2.86−3.00 (m, 4 H).

2′-(1-Methyl-1*H*-pyrazol-3-ylamino)-6-piperazin-1-yl[2,4′]bipyridinyl-4-carbonitrile (12k). The title compound was prepared by a similar method as described for **12i**: MS (ESI) *m/z* 361.2 (M + 1); ^{1}H NMR (400 MHz, MeOH-*d$_4$*) δ ppm 8.16 (d, *J* = 5.3 Hz, 1 H), 7.92 (s, 1 H), 7.46 (d, *J* = 2.0 Hz, 1 H), 7.40 (s, 1 H), 7.32 (dd, *J* = 5.3, 1.5 Hz, 1 H), 7.14 (s, 1 H), 6.25 (d, *J* = 2.3 Hz, 1 H), 3.82 (s, 3 H), 3.64−3.73 (m, 4 H), 2.89−3.01 (m, 4 H).

4-(2′-Fluoro-4-nitro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (14a). A mixture of 2,6-dibromo-4-nitropyridine (5.0 g, 17.8 mmol), piperazine-1-carboxylic acid *tert*-butyl ester (4.0 g, 21.4 mmol), triethylamine (5 mL, 35.6 mmol), and dioxane (60 mL) was heated to 110 °C for 4 h. The mixture was then allowed to cool to room temperature, diluted with CH$_2$Cl$_2$, washed with saturated NaHCO$_3$, brine and then dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 10−30% EtOAc/heptane gradient) to give 4-(6-bromo-4-nitropyridin-2-yl)piperazine-1-carboxylic acid *tert*-butyl ester: MS (ESI) *m/z* 386.9, 388.9 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.42 (d, *J* = 1.5 Hz, 1 H), 7.22 (d, *J* = 1.5 Hz, 1 H), 3.64−3.69 (m, 4 H), 3.55−3.60 (m, 4 H), 1.50 (s, 9 H).

A mixture of 4-(6-bromo-4-nitropyridin-2-yl)piperazine-1-carboxylic acid *tert*-butyl ester (1.9 g, 4.9 mmol), 2-fluoropyridine-4-boronic acid (0.9 g, 6.37 mmol), Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ (0.2 g, 0.245 mmol), an aqueous solution of Na$_2$CO$_3$ (5.0 mL, 2.0 M), and DME (45 mL) was sparged with argon for 10 min and then heated to 90 °C for 3 h under argon. The mixture was then allowed to cool to room temperature, diluted with CH$_2$Cl$_2$, washed with saturated NaHCO$_3$ (×2), dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 20−30% EtOAc/heptane gradient) to give the title compound: MS (ESI) *m/z* 404.0 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.36 (d, *J* = 5.3 Hz, 1 H), 7.75−7.81 (m, 2 H), 7.56−7.60 (m, 1 H), 3.78 (dd, *J* = 6.3, 4.0 Hz, 4 H), 3.60−3.67 (m, 4 H), 1.51 (s, 9 H).

4-(2′-Cyclohexylamino-4-nitro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (15a). A mixture of **14a** (2.5 g, 6.2 mmol) and cyclohexylamine (250 mL) was heated to 107 °C for 62 h. The mixture was then cooled and concentrated. The residue was purified via flash chromatography (SiO$_2$, 30−40% EtOAc/heptane gradient) to give the title compound: MS (ESI) *m/z* 483.1 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.18−8.21 (m, 1 H), 7.72 (d, *J* = 1.5 Hz, 1 H), 7.35 (d, *J* = 1.5 Hz, 1 H), 7.10 (dd, *J* = 5.3, 1.5 Hz, 1 H), 6.96−6.99 (m, 1 H), 4.57 (d, *J* = 8.3 Hz, 1 H), 3.65−3.78 (m, 5 H), 3.62 (dd, *J* = 6.3, 4.0 Hz, 4 H), 2.06−2.15 (m, 2 H), 1.74−1.85 (m, 2 H), 1.62−1.73 (m, 1 H), 1.51 (s, 9 H), 1.38−1.49 (m, 2 H), 1.19−1.37 (m, 3 H).

4-[2′-(*tert*-Butoxycarbonylcyclohexylamino)-4-nitro[2,4′]bipyridinyl-6-yl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (16a). A mixture of **15a** (1.2 g, 2.49 mmol), BOC anhydride (2.72 g,

12.4 mmol), and DMAP (0.061 g, 0.498 mmol) in acetonitrile (50 mL) and CH$_2$Cl$_2$ (5 mL) was heated to 85 °C for 4.5 h. The mixture was then cooled and concentrated under reduced pressure. The residue was taken up in CH$_2$Cl$_2$ and washed with saturated NaHCO$_3$ and brine, respectively, and then dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 0–25% EtOAc/hexanes gradient) to give the title compound: MS (ESI) *m/z* 583.2 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.58–8.62 (m, 1 H), 7.72–7.79 (m, 3 H), 7.39 (d, *J* = 1.5 Hz, 1 H), 4.09–4.20 (m, 1 H), 3.77 (dd, *J* = 6.3, 4.0 Hz, 4 H), 3.62 (dd, *J* = 6.3, 4.0 Hz, 4 H), 1.91–2.00 (m, 2 H), 1.73–1.83 (m, 2 H), 1.54–1.65 (m, 3 H), 1.48–1.54 (m, 9 H), 1.42–1.45 (m, 9 H), 1.25–1.42 (m, 2 H), 0.98–1.12 (m, 1 H).

4-[2′-(*tert*-Butoxycarbonylcyclohexylamino)-4-hydroxy[2,4′]bipyridinyl-6-yl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (17a). A mixture of **16a** (1.9 g, 3.26 mmol), KOH (1.8 g, 32.6 mmol), and DMSO (65 mL) was stirred at room temperature for 1 h and then diluted with CH$_2$Cl$_2$, washed with H$_2$O (2×) and then brine, dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 0–50% EtOAc/heptane gradient) to give the title compound: MS (ESI) *m/z* 554.2 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.49 (d, *J* = 5.3 Hz, 1 H), 7.65–7.70 (m, 1 H), 7.61–7.65 (m, 1 H), 6.58–6.61 (m, 1 H), 6.01–6.05 (m, 1 H), 4.00–4.14 (m, 1 H), 3.49–3.59 (m, 8 H), 1.92–2.01 (m, 2 H), 1.70–1.80 (m, 2 H), 1.54–1.63 (m, 1 H), 1.50 (s, 9 H), 1.22–1.48 (m, 14 H), 0.91–1.09 (m, 1 H).

***tert*-Butyl 4-[4-Bromo-2′-(cyclohexylamino)-2,4′-bipyridin-6-yl]piperazine-1-carboxylate (18a).** A mixture of **17a** (1.2 g, 2.17 mmol) and POBr$_3$ (3.7 g, 13 mmol) was placed in flask with HBr receiver and heated to 130 °C for 1 h. The mixture was cooled to 0 °C, quenched with MeOH, and concentrated. The resulting slurry was diluted with a saturated aqueous solution of NaH-CO$_3$, extracted with CH$_2$Cl$_2$ (×5). The combined organic layer was concentrated. The residue was purified via semipreparative HPLC (25–55% CH$_3$CN/H$_2$O gradient with 0.1% NH$_4$OH in 17 min) to give (4-bromo-6-piperazin-1-yl[2,4′]bipyridinyl-2′-yl)cyclohexylamine: MS (ESI) *m/z* 451.9, 417.9 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 9.06 (br s, 2 H), 8.01 (d, *J* = 5.6 Hz, 1 H), 7.46 (s, 1 H), 7.27 (s, 1 H), 7.21 (br s, 1 H), 7.09 (br s, 1 H), 3.85–3.90 (m, 4 H), 3.70–3.81 (m, 1 H), 3.18–3.25 (m, 4 H), 1.89–1.98 (m, 2 H), 1.69–1.78 (m, 2 H), 1.57–1.66 (m, 1 H), 1.28–1.42 (m, 2 H), 1.13–1.28 (m, 3 H).

A mixture of (4-bromo-6-piperazin-1-yl[2,4′]bipyridinyl-2′-yl)-cyclohexylamine (crude, 0.362 mmol), BOC anhydride (0.4 g, 1.81 mmol), and triethylamine (0.252 mL, 1.81 mmol) in CH$_2$Cl$_2$ (25 mL) was stirred at room temperature. After 0.5 h, the mixture was diluted with CH$_2$Cl$_2$, washed with a saturated aqueous solution of NaHCO$_3$ (×2) and then brine, dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 15–25% EtOAc/heptane gradient) to give the title compound: ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.13–8.16 (m, 1 H), 7.22 (d, *J* = 1.3 Hz, 1 H), 7.02 (dd, *J* = 5.4, 1.5 Hz, 1 H), 6.92–6.95 (m, 1 H), 6.81 (d, *J* = 1.3 Hz, 1 H), 4.49–4.56 (m, 1 H), 3.60–3.73 (m, 5 H), 3.55–3.60 (m, 4 H), 2.04–2.13 (m, 2 H), 1.71–1.83 (m, 2 H), 1.62–1.71 (m, 1 H), 1.50 (s, 9 H), 1.38–1.47 (m, 2 H), 1.17–1.35 (m, 3 H).

Cyclohexyl-[6-piperazin-1-yl-4-(1*H*-pyrazol-4-yl)[2,4′]bipyridinyl-2′-yl]amine (19a). A mixture of **18a** (0.22 g, 0.426 mmol), 1*H*-pyrazole-4-boronic acid (0.29 g, 2.4 mmol), Pd(dppf)Cl$_2$·CH$_2$Cl$_2$ (0.007 g, 0.085 mmol), 2 M Na$_2$CO$_3$ (2.4 mL), and DME (5 mL) was sparged with argon for 10 min. The vessel was sealed and treated with microwave at 130 °C for 20 min. The mixture was diluted with CH$_2$Cl$_2$, washed with saturated NaH-CO$_3$, brine, dried (Na$_2$SO$_4$), filtered, and concentrated. The residue was purified via flash chromatography (SiO$_2$, 70–100% EtOAc/heptane gradient) to give an intermediate of BOC-protected title compound [MS (ESI) *m/z* 504.0 (M + 1)]. The intermediate was then treated with 50% TFA in CH$_2$Cl$_2$ at room temperatue for 1 h and concentrated. The resulting residue was

mixed with 2 N NH$_3$ in MeOH and concentrated again and then separated via semipreparative HPLC (10–55% CH$_3$CN/H$_2$O gradient with 0.1% NH$_4$OH in 17 min) to give the title compound: MS (ESI) *m/z* 404.0 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 13.07 (br s, 1 H), 8.45 (br s, 1 H), 8.19 (br s, 1 H), 8.00 (d, *J* = 5.3 Hz, 1 H), 7.39–7.41 (m, 1 H), 7.17–7.18 (m, 1 H), 7.08 (dd, *J* = 5.4, 1.5 Hz, 1 H), 7.02–7.04 (m, 1 H), 6.40 (d, *J* = 7.7 Hz, 1 H), 3.69–3.79 (m, 1 H), 3.53–3.58 (m, 4 H), 2.80–2.85 (m, 4 H), 1.89–1.99 (m, 2 H), 1.68–1.77 (m, 2 H), 1.55–1.65 (m, 1 H), 1.26–1.39 (m, 2 H), 1.13–1.26 (m, 3 H).

Cyclohexyl-[6-piperazin-1-yl-4-(2*H*-pyrazol-3-yl)[2,4′]bipyridinyl-2′-yl]amine (19b). The title compound was prepared by a similar method to **19a**: MS (ESI) *m/z* 404.0 (M + 1); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ ppm 13.09 (br s, 1 H), 8.01 (d, *J* = 5.3 Hz, 1 H), 7.84 (br s, 1 H), 7.58 (br s, 1 H), 7.16–7.21 (m, 2 H), 7.06 (dd, *J* = 5.4, 1.5 Hz, 1 H), 6.97 (d, *J* = 2.0 Hz, 1 H), 6.45 (d, *J* = 7.6 Hz, 1 H), 3.69–3.82 (m, 1 H), 3.53–3.61 (m, 4 H), 2.82–2.88 (m, 4 H), 1.90–1.99 (m, 2 H), 1.67–1.78 (m, 2 H), 1.54–1.65 (m, 1 H), 1.26–1.41 (m, 2 H), 1.11–1.26 (m, 3 H).

4-(2′-Chloro-4-nitro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (14b). The title compound was prepared by a similar method to **14a**: MS (ESI) *m/z* 420.0, 422.0 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.52 (dd, *J* = 5.2, 0.6 Hz, 1 H), 7.95 (dd, *J* = 1.5, 0.6 Hz, 1 H), 7.81 (dd, *J* = 5.3, 1.5 Hz, 1 H), 7.77 (d, *J* = 1.5 Hz, 1 H), 7.42 (d, *J* = 1.5 Hz, 1 H), 3.75–3.80 (m, 4 H), 3.61–3.66 (m, 4 H), 1.51 (s, 9 H).

4-(2′-Isopropylamino-4-nitro[2,4′]bipyridinyl-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (15b). After a solution of **14b** (0.8 g, 1.91 mmol) in dioxane (75 mL) was sparged with argon, isopropylamine (3.25 mL, 38.14 mmol) was added followed by Pd(*t*-Bu$_3$P)$_2$ and cesium carbonate (1.87 g, 5.73 mmol). The vessel was sealed and heated at 110 °C for 5 h. The mixture was then allowed to cool and then filtered and concentrated. The residue was purified via flash chromatography (SiO$_2$, 25–55% EtOAc/hexanes gradient) to give the title compound: MS (ESI) *m/z* 443.1 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.19 (dd, *J* = 5.4, 0.6 Hz, 1 H), 7.71 (d, *J* = 1.6 Hz, 1 H), 7.33 (d, *J* = 1.5 Hz, 1 H), 7.10 (dd, *J* = 5.3, 1.5 Hz, 1 H), 6.93–6.98 (m, 1 H), 3.96–4.11 (m, 1 H), 3.95–4.10 (m, 1 H), 3.70–3.78 (m, 4 H), 3.61 (dd, *J* = 6.4, 4.0 Hz, 4 H), 1.50 (s, 9 H), 1.29 (d, *J* = 6.3 Hz, 6 H).

***tert*-Butyl 4-{2′-[(*tert*-Butoxycarbonyl)(isopropyl)amino]-4-nitro-2,4′-bipyridin-6-yl}piperazine-1-carboxylate (16b).** The title compound was prepared by a similar method to compound **16a**: MS (ESI) *m/z* 543.3 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.58 (dd, *J* = 5.2, 0.6 Hz, 1 H), 7.76–7.80 (m, 2 H), 7.73 (dd, *J* = 5.2, 1.6 Hz, 1 H), 7.38 (d, *J* = 1.5 Hz, 1 H), 4.53–4.65 (m, 1 H), 3.74–3.79 (m, 4 H), 3.62 (dd, *J* = 6.3, 4.0 Hz, 4 H), 1.51 (s, 9 H), 1.46 (s, 9 H), 1.32 (d, *J* = 6.8 Hz, 6 H).

***tert*-Butyl 4-{2′-[(*tert*-Butoxycarbonyl)(isopropyl)amino]-4-hydroxy-2,4′-bipyridin-6-yl}piperazine-1-carboxylate (17b).** The title compound was prepared by a similar method to **17a**: MS (ESI) *m/z* 514.3 (M + 1).

***tert*-Butyl 4-(2′-[(*tert*-Butoxycarbonyl)(isopropyl)amino]-4-{[(trifluoromethyl)sulfonyl]oxy}-2,4′-bipyridin-6-yl)piperazine-1-carboxylate (18b).** To a solution of **17b** (0.379 g, 0.739 mmol) and triethylamine (0.7 mL, 3.70 mmol) in CH$_2$Cl$_2$ (12 mL), 2-(*N,N*-bis(trifluoromethylsulfonyl)amino)pyridine (0.265 g, 0.739 mmol) was added portionwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5 h and concentrated. The residue was diluted with CH$_2$Cl$_2$, washed with saturated NaHCO$_3$ (2×) and brine, and then dried (Na$_2$SO$_4$), filtered, and concentrated. The title compound was obtained and was used without further purification: MS (ESI) *m/z* 646.2 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.55 (dd, *J* = 5.2, 0.5 Hz, 1 H), 7.71 (d, *J* = 0.9 Hz, 1 H), 7.65 (dd, *J* = 5.2, 1.6 Hz, 1 H), 7.00 (d, *J* = 1.6 Hz, 1 H), 6.50 (d, *J* = 1.8 Hz, 1 H), 4.53–4.65 (m, 1 H), 3.66–3.71 (m, 4 H), 3.57–3.63 (m, 4 H), 1.50 (s, 9 H), 1.45 (s, 9 H), 1.31 (d, *J* = 6.8 Hz, 6 H).

Isopropyl-[6-piperazin-1-yl-4-(1*H*-pyrazol-4-yl)[2,4′]bipyridinyl-2′-yl]amine (19c). The title compound was prepared from **18b** by a similar method to **19a**: MS (ESI) *m/z* 364.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.11 (br s, 1 H), 8.41 (br s, 2 H), 8.02 (d, *J* = 5.3 Hz, 1 H), 7.41 (s, 1 H), 7.16 (s, 1 H), 7.10 (dd, *J* = 5.3, 1.3 Hz, 1 H), 7.04 (s, 1 H), 6.37 (d, *J* = 7.6 Hz, 1 H), 3.98−4.13 (m, 1 H), 3.52−3.59 (m, 4 H), 2.80−2.87 (m, 4 H), 1.16 (d, *J* = 6.6 Hz, 6 H).

[6-Piperazin-1-yl-4-(1*H*-pyrazol-4-yl)[2,4′]bipyridinyl-2′-yl]-(tetrahydropyran-4-yl)amine (19c). The title compound was prepared by a similar method to **19c**: MS (ESI) *m/z* 406.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.17 (br s, 1 H), 8.99 (br s, 2 H), 8.49 (br s, 1 H), 8.21 (br s, 1 H), 8.03 (d, *J* = 5.6 Hz, 1 H), 7.55 (s, 1 H), 7.25 (br s, 1 H), 7.17−7.23 (m, 2 H), 3.94−4.06 (m, 1 H), 3.85−3.93 (m, 6 H), 3.39−3.47 (m, 2 H), 3.21−3.26 (m, 4 H), 1.91 (d, *J* = 15.3 Hz, 2 H), 1.39−1.53 (m, 2 H).

Cyclohexyl-(4-trimethylstannanylpyridin-2-yl)amine (21). 2-Fluoro-4-iodopyridine (4.0 g, 17.9 mmol) and cyclohexylamine (5.1 mL, 44.8 mmol) were sealed in a pressure vessel and heated to 120 °C for 3 h. After cooling, the mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (from 10% to 20% to 30% EtOAc/hexanes) to yield 5.1 g of 2-cyclohexylamino-4-iodopyridine.

To a reaction containing the 2-cyclohexylamino-4-iodopyridine prepared above (4.9 g, 16.2 mmol) dissolved in toluene (175 mL) was added Me₃SnSnMe₃ (7.93 g, 24.2 mmol). The solution was degassed with N₂ for 10 min, Pd(Ph₃P)₄ (1.87 g, 1.6 mmol) was added, and the mixture was heated to 100 °C on. Upon cooling, the mixture was filtered over Celite, concentrated under reduced pressure, and partitioned between EtOAc and a saturated aqueous solution of KF. The separated organic phase was washed with a saturated aqueous solution of NaCl, dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by flash chromatography (from 10% to 25% to 30% EtOAc/hexanes) to afford the title compound (3.8 g, 69%) as a white solid: ^{1}H NMR (400 MHz, CDCl₃) δ ppm 0.29 (s, 7.54 H), 0.29 (d, *J* = 55.7 Hz, 0.77 H), 0.29 (d, *J* = 53.3 Hz, 0.69 H), 1.14−1.31 (m, 3 H), 1.35−1.50 (m, 2 H), 1.60−1.66 (m, 1 H), 1.71−1.80 (m, 2 H), 1.99−2.09 (m, 2 H), 3.55−3.68 (m, 1 H), 4.25−4.34 (d, *J* = 7.7 Hz, 1 H), 6.45 (s, 0.84 H), 6.45 (d, *J* = 49.6 Hz, 0.16 Hz), 6.61 (d, *J* = 4.8 Hz, 0.84 H), 6.61 (dd, *J* = 39.8, 4.8 Hz, 0.16 Hz), 7.96−8.03 (m, 1 H).

6-Chloro-2′-cyclohexylamino[2,4′]bipyridinyl-4-carboxylic Acid Methyl Ester (23). The title compound was prepared from 2,6-dichloroisonicotinic acid methyl ester and cyclohexyl-(4-trimethylstannanylpyridin-2-yl)amine by analogy to the Stille coupling method outlined above: ^{1}H NMR (400 MHz, CDCl₃) δ ppm 1.17−1.32 (m, 2 H), 1.38−1.52 (m, 3 H), 1.60−1.70 (m, 1 H), 1.71−1.82 (m, 2 H), 2.02−2.11 (m, 2 H), 3.69−3.80 (m, 1 H), 4.00 (s, 3 H), 4.58−4.68 (m, 1 H), 7.03 (s, 1 H), 7.05−7.10 (m, 1 H), 7.87 (d, *J* = 1.1 Hz, 1 H), 8.16−8.21 (m, 2 H).

2′-Cyclohexylamino-6-(3,3-dimethylpiperazin-1-yl)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24h). Compound **23** was converted to 6-(3,3-dimethylpiperazin-1-yl)-2′-cyclohexylamino-[2,4′]bipyridinyl-4-carboxylic acid methyl ester by a method similar to that described above for **10**. The resulting product 6-(3,3-dimethylpiperazin-1-yl)-2′-cyclohexylamino[2,4′]bipyridinyl-4-carboxylic acid methyl ester (61 mg, 0.14 mmol) and a solution of 7 M NH₃ in MeOH (10 mL) were placed in a pressure vessel and heated to 90 °C on. The mixture was cooled and concentrated under reduced pressure. The residue was purified by HPLC to give the title compound: MS (ESI) *m/z* 409.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.23 −1.24 (s, 6 H), 1.25−1.32 (m, 2H), 1.37−1.53 (m, 2 H), 1.68 (d, *J* = 13.9 Hz, 1 H), 1.80 (d, *J* = 13.9 Hz, 2 H), 2.05 (d, *J* = 10.9 Hz, 2 H), 2.96−3.03 (m, 2 H), 3.52 (s, 2 H), 3.64−3.72 (m, 3 H), 7.12 (dd, *J* = 5.7, 1.6 Hz, 1 H), 7.20 (d, *J* = 1.8 Hz, 2 H), 7.50 (s, 1 H), 7.97 (d, *J* = 5.6 Hz, 1 H).

2′-Cyclohexylamino-6-((*R*)-3-methylpiperazin-1-yl)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24c). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 395.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.20 (d, *J* = 6.32 Hz, 3 H), 1.24−1.33 (m, 3 H), 1.39−1.48 (m, 2 H), 1.65−1.72 (m, 1H), 1.80 (d, *J* = 13.9 Hz, 2 H), 2.04 (d, *J* = 9.9 Hz, 2 H), 2.58−2.66 (m, 1 H), 2.85−2.98 (m, 3 H), 3.07−3.15 (m, 1 H), 3.62−3.72 (m, 1 H), 4.39 (d, *J* = 12.9 Hz, 2 H), 7.12 (dd, *J* = 5.7, 1.4 Hz, 1 H), 7.22 (d, *J* = 6.8 Hz, 2 H), 7.54 (s, 1 H), 7.97 (d, *J* = 5.6 Hz, 1 H).

2′-Cyclohexylamino-6-((*S*)-3-methylpiperazin-1-yl)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24d). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 395.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.19 (d, *J* = 6.3 Hz, 3 H), 1.23−1.33 (m, 3 H), 1.39−1.52 (m, 2 H), 1.68 (d, *J* = 11.9 Hz, 1 H), 1.80 (d, *J* = 13.1 Hz, 2 H), 2.04 (d, *J* = 12.4 Hz, 2 H), 2.57−2.65 (m, 1 H), 2.84−3.00 (m, 3 H), 3.10 (d, *J* = 10.9 Hz, 1 H), 3.63−3.71 (m, 1 H), 4.38 (d, *J* = 10.9 Hz, 2 H), 7.12 (dd, *J* = 5.7, 1.6 Hz, 1 H), 7.22 (d, *J* = 3.8 Hz, 2 H), 7.53 (s, 1 H), 7.97 (d, *J* = 5.6 Hz, 1 H).

2′-Cyclohexylamino-6-morpholin-4-yl[2,4′]bipyridinyl-4-carboxylic Acid Amide (24i). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 382.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.12−1.26 (m, 3 H), 1.27−1.40 (m, 2 H), 1.54−1.66 (m, 1 H), 1.68−1.78 (m, 2 H), 1.87−1.98 (m, 2 H), 3.56−3.63 (m, 4 H), 3.71−3.78 (m, 5 H), 6.47 (d, *J* = 7.8 Hz, 1 H), 7.04 (dd, *J* = 5.3, 1.5 Hz, 1 H), 7.16 (s, 1 H), 7.24 (s, 1 H), 7.58 (s, 1 H), 7.63 (s, 1 H), 8.02 (d, *J* = 5.3 Hz, 1 H), 8.18 (s, 1 H).

2′-Cyclohexylamino-6-((*R*)-pyrrolidin-3-ylamino)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24a). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 381.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.27 (q, *J* = 12.9 Hz, 3 H), 1.39−1.52 (m, 2 H), 1.69 (d, *J* = 14.2 Hz, 1 H), 1.80 (d, *J* = 13.4 Hz, 2 H), 1.85−1.94 (m, 1 H), 2.05 (d, *J* = 12.4 Hz, 2 H), 2.29 (dd, *J* = 12.5, 7.7 Hz, 1 H), 2.96−3.02 (m, 1 H), 3.04−3.12 (m, 1 H), 3.16−3.25 (m, 1 H), 3.36 (dd, *J* = 11.8, 6.19 Hz, 1 H), 3.63−3.71 (m, 1 H), 4.48−4.57 (m, 1 H), 6.93 (d, *J* = 1.0 Hz, 1 H), 7.13 (dd, *J* = 5.6, 1.5 Hz, 1 H), 7.18 (s, 1 H), 7.43 (d, *J* = 1.3 Hz, 1 H), 7.96 (d, *J* = 5.6 Hz, 1 H).

2′-Cyclohexylamino-6-((*S*)-pyrrolidin-3-ylamino)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24b). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 381.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.21−1.33 (m, 3 H), 1.46 (d, *J* = 12.4 Hz, 2 H), 1.68 (d, *J* = 10.4 Hz, 1 H), 1.75−1.84 (m, 2 H), 1.84−1.93 (m, 1 H), 2.05 (d, *J* = 11.6 Hz, 2 H), 2.23−2.34 (m, 1 H), 2.97 (dd, *J* = 12.0, 4.4 Hz, 1 H), 3.02−3.11 (m, 1 H), 3.14−3.23 (m, 1 H), 3.36−3.39 (m, 1 H), 3.63−3.71 (m, 1 H), 4.48−4.57 (m, 1 H), 6.93 (d, *J* = 1.3 Hz, 1 H), 7.13 (dd, *J* = 5.6, 1.5 Hz, 1 H), 7.18 (d, *J* = 2.3 Hz, 1 H), 7.43 (d, *J* = 1.0 Hz, 1 H), 7.96 (d, *J* = 5.7, 0.6 Hz, 1 H).

2′-Cyclohexylamino-6-(4-methylpiperazin-1-yl)[2,4′]bipyridinyl-4-carboxylic Acid Amide (24f). The title compound was prepared by a method similar to that described for **24h**: MS (ESI) *m/z* 493.1 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.17 (br s, 1 H), 8.01 (d, *J* = 5.3 Hz, 1 H), 7.60 (br s, 1 H), 7.53 (s, 1 H), 7.23 (s, 1 H), 7.15 (s, 1 H), 7.02 (dd, *J* = 5.4, 1.4 Hz, 1 H), 3.69−3.80 (m, 1 H), 3.60−3.66 (m, 4 H), 2.41−2.47 (m, 4 H), 2.24 (s, 3 H), 1.89−1.97 (m, 2 H), 1.67−1.78 (m, 2 H), 1.54−1.65 (m, 1 H), 1.25−1.40 (m, 2 H), 1.11−1.26 (m, 3 H).

Aminomethyl-2″-cyclohexylamino-3,4,5,6-tetrahydro-2*H*-[1,2′;6′,4″]terpyridine-4′-carboxylic Acid Amide (24g). The title compound was prepared by a method similar to that described for **24h**. The BOC protecting group was removed by treatment of the intermediate with TFA/DCM at room temperature: MS (ESI) *m/z* 409.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 7.94−7.98 (m, 1 H), 7.47−7.50 (m, 1 H), 7.18−7.25 (m, 2 H), 7.13 (dd, *J* = 5.7, 1.5 Hz, 1 H), 4.54−4.65 (m, 2 H), 3.62−3.74 (m, 1 H), 2.89−3.02 (m, 2 H), 2.64 (d, *J* = 6.8 Hz, 2 H), 1.99−2.10 (m, 2 H), 1.84−1.92 (m, 2 H), 1.63−1.84 (m, 4 H), 1.38−1.54 (m, 2 H), 1.21−1.35 (m, 5 H).

3-Amino-2″-cyclohexylamino-3,4,5,6-tetrahydro-2*H*-[1,2′;6′,4″]-terpyridine-4′-carboxylic Acid Amide (24e). The title compound

was prepared by a method similar to that described for **24h**. BOC group removed by treatment of intermediate with TFA/DCM at room temperature: MS (ESI) m/z 395.1 (M + 1); ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.15 (d, J = 5.3 Hz, 1 H), 7.20−7.25 (m, 1 H), 7.05−7.12 (m, 2 H), 7.02 (br s, 1 H), 6.18 (br s, 1 H), 5.68 (br s, 1 H), 4.56 (d, J = 7.8 Hz, 1 H), 4.31−4.38 (m, 1 H), 4.15−4.25 (m, 1 H), 3.61−3.77 (m, 1 H), 3.06−3.16 (m, 1 H), 2.82−2.99 (m, 2 H), 1.99−2.15 (m, 3 H), 1.73−1.91 (m, 3 H), 1.60−1.72 (m, 2 H), 1.32−1.48 (m, 3 H), 1.18−1.32 (m, 3 H).

4-(6-Chloro-4-difluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (26b). A solution of 2,6-dichloro-4-difluoromethylpyridine (0.1 g, 0.5 mmol), piperazine-1-carboxylic acid *tert*-butyl ester (0.94 g, 0.5 mmol), and Et$_3$N (0.28 mL, 2.0 mmol) in dioxane (3 mL) was heated in a sealed tube at 90 °C for 48 h. After cooling, the mixture was concentrated in vacuo. The residue was purified by flash chromatography (10−30% EtOAc/heptanes) to give the title compound as a clear oil: ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 1.48 (s, 9 H), 3.49−3.63 (m, 8 H), 6.47 (t, J = 55.7 Hz, 1 H), 6.56 (s, 1 H), 6.72 (s, 1 H).

Cyclohexyl-(4-difluoromethyl-6-piperazin-1-yl[2,4′]bipyridinyl-2′-yl)amine (24k). A solution of 4-(6-chloro-4-difluoromethylpyridin-2-yl)piperazine-1-carboxylic acid *tert*-butyl ester (0.06 g, 0.17 mmol) and cyclohexyl-(4-trimethylstannanylpyridin-2-yl)amine (0.065 g, 0.19 mmol) in dioxane (4 mL) was degassed with N$_2$. CsF (0.059 g, 0.39 mmol) and Pd(t-Bu$_3$P)$_2$ were added. The mixture was heated under N$_2$ to 100 °C for 5 h. The mixture was cooled to room temperature, filtered through Celite, rinsed with fresh dioxane, and concentrated. The residue was purified by flash chromatography (10−50% EtOAc/heptanes) to give 6-(4-*tert*-butoxycarbonylpiperazin-1-yl)-2′-cyclohexylamino[2,4′]bipyridinyl-4-difluoromethane as a white foam: ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 1.18−1.32 (m, 2 H), 1.36−1.53 (m, 11 H), 1.59−1.71 (m, 2 H), 1.73−1.83 (m, 2 H), 2.05−2.14 (m, 2 H), 3.53−3.73 (m, 9 H), 4.50−4.58 (m, 1 H), 6.59 (t, J = 56.0 Hz, 1 H), 6.71−6.77 (m, 1 H), 6.98 (s, 1 H), 7.07 (dd, J = 5.3, 1.3 Hz, 1 H), 7.15 (s, 1 H), 8.14 (d, J = 5.3 Hz, 1 H).

TFA was added dropwise to a solution of 6-(4-*tert*-butoxycarbonylpiperazin-1-yl)-2′-cyclohexylamino[2,4′]bipyridinyl-4-difluoromethane (110 mg, 0.23 mmol) in CH$_2$Cl$_2$ (5 mL) until TLC showed complete consumption of the starting material. A 3 N solution of NH$_3$ in MeOH was added, and the mixture was concentrated under reduced pressure. The residue was purified by reverse phase HPLC to yield the title compound as a white solid: MS (ESI) m/z 388.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.19−1.33 (m, 3 H), 1.37−1.53 (m, 2 H), 1.68 (m, 1 H), 1.75−1.85 (m, 2 H), 1.98−2.08 (m, 2 H), 2.92−3.00 (m, 4 H), 3.62−3.73 (m, 5 H), 6.74 (t, J = 55.8 Hz, 1 H), 6.93 (s, 1 H), 7.09 (dd, J = 5.6, 1.5 Hz, 1 H), 7.20 (s, 1 H), 7.26 (s, 1 H), 7.96 (dd, J = 5.6, 0.76 Hz, 1 H).

Cyclohexyl-(6-piperazin-1-yl-4-trifluoromethyl[2,4′]bipyridinyl-2′-yl)amine (24j). The title compound was prepared by a method similar to that described for **24k**: MS (ESI) m/z 406.2 (M + 1); ^{1}H NMR (400 MHz, MeOD) δ ppm 1.19−1.33 (m, 3 H), 1.38−1.51 (m, 2 H), 1.63−1.72 (m, 1 H), 1.74−1.84 (d, 2 H), 1.99−2.08 (m, 2 H), 2.94−2.99 (m, 4 H), 3.68−3.73 (m, 5 H), 7.02 (s, 1 H), 7.09 (dd, J = 5.6, 1.5 Hz, 1 H), 7.18−7.20 (m, 1 H), 7.31 (s, 1 H), 7.98 (dd, J = 5.6, 0.8 Hz, 1 H).

4-(6-Chloro-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-4-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (28) and 4-(4-Chloro-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-6-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (29). To a mixture of 2,6-dichloro-3-methylisonicotinic acid ethyl ester (10.0 g, 42.7 mmol) and acetic acid (2.69 g, 44.9 mmol) in carbon tetrachloride (147 mL) were added *N*-bromosuccinimide (8.36 g, 47.0 mmol) and then benzoyl peroxide (1.03 g, 4.27 mmol). The mixture was stirred in an oil bath at 60 °C under a heat lamp for 5 h. The mixture was then cooled to room temperature. About half of the solvent was removed by rotary evaporation. The white succinimide solid was removed by filtration. The filtrate was

concentrated under reduced pressure and used immediately for the next step: MS(ESI) m/z 313.99; ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.72 (s, 1 H), 4.99 (s, 2 H), 4.48 (q, J = 7.16 Hz, 2 H), 1.46 (t, J = 7.07 Hz, 3 H).

A mixture of 3-bromomethyl-2,6-dichloroisonicotinic acid ethyl ester (13.4 g, 42.7 mmol), tetrahydrofuran (100 mL), and ammonium hydroxide (300 mL of 28−30% ammonia) was stirred at room temperature for 18 h. The solvents were then removed by rotary evaporation. The nearly dry solid was treated with a small amount of water. The salmon-colored solid was isolated by filtration, washed with small amounts of water and then diethyl ether, and dried under vacuum. Filtration of the cooled filtrate yields additional product (7.47 g, 36.8 mmol, 86%): MS(ESI) m/z 203.2 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.29 (br s, 1 H), 7.83 (s, 1 H), 4.45 (s, 2 H).

4,6-Dichloro-2,3-dihydropyrrolo[3,4-*c*]pyridin-1-one (5.63 g, 27.7 mmol), *tert*-butyl piperazine-1-carboxylate (7.75 g, 41.6 mmol), triethylamine (14.0 g, 139 mmol), and dioxane (50 mL) were stirred at 120 °C in a 350 mL sealed tube for 16 h. To the cooled down reaction mixture were then added more *tert*-butylpiperazine 1-carboxylate (5.2 g, 27.7 mmol) and triethylamine (2.83 g, 28.0 mmol). The vessel was sealed again and stirred at 120 °C for 24 h. The reaction mixture was then cooled to ambient temperature, and **28** was isolated by filtration (6.18 g, 17.5 mmol, 63%): MS(ESI) m/z 353.15 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.04 (s, 1 H), 6.89 (s, 1 H), 4.57 (s, 2 H), 3.61−3.54 (m, 4 H), 3.47−3.41 (m, 4 H), 1.42 (s, 9 H).

Intermediate **29** was obtained following concentration of the above filtrate. The solid was treated with methanol and filtered to give **29** as light-yellow solid: MS(ESI) m/z 353.30 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.93 (s, 1 H), 7.01 (s, 1 H), 4.28 (s, 2 H), 3.58−3.53 (m, 4 H), 3.45−3.40 (m, 4 H), 1.42 (s, 9 H).

6-(2-Cyclohexylaminopyridin-4-yl)-4-piperazin-1-yl-2,3-dihydropyrrolo[3,4-*c*]pyridin-1-one (30). To an argon-degassed mixture of 4-(6-chloro-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-4-yl)piperazine-1-carboxylic acid *tert*-butyl ester (0.997 g, 2.83 mmol), cyclohexyl-(4-trimethylstannanylpyridin-2-yl)amine (1.15 g, 3.39 mmol), and cesium fluoride (0.988 g, 6.50 mmol) in dioxane (100 mL) was added bis(tri-*tert*-butylphosphine)palladium(0) (0.116 g, 0.226 mmol). The reaction mixture was stirred at 100 °C for 18 h. The room temperature reaction mixture was then filtered through Celite and concentrated down to dryness. Treatment of the brown residue with diethyl ether (50 mL) yields an off-white solid which was isolated by filtration (1.37 g, 2.78 mmol, 98%). The yield was slightly inflated because the product was less than 95% pure: MS(ESI) m/z 493.29 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.97 (s, 1 H), 8.02 (d, J = 5.56 Hz, 1 H), 7.44 (s, 1 H), 7.20 (s, 1 H), 7.08 (d, J = 7.07 Hz, 1 H), 6.42 (d, J = 7.83 Hz, 1 H), 4.61 (s, 2 H), 3.85−3.70 (m, 1 H), 3.70−3.58 (m, 4 H), 3.57−3.43 (m, 4 H), 2.02−1.87 (m, 2 H), 1.81−1.66 (m, 2 H), 1.65−1.55 (m, 1 H), 1.44 (s, 9 H), 1.40−1.27 (m, 2 H), 1.27−1.13 (m, 3 H).

To a suspension of 4-[6-(2-cyclohexylaminopyridin-4-yl)-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-4-yl]piperazine-1-carboxylic acid *tert*-butyl ester (1.37 g, 2.78 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (5 mL, 7.4 g, 65 mmol). The solution was stirred at ambient temperature for 2 h. The solvents were removed by rotary evaporation. The crude residue was treated with triethylamine (5 mL) and dichloromethane (200 mL) and then washed with water (40 mL). After separation of the layers, the organic layer was dried over sodium sulfate, filtered, and concentrated down to dryness by rotary evaporation. The tan solid was then treated with hot 2-propanol (50 mL). After cooling of the suspension to room temperature, a light-yellow solid was isolated by filtration and dried under reduced pressure (0.616 g, 1.57 mmol, 56%). MS(ESI) m/z 393.24 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.98 (s, 1 H), 8.02 (d, J = 5.30 Hz, 1 H), 7.45 (s, 1 H), 7.17 (s, 1 H), 7.09 (d, J = 5.56 Hz, 1 H), 6.42 (d, J = 8.08 Hz, 1 H), 4.60 (s, 2 H), 3.88−3.59 (m, 5 H), 3.15−2.94 (m, 4 H), 2.03−1.86 (m, 2 H),

1.80−1.67 (m, 2 H), 1.65−1.54 (m, 1 H), 1.42−1.26 (m, 2 H), 1.26−1.13 (m, 3 H).

4-(2-Cyclohexylaminopyridin-4-yl)-6-piperazin-1-yl-2,3-dihydropyrrolo[3,4-*c*]pyridin-1-one (31). To a nitrogen-degassed mixture of 4-(4-chloro-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-6-yl)piperazine-1-carboxylic acid *tert*-butyl ester (0.103 g, 0.2919 mmol) and cyclohexyl-(4-trimethylstannanylpyridin-2-yl)-amine (0.114 g, 0.336 mmol) in toluene (10 mL) was added *trans*-dichlorobis(triphenylphosphine)palladium(II) (0.021 g, 0.029 mmol). The reaction mixture was stirred under nitrogen at 110 °C for 16 h. The mixture was cooled to room temperature and the solvent was removed by rotary evaporation. The crude was purified through two successive silica gel columns (first solvent system, ethyl acetate and then 95:5 ethyl acetate/methanol; second solvent system, 98:2:0.5 and then 96:4:0.9 dichloromethane/methanol/ammonium hydroxide). A third column was used for contaminated fractions. A yellow solid was obtained as a result (0.043 g, 0.087 mmol, 30%): MS(ESI) m/z 493.33 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.94 (s, 1 H), 8.04 (d, J = 5.43 Hz, 1 H), 7.08 (s, 1 H), 6.98 (s, 1 H), 6.95 (d, J = 5.43 Hz, 1 H), 6.50 (d, J = 7.58 Hz, 1 H), 4.59 (s, 2 H), 3.77−3.69 (m, 1 H), 3.67−3.59 (m, 4 H), 3.50−3.44 (m, 4 H), 1.95 (d, J = 10.11 Hz, 2 H), 1.77−1.69 (m, 2 H), 1.64−1.56 (m, 1 H), 1.43 (s, 9 H), 1.39−1.31 (m, 2 H), 1.25−1.17 (m, 3 H).

A solution of 4-[4-(2-cyclohexylaminopyridin-4-yl)-1-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-6-yl]piperazine-1-carboxylic acid *tert*-butyl ester (0.042 g, 0.085 mmol) in dichloromethane (5 mL) and trifluoroacetic acid (3.0 mL, 4.4 g, 39 mmol) was stirred for 2 h. The solvents were removed by rotary evaporation. The residue was then dissolved into dichloromethane and washed with 2 N aqueous sodium hydroxide solution and then brine. The aqueous layers were extracted three times with fresh dichloromethane. The combined organic layers were dried over sodium sulfate, filtered, concentrated by rotary evaporation, and dried in vacuo to yield a yellow solid (0.030 g, 0.077 mmol, 90%): MS (ESI) m/z 393.24 (M + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.94 (s, 1 H), 8.04 (d, J = 4.55 Hz, 1 H), 7.05 (s, 1 H), 6.97 (s, 1 H), 6.94 (d, J = 4.29 Hz, 1 H), 6.52 (d, J = 7.58 Hz, 1 H), 4.59 (s, 2 H), 3.73 (br s, 1 H), 3.62 (s, 4 H), 2.92 (s, 4 H), 1.94 (d, J = 9.85 Hz, 2 H), 1.72 (d, J = 10.36 Hz, 2 H), 1.59 (d, J = 9.35 Hz, 1 H), 1.36−1.27 (m, 2 H), 1.25−1.14 (m, 3 H).

Supporting Information Available: A table of additional comparative kinase selectivity for compounds **1** and **12a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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