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Article

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Ligand-Protein Interactions of Selective Casein Kinase 1δ inhibitors.

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ABSTRACT

Casein Kinase 1 (CK1) δ and ε are believed necessary enzymes for the regulation of circadian rhythms in all mammals. Based on our previously published work demonstrating that a CK1ε preferring compound to be ineffective circadian clock modulator, we have synthesized a series of pyrazole-substitued pyridine inhibitors, selective for the CK1δ isoform. Additionally, using structure-based drug design, we have been able to exploit differences in the hinge region

between CK1δ and p38 to find selective inhibitors that have minimal p38 activity. The SAR, brain exposure and the effect of these inhibitors on mouse circadian rhythms are described. The *in vivo* evaluation of these inhibitors demonstrates that selective inhibition of CK1δ at sufficient central exposure levels is capable of modulating of circadian rhythms.

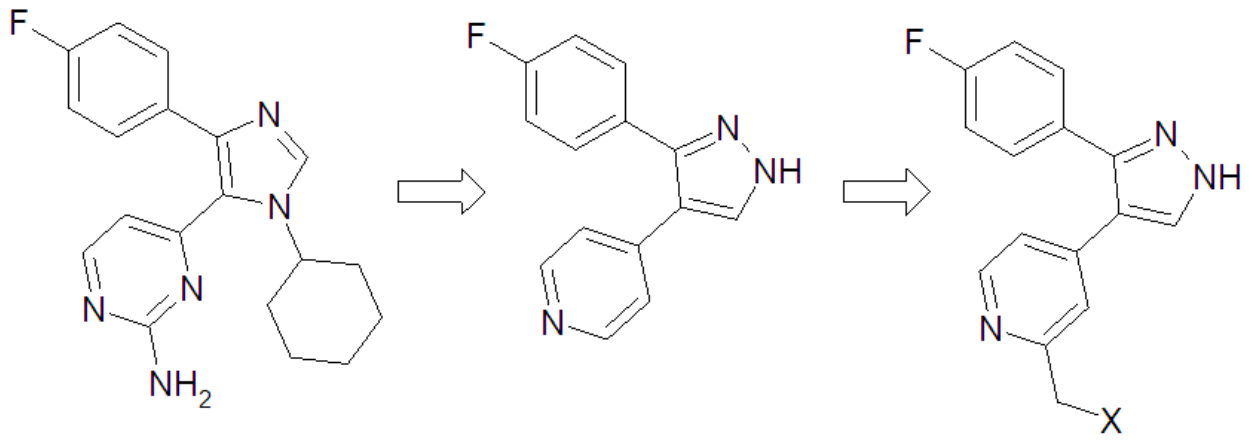
INTRODUCTION

Casein kinase 1 protein kinases (CK1s) function as regulators of signal transduction pathways and are involved in Wnt signaling and circadian rhythms.[1-4] We have previously demonstrated that dual CK1δ/ε inhibitors, such as PF-670462 (compound **1**, chart 1), have robust phase delay in animal models of circadian rhythm,[5] while selective CK1ε compounds have been shown to be ineffective clock modulators.[6] As such, CK1δ potency appears to be a key factor in the design of a circadian rhythm modulating agent.

A number of aspects can potentially confound the design of a successful CNS-active CK1δ inhibitor. These include poor whole-cell potency, a lack of selectivity and incorrectly aligned ADMET properties. Targeting a kinase for CNS indications may be particularly challenging, due to the balancing of free drug levels necessary to achieve central exposure with potential peripheral safety considerations. In fact, due to the polar nature of many kinase scaffolds, peripheral free drug exposure may be as much as 2-3-fold higher than the relevant free central exposure. Since many approved kinase drugs are known to have cardiotoxicity,[7,8] imbalance between peripheral and central drug levels is a clear area of concern. Leveraging the CNS drug analysis and CNS MPO algorithm, molecules were designed in high desirability space (CNS MPO > 4) to increase the odds of identifying compounds with good CNS exposure and aligned ADME attributes.[9,10] Although many have highlighted the potential benefits of drug

promiscuity,[11,12] we share in the opinion that overly promiscuous compounds are "forbiddingly dangerous…without a rational strategy to control specificity…"[13] and would be a higher risk during clinical assessment.

While the previously described compound **1** has proven to be a useful tool for demonstrating the role of CK1δ inhibitors for modulation of circadian rhythms *in vivo*, it suffers from poor off-target pharmacology. Recent structural examinations of 1 bound to CK1δ and CK1ε, suggest a very similar binding to both isoforms[14,15], and help explain why 1 is only slightly CK1δ preferring. In addition to both CK1δ and CK1ε, 1 was active (>50% Inh at 1uM) against 6 kinases: PKAα, p38, MAP4K4, CK1α, LCK and EGFR. This non-optimal selectivity profile necessitated the need for modification to build in greater selectivity and a more favorable CNS profile with the ultimate goal of advancing a selective CK1δ inhibitor to the clinic to test the circadian rhythm shift mechanism of action (MOA). We began our pursuit to identify a selective CK1δ inhibitor by screening our internal compound collection for viable hits. Compounds **1** and **2** were identified from this HTS.



1 **PF-670462** **2** **3** X = OH **4** X = NHMe

Chart 1.

This paper describes the use of structure-based drug design to develop ATP-competitive CK1δ preferring inhibitors. We report the x-ray structures of 4 pyrazole-substituted pyridine compounds bound to CK1δ and compare their binding characteristics to those of a similar inhibitor bound to p38, as this enzyme proved to be the most challenging of the off-target kinase activities. We find that the binding mode of the more selective inhibitors is characterized by an interaction with a glycine residue (Gly110) of the kinase. Although this residue is conserved in p38 and CK1δ, the adjacent hinge residue, Pro87, results in a framework that is both rigid and unique. These inhibitors represent the first such CK1δ preferring ligands designed with a clear effort toward gaining both enzyme activity and central exposure.

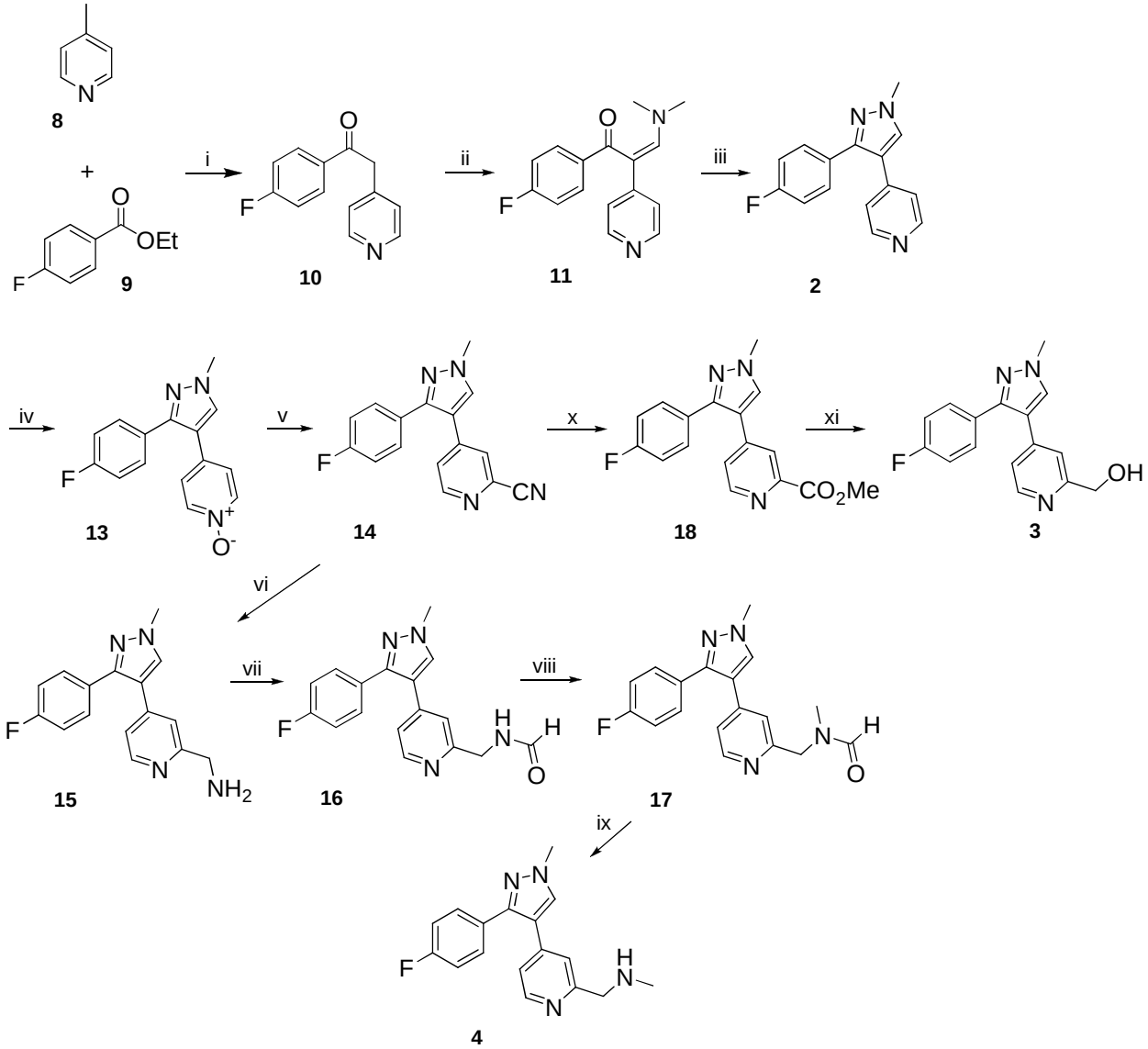
Chemistry

Scheme 1 illustrates the synthesis of CK1δ preferring inhibitors, **2**-**4**. Commercially available pyridine **8** and ethyl 4-fluorobenzoate **9** were condensed in presence of LHMDS to give ketone **10** which was directly treated with dimethylformamide dimethyl acetal (DMF-DMA) to afford enamine **11** as a mixture of *E*,*Z* isomers in ~1:8 ratio. Reacting **11** with methyl hydrazine at 0^{o} C resulted in a mixture of *N*-methylpyrazole regio-isomers which, after trituration with 1:1 ether/heptane, gave compound **2** in 31% isolated yield over 3 steps.

Oxidation of **2** with *m*CPBA afforded *N*-oxide **13** in 95% yield which upon cyanation, with trimethylsilylcyanide (TMSCN), resulted in an 86% yield of pyridylnitrile **14**. Catalytic hydrogenation using sponge nickel and 7N ammonia in MeOH gave amine **15** in 99% yield, which upon heating with ethyl formate produced formamide **16**. *N*-Methylation, using MeI, resulted in **17** in 90% yield. Subsequent hydrolysis using methanolic hydrochloric acid provided the target compound **4** in reasonable yield (56%).

The desired target compound **3** could be prepared in 2 steps via standard reaction conditions. Namely, pyridyl nitrile **14** could be hydrolyzed to methyl ester **18** using acidic methanol in 55% yield. Alcohol **3** could then be obtained by LAH reduction of **18** in 20% yield.

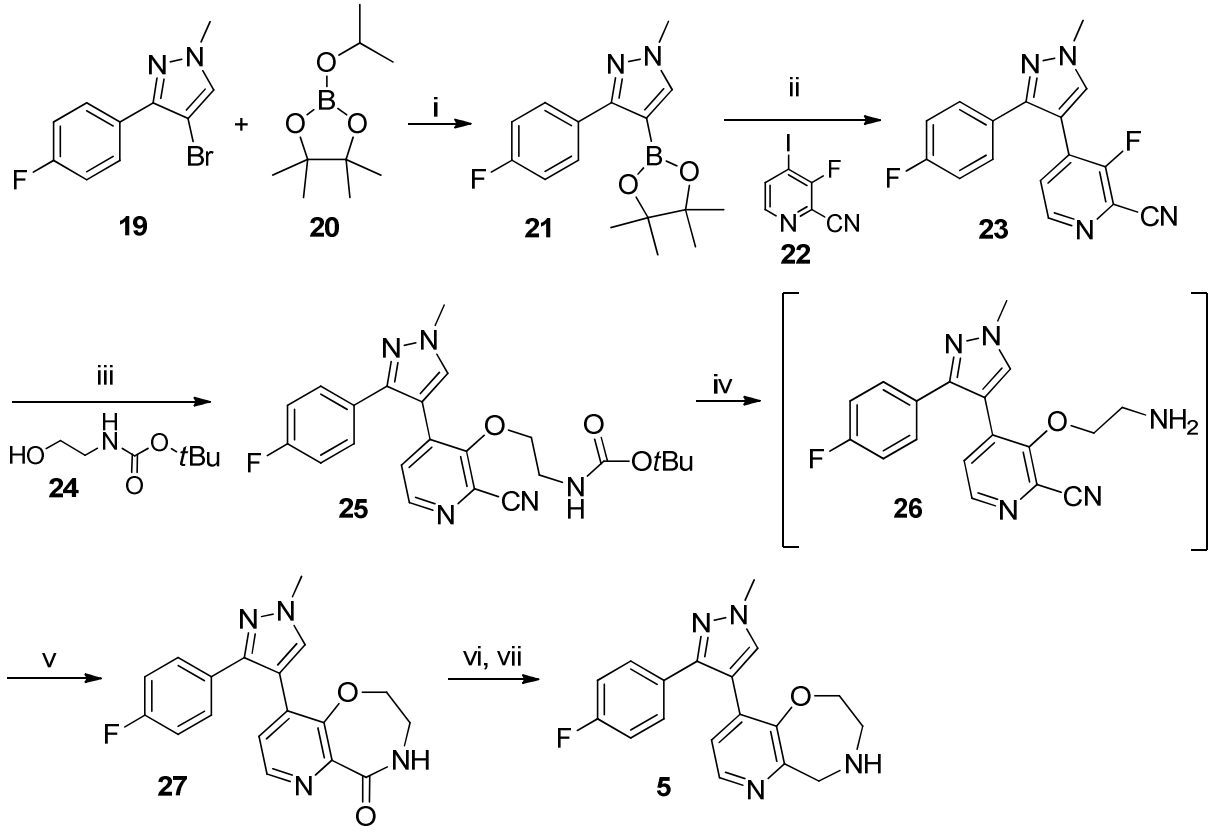
Scheme 1.[a] Synthesis of 2-substituted pyridyl anlogs **2-4**.



[a] Reagents and conditions: i) 2 Eq. LHMDS, THF, 0 ◦C to rt, 3.75 h (crude); ii) 1.1 eq DMF-DMA, reflux, 72 h (crude); iii) 1.2 eq methylhydrazine, EtOH, 0 ºC, 3h (31% over 3 steps); iv) 1.2 eq mCPBA, CH_2Cl_2, 0 ºC to rt, 18h (99%); v) 1.3 eq TMS-CN, 1.2 eq dimethylcarbamoyl

chloride, CH_2Cl_2, rt, 16h (86%); vi) H_2 (50 psi), catalytic sponge Ni, 7N ammonia in MeOH, THF, rt, 2h (99%); vii) ethyl formate, 70 ºC, 3h (80%); viii) 1.5 eq MeI, 1.2 eq NaH, THF, rt, 18h (90%); ix) MeOH/12N HCl (10:1 v/v), reflux, 90 min, followed by ISOPROPYL ALCOHOL/MeOH recryst 56%); x) 1.25M HCl/MeOH, reflux 20h, (55%); xi) 1M LAH/THF, rt, 30 min (25%)

Scheme 2.[a] Synthesis of fused oxazepine **5**.

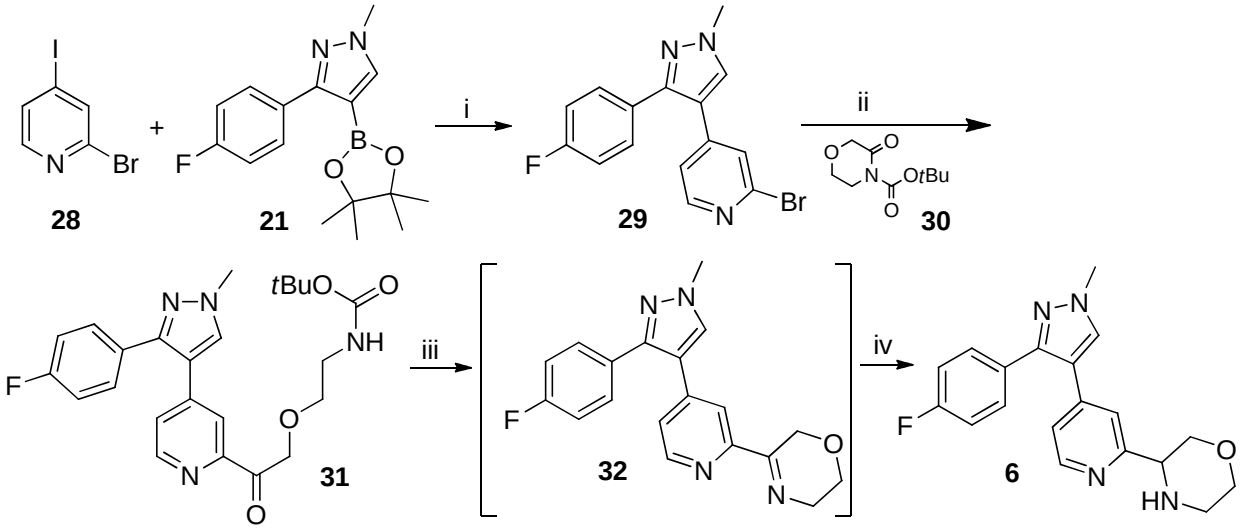


[b] Reagents and conditions: i) 2 eq n-BuLi, THF, -70 ºC then 1.5 eq **20**, 1.5h (44%): ii) 0.9 eq **21**, 2 mol% $Pd(OAc)_2$, 4 mol% Xphos, 1.2 eq Cs_2CO_3, ACN/H_2O (5:1), reflux, 3h (80%); iii) 1.4 eq **24**, 1.7 eq NaH, THF, -20 to -5 ºC, 4h, (70%); iv) H_2SO_4 conc, 110° C, 10 min v) 5N NaOH, pH 7.0 phosphate buffer, 110 °C, 3 h, 80% from **25**; vi) 2.25 eq LAH/THF, 50 ºC, 2h; vii) 2 eq *p*-toluenesulfonic acid, MeOH, 42% from **26**.

Scheme 2 illustrates the synthesis of CK1δ/ε inhibitor **5**. Pinacol borane **21** was prepared by lithiation of bromopyrazole **19** followed by borolation with **20** in 44% yield. Subsequent Suzuki coupling of iodide **22** with **21** using cesium carbonate, Xphos, and $Pd(OAc)_2$ yielded

diarylpyrazole **23** in 80% yield. S_NAr displacement using deprotonated *N*-Boc ethanolamine **24** generated pyridylether **25** in 81% yield. Removal of the Boc protecting group afforded amine **26** and subsequent one-pot cyano-hydrolysis and ring closure yielded oxazepinone **27** in 80% over 2 steps. The desired oxazepine **5** was then prepared by reduction of **27** using lithium aluminum hydride followed by conversion to the tosylate salt in 42% yield

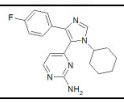
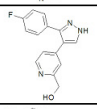
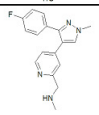
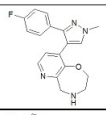
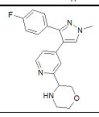
Scheme 3.[a] Synthesis of morpholine **6**.



Scheme 3[c] [c] Reagents and conditions: i) 5 mol% $Pd(dppf)_2Cl_2$, 2 eq CsF, DMF, 140 °C, 2h(80%); ii) 1 eq. *n*-BuLi, toluene, 1eq **30**, -78 ºC, 1.5h (22%iii) 10 eq. 1.0 M HCl/EtOH, EtOH, rt, 12h (assumed 100%); iv) 4 eq. $NaBH_4$, MeOH, -40 ºC to rt, 30 min (26%)

Scheme 3 illustrates the synthesis of CK1δ/ε inhibitor **6**. The synthesis started with Suzuki coupling of a commercially available pyridyl iodide **28** and boronate intermediate **21** that resulted in 80% yield of intermediate **29** as a white solid. Temperature controlled halogen-metal exchange of **29** followed by addition of morpholinone **30** at -78° C yielded keto-ether intermediate **31** as an off-white foam which was subjected to acidic hydrolysis conditions and subsequent ring closure to yield imine **32**. Reduction of the imine with sodium borohydride resulted in the isolation of racemic morpholine as white foam in 26% yield. Preparative chiral HPLC was then used to separate the (-) antipode of **6** from its (+) antipode.

Table 1. Structure, pharmacology and exposure data.

			Kinase Potency[a] [nM]				Safety		Exposure[b] [uM]	
Cmpd	Structure	ACD pKa	CK1δ	CK1ε	CK1d WC	P38	Selectivity	Dof/herg [uM]	$C_{b,u}$	$C_{p,u}$
1			7.8	29.0	80.5	39.2	6/34	27.7/ 27.0 (n = 1)	0.241[c]	0.752[c]
2			48.1	305	545	126	9/103	-- / >100 (n = 1)		
3			14.2 (n = 2)	81.6 (n = 2)	237 (n=2)	42%@1uM	1/36	6% @ 10uM/ --		
4		8.09	12.9	67.2	63.8	3040 (n=1)	0/43	38.2/3.99 (n = 1)	7.53	9.66
5		5.93	6.0	27.0	42.1	654	3/43	>79.6/25.7 (n = 1)	2.97	8.82
6		7.25	6.6	28.2	90.0 (n = 2)	>1000 (n = 1)	1/39	25.9/4.43 (n = 1)	3.70	1.95

[a] IC_{50} values reported as geometric means of n > 2 independent experiments, unless otherwise noted.

[b] Exposures are maximum concentrations at a 56 mg/kg dose, unless otherwise noted.

[c] 32 mg/kg dose.

RESULTS AND DISCUSSION

Structure Based Drug Design. Compound **2** was a high throughput screening hit that was previously co-crystallized with the p38 enzyme.[16,17] The X-ray structure of **2** bound to p38 (Figure 1) shows the pyridine core interacting as a single point binder with the hinge (Met109), the pyrazole nitrogen interacting through a water molecule with the catalytic Lys38, and the para-fluoro aryl ring occupying the hydrophobic selectivity pocket of p38. In our hands, **2** was found to also be a potent CK1δ inhibitor (IC_{50} = 48 nM), with moderate whole-cell activity (EC_{50}

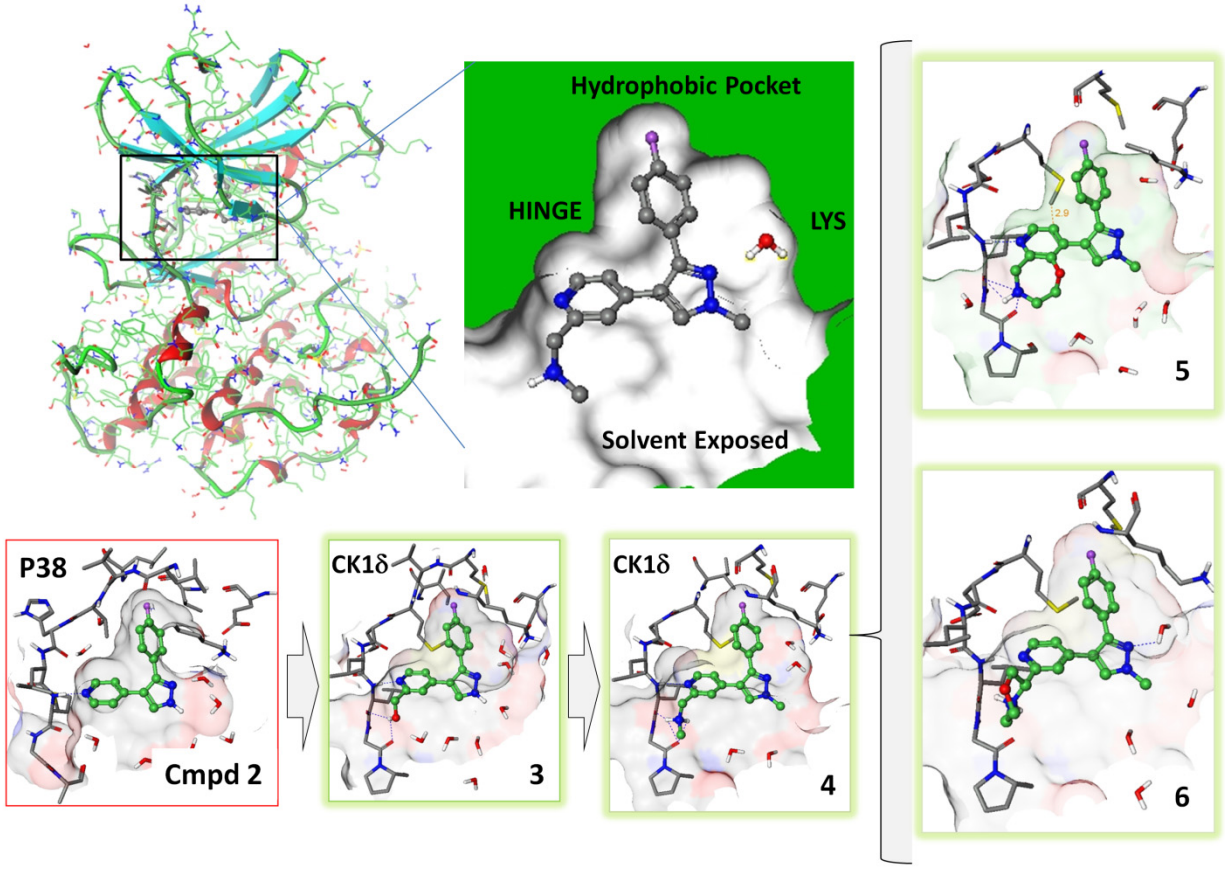


Figure 1. Binding of inhibitors to p38a (red box; compound **2**) and CK1δ (ribbon diagram and green boxes; compounds **3-6**). All structures share a common interaction of the pyridine core interacting as a single point binder with the hinge (Met109/Leu85) of p38/CK1δ. Also conserved in all structures is the pyrazole nitrogen interacts through a water molecule with the catalytic Lys38, as well as the para-fluoro aryl ring occupied the hydrophobic selectivity pocket of p38. Selective CK1δ inhibitors (**3-6**) make additional hinge interaction with the proline carbonyl of CK1δ. Compound **3-6** also hydrogen bond to the carbonyl oxygens of both residues Leu85 and Gly86.

= 545 nM). Evaluation of 2 at a 1 μM concentration against a larger selectivity panel, consisting of 103 kinases, found that **2** inhibited 9 kinases at greater than 50%: ABL (61); LYN (70.5); MAPK14 (55); p38 (85); JNK2/3 (48); ROCK2 (52); EGFR (80); MAP4K4 (66); SAPK2A (96).

The relative selectivity of compound **2** over this subset of the kinase pharmacology can be attributed largely to the *para*-fluoro aryl group which interacts with the so-called 'hydrophobic'

selectivity pocket. This feature exists in both the CK1δ and p38 enzymes despite differences in primary sequence; in p38 this is comprised of Thr-Val-Leu residues, while in CK1δ/ε it is comprised of Met-Val-Met residues.

An obvious and important difference between these kinases was the proline residue (Pro87) of the backbone of CK1δ that is not present in p38. This difference was targeted in the design of novel and selective CK1δ inhibitors as exemplified by compound **3** (Figures 1 and 2). The primary hydroxyl group of compound **3** makes a selective interaction with the proline (Pro87) carbonyl of CK1δ that was in an altered conformation compared to p38 (Ala111). Although the same carbonyl exists in both enzymes, the flexible hinge of p38 positioned this functionality in a different orientation relative to CK1δ; shown in Figure 2. Previous studies have shown that p38 ligands containing the pyridine hinge binding motif can induce a peptide flip in the hinge region by introduction of an amino group *ortho* to the pyridine nitrogen.[18] With these ligands, the amino group H-bonds to the backbone carbonyl of the hinge residue (Met109 in p38), yielding improved potency and p38 selectivity. In contrast, the proline of CK1δ, holds that same carbonyl at a different angle and renders the ligand-induced 'peptide flipping' all but impossible. Compound **3** adds an additional methyl spacer between the pyridine acceptor and the putative donor in an attempt to gain selectivity. Indeed, **3** proved to increase the p38 selectivity to roughly 50x; whereas **2** was only 3x. However, the hydroxyl group was a concern due to its potential for rapid metabolism.

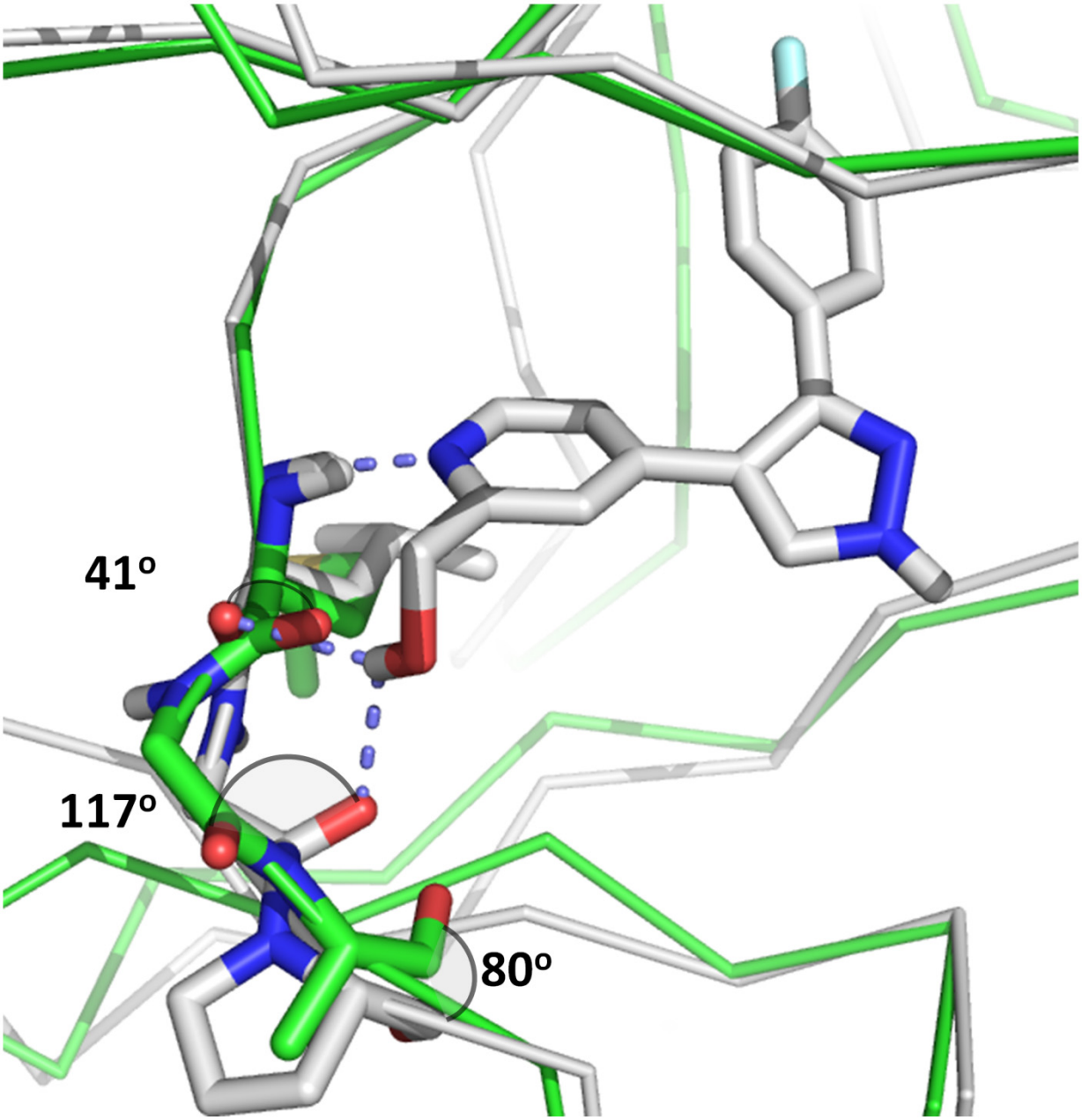


Figure 2. View of backbone carbonyls of CK1δ (white wire with compound 3) and p38 (green wire). Comparing the angle between the carbonyl groups (C=O vector) gives values of 41 for Leu85 (Met109 in p38), 117 for the Gly86 (Gly110 in p38) and 80 for Pro87 (Ala111 in p38).

Compound **4** was similarly designed to retain the new-found selectivity handle and maintain or improve potency at reasonable levels of central exposure.[19] This was accomplished through inclusion of a methyl-amine group which also displaced nearby water molecules and shielded the H-bond to the carbonyl.[20] The potency of **4** improved roughly 2x over compound **3** and the

selectivity over p38 widened to 230x. Importantly, compound **4** was completely selective in our kinase panel (0/43, table 1) and showed large improvements in cellular activity. Additionally, **4**, when dosed subcutaneously (SC), possessed good central exposure (7.5 μM free brain exposure; $C_{b,u}/C_{p,u}$ = 0.78). However, the addition of a basic center was found to increase hERG-liability.

The next round of designs produced compounds **5-6** which have in common the inclusion of a group designed to lower the basicity of the amine; a strategy which has been used to reduce the affinity of basic compounds to the hERG channel.[21-23] With the inclusion of the oxazepine ring, compound **5** had improved CK1δ potency to 6.5 nM and also proved to be the most potent compound in the whole cell assay at 39 nM. hERG activity of compound **5** was reduced ~6x relative to compounds **4**. Published hERG models have suggested the importance of ligand properties on channel block such as basicity, hydrophobicity and ligand flexibility.[25] Conceptually, compounds possessing a basic center are thought to interact with aromatic residues (Phe656), leaving polar heterocycles, such as the pyrazole group of our ligands, to interact with the channel via specific H-bonding interactions to a serine residue (Ser624) at the top of the pore.[25,26] The oxazepene, **5**, may benefit from both the reduction of the pKa (5.93 versus 7-8), as well as conformational restriction which may hinder the spacing between pharmacophoric elements.[26] Although **5** maintained 100x selectivity over p38, at 654 nM, it was markedly more potent versus p38 than **4**. In addition, two additional kinase activities were detected in the kinase panel (CK1α and MAP4K4). While the free plasma concentration ($C_{p,u}$ following SC dosing) of **5** was comparable to **4**, free brain levels were now disproportionally lower by roughly 2.5x ($C_{b,u}/C_{p,u}$ = 0.33), narrowing the gains made from the reduction of hERG activity.

Morpholines are also well known to reduce basicity via a negative inductive effect. This concept was introduced into the scaffold of compound **2** to produce compound **6**. Docking[27] of **6** into the CK1δ X-ray structure also suggested the ability to increase the potency of the ligand on the receptor for this series through the creation of additional interactions with Ile15. This compound was the most potent compound measured in the enzyme assay (5.3 nM) and very potent in the whole cell assay (89.9 nM). Compound **6** also maintained good p38 and overall kinase selectivity versus our panel.

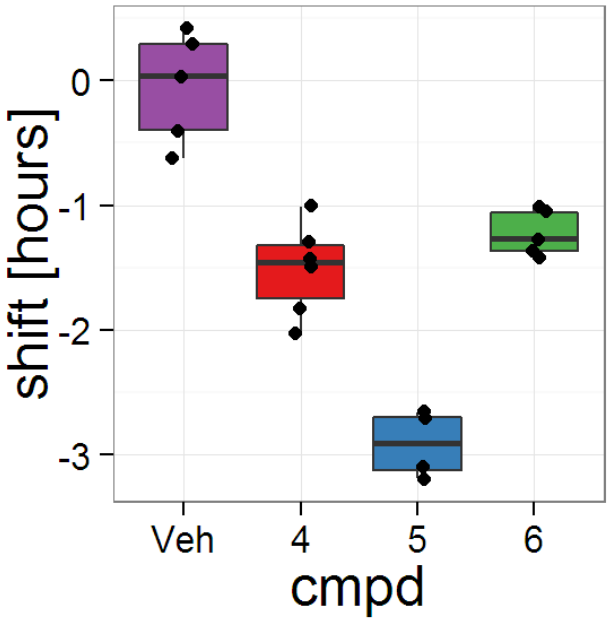


Figure 3. Mouse circadian rhythm shift (y-axis, in hours) versus compound (x-axis). Top and lower bounds of box refer to 75th and 25th percentile values. Mean shifts are -1.5, -2.9 and -1.2 hours ($p < 0.001$ for all three) for compounds 4, 5 and 6 respectively.

Selected compounds from table 1 were screened in an *in vivo* mouse circadian rhythm model.[6] Figure 3 illustrates the magnitude of circadian rhythm shift observed in mice following 3 days of drug treatment (56 mg/kg subcutaneous dose). For the mice treated in these studies, the shifts refer to the difference between the start of the active period after dosing versus before

dosing. All compounds demonstrate significant ($p < 0.001$) phase shift delay, with **5** showing the largest mean shift of 2 hours and 55 minutes.

CONCLUSIONS

We have illustrated that compounds **3-6** are potent CK1δ inhibitors that gain a large degree of selectivity over p38 via a unique H-bonding interaction with the hinge region. In particular, placement of a CH_2-spaced heteroatom capable of donating a H-bond to the carbonyl of the hinge Pro in CK1δ. Larger cyclic amines, oxazepine and morpholine, were designed to maintain this motif and have been shown to shift mouse circadian rhythms *in vivo*. These analogues demonstrate the utility of structure-based drug design to address issues of potency and selectivity. However, these analogues also suffer mild hERG pharmacology. For instance, **5**, though active *in vivo*, is present at 8.8 μM maximum free plasma concentration at this dose; which translates to an approximate 3-fold hERG TI (hERG $EC_{50}/C_{p,u}$). Future publications will highlight compounds designed to address this issue, while maintaining *in vivo* efficacy.

EXPERIMENTAL SECTION

General Information. All solvents and reagents were obtained from commercial sources and were used as received. Compound **1** is commercially available (Tocris), **2** was prepared using published methodology[28], and **3-6** were prepared as discussed below. All reactions were followed by TLC (TLC plates F254, Merck) or LCMS (liquid chromatography-mass spectrometry) analysis. Varian 300, 400 or 500 MHz instruments were used to obtain ^1H and ^{13}C NMR spectra in deuterated solvents. All proton shifts are reported in δ units (ppm) and were measured relative to signals for chloroform (7.26 ppm) or methanol (3.30 ppm). All ^{13}C shifts

are reported in δ units (ppm) relative to the signals for chloroform (77 ppm) or methanol (49 ppm) with ^{1}H decoupling. All coupling constants (*J* values) are reported in Hertz (Hz). GCMS were recorded on an HP 6890 GC system equipped with a 5973 mass selective detector (Stationary phase: HP-1, fused silica, 12 m x 0.202 mm x 0.33 μm, temperature limits: -60 ºC to 325 ºC, ramp rate = 30 ºC/ min, solvent delay = 0.4 min). Analytical analyses by UPLC were performed on a Waters Acquity system with PDA detection (UV 210 nm) at 45º C, flow rate 0.5 mL/min, with a gradient of 95/5 buffer/acetonitrile (0 to 7.55 min), 10/90 buffer/acetonitrile (7.55 to 7.85 min), 95/5 buffer/acetonitrile (8.10 to 10.30 min) using the following columns and buffers: Waters BEH C8 column (2.1 x 100 mm, 1.7 um) with 50 mM sodium perchlorate/0.1% phosphoric acid or 10 mM ammonium bicarbonate as buffer; Waters BEH RP C18 column (2.1 x 100 mm, 1.7 um) or Waters HSS T3 (2.1 x 100 mm, 1.8 um) column with 0.1% methanesulfonic acid buffer. Mass spectra were recorded on a Micromass ADM atmospheric pressure chemical ionization instrument (MS, APCI). High resolution mass spectra were obtained on an Agilent 6620 LC-MS TOF equipped with a Zorbax Eclipse column (50 mm x 4.6 mm, 1.8 micron XDB-C18) using 0.1% formic acid aqueous as mobile phase A1 and acetonitrile containing 0.1% formic acid as mobile phase B1. Column chromatography was carried out on silica gel 60 (32-60 mesh, 60 Å) or on pre-packed Biotage™ columns. The purities of final compounds 3-6 as measured by UPLC were found to be above 95%.

1-(4-Fluorophenyl)-2-(pyridine-4-yl)ethanone (10). To a solution of 4-methylpyridine (48g, 500 mmol) in THF (100 mL) under N_2 at 0 ^{o}C was slowly added LHMDS (1.0M in THF, 1,030 mL,1030 mmol). Once addition was complete, a dark brown solution resulted and this was stirred at 0 ^{o}C for 1h. Ethyl 4-fluorobenzoate (85g, 74 mL, 500 mmol) in THF (100 mL) was added slowly via an addition funnel and stirring was continued at 0 ^{o}C for another 45 min before

the reaction was slowly warmed to rt. After stirring for 2 h at rt, the reaction was quenched with 50 mL of water. This reaction mixture was cooled to 0 ^{o}C, the pH adjusted to 7.0 by the addition of ~50 mL of 6N HCl and extracted with EtOAc (2x400 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. The crude solid was triturated with 50 mL heptanes to afford the title compound (108g) as a light yellow solid. This material was used without purification in the subsequent step. A portion of this solid was subjected to silica gel chromatography using 100% EtOAc to afford ~95% pure title compound as a light yellow colored solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.45 (dd, J = 1.6, 4.3, 2H), 7.90-7.95 (m, 2H), 7.09 (dd, J = 1.6, 4.3, 2H), 7.01-7.07 (m, 2H), 4.13 (s, 2H). ^{13}C ($CDCl_3$, 100 MHz) δ 194.3, 166.6 (d, J_{F-C} = 255.5), 149.9, 143.3, 132.6 (d, J_{F-C} = 3.7), 130.6 (d, J_{F-C} = 9.6), 124.9, 113.6 (d, J_{F-C} = 22.1), 44.4. HRMS calcd for $C_{13}H_{10}FNO$ 216.0819 $(M+H)^+$, found 216.0816.

(Z)-(Dimethylamino)-1-(4-fluorophenyl)-2-(pyridin-4-yl)prop-2-en-1-one (11). To a 500 solution of ketone **10** (106g, 500 mmol) in THF (500 mL) was added dimethylformamide dimethyl acetal (76 mL, 572 mmol) and the mixture was refluxed at 75 ^{o}C for 72 h. The reaction was cooled to rt and concentrated down under reduced pressure to afford the title compound as a reddish gum which was taken crude to the next step. A portion of this material was purified by silica gel chromatography using 5% MeOH/EtOAc to afford 95% pure title compound as light yellow solid. ^{1}H NMR showed a ~1:8 mixture of E, Z isomers. ^{1}H NMR ($CDCl_3$, 400 MHz), major Z-isomer : δ 8.44 (dd, J = 1.6, 4.5, 2H), 7.36-7.41 (m, 2H), 7.31 (s, 1H), 7.01 (dd, J = 1.6, 4.5, 2H), 6.88-6.95 (m, 2H), 2.72 (s, 6H). ^{13}C ($CDCl_3$, 100 MHz) δ 192.4, 161.2 (d, J_{F-C} = 250.3), 154.5, 148.8, 145.7, 137.0 (d, J_{F-C} = 3.7), 130.7 (d, J_{F-C} = 8.8), 126.7, 114.7 (d, J_{F-C} = 22.1), 108.5, 44.4. HRMS calcd for $C_{16}H_{15}FN_2O$ 271.1241$(M+H)^+$, found 271.1240.

4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridine (2). To a solution of enamine **11** (140g, 520 mmol) in EtOH (200 mL) at 0 ^{o}C was added dropwise a solution of methylhydrazine (33 mL, 624 mmol) in EtOH (70 mL). After 3 h stirring at 0 ^{o}C, the reaction was concentrated under reduced pressure, re-dissolved in 500 mL EtOAc, and washed with 75 mL water and 50 mL satd. brine. The organics were dried over Na_2SO_4, filtered and the concentrate was passed through a silica plug and rinsed with 2L each EtOAc and 10% MeOH/EtOAc. The filtrate was concentrated under reduced pressure and triturated with 1:1 heptanes/Et_2O (200 mL). The title compound (42 g, 31%) was recovered as a white solid after filtration and drying. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49-8.52 (m, 2H), 7.60 (s, 1H), 7.42-7.47 (m, 2H), 7.14-7.17 (m, 2H), 7.02-7.09 (m, 2H), 4.00 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.9 (d, J_{F-C} = 247.2), 150.3, 148.9, 141.2, 131.9 (d, J_{F-C} = 8.0), 130.9, 129.0 (d, J_{F-C} = 3.2), 122.9, 118.4, 114.7 (d, J_{F-C} = 21.8), 39.5. HRMS calcd for $C_{15}H_{12}FN_3$ 254.1088 $(M+H)^+$, found 254.1080.

4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridine 1-oxide (13). To a 0^{o} C solution of of diarylpyrazole **2** (42.0 g, 163.9 mmol) in 300 mL of DCM was added *m*CPBA (42 g, 197 mmol). The reaction was warmed to rt and stirred 18h then purified directly by silica gel chromatography using 5-50% MeOH/EtOAc to afford 44 g (99%) of the title compound as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04-8.08 (m, 2H), 7.57 (s, 1H), 7.36-7.42 (m, 2H), 7.07-7.11 (m, 2H), 7.00-7.06 (m, 2H), 3.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.7 (d, J_{F-C} = 248.7), 148.3, 139.0, 131.5, 130.2, 130.1(d, J_{F-C} = 8.1), 128.5 (d, J_{F-C} = 3.7), 124.6, 116.5, 115.7 (d, J_{F-C} = 22.1), 39.2. HRMS calcd for $C_{15}H_{12}FN_3O$ 270.1037 $(M+H)^+$, found 270.1032.

4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)picolinonitrile (14). To a 0^{o} C suspension of *N*-oxide **13** (57 g, 212 mmol) and trimethylsilyl cyanide (28 g, 275 mmol) in 250 mL DCM was added dropwise dimethyl carbamoyl chloride (24 mL, 254 mmol). Once addition was complete,

the resulting clear solution was stirred at rt for 16 h. The reaction was treated with 150 mL sat. K_2CO_3 and diluted with 250 mL of DCM. After one more extraction of the aqueous phase with 200 mL DCM, the combined organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The concentrate was slurried with 200 mL Et_2O, and filtered to recover 51g (86%) of the title compound as a light yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54 (dd, J = 5.3, 0.6, 1H), 7.68 (s, 1H), 7.56 (dd, J = 1.68, 0.8, 1H), 7.38-7.43 (m, 2H), 7.32 (dd, J = 5.1, 1.8, 1H), 7.05-7.12 (m, 2H), 4.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.6 (d, J_{F-C} = 248.1), 151.2, 148.9, 142.5, 134.2, 130.9, 130.1 (d, J_{F-C} = 8.1), 128.3 (d, J_{F-C} = 3.7), 126.8, 125.0, 117.2, 116.1, 115.8 (d, J_{F-C} = 21.4), 39.4. HRMS calcd for $C_{16}H_{11}FN_4$ 279.1041 $(M+H)^+$, found 279.1036.

(4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)methanamine (15). To a nitrogen sparged 2L pressure autoclave was added sponge nickel (Johnson Matthey type A-4000, 23g, 387 mmol), nitrile **14** (35 g, 125.8 mmol), and 7N ammonia in methanol (1225 mL, 8.58 mol). The vessel was purged with nitrogen (pressurized to 50 psi and then vented) 3x, then purged with hydrogen (pressurized to 50 psi and vented) 3x. The mixture was pressurized to 50 psi with hydrogen and shaken for 2 h. After purging with nitrogen 3x and the reaction was filtered (celite) and washed methanol (350 mL). The filtrate was concentrated under reduced pressure to recover 36 g (99%) of the title compound as a pink gum. ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.47 (d, J = 5.1, 1H), 8.02 (s, 1H), 7.40-7.46 (m, 2H), 7.31 (br s, 1H), 7.21 (dd, J = 5.3, 1.8, 1H), 7.10-7.16 (m, 2H), 4.17 (s, 2H), 3.98 (s, 3H). ^{13}C NMR (MeOH-d_4, 100 MHz) δ 165.7 (d, J_{F-C} = 245.9), 154.1, 150.7, 150.0, 144.0, 133.5, 131.9 (d, J_{F-C} = 8.1), 130.6 (d, J_{F-C} = 3.0), 123.5, 122.1, 118.7, 116.7 (d, J_{F-C} = 22.1), 44.2, 39.4. HRMS calcd for $C_{16}H_{15}FN_4$ 283.1354 $(M+H)^+$, found 283.1349.

N-((4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)methyl)formamide (16). Amine **15** (35 g, 124 mmol) in ethyl formate (500 mL) was heated to 70 ^{o}C. After 3 h heating, the reaction was cooled to RT, concentrated under reduced pressure, passed through a 3.5"x5" silica gel plug flushing first with 100% EtOAc to remove the less polar impurities and then eluting with 25% MeOH in EtOAc to yield 31 g (80%) of the title compound as light yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) showed a ~9:1 mixture of rotamers; major isomer - δ 8.36 (d, J = 5.3, 1H), 8.27 (s, 1H), 7.61 (s, 1H), 7.37-7.43 (m, 2H), 7.14 (s, 1H), 7.00-7.06 (m, 4H), 4.52 (d, J = 5.3, 2H), 3.97 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.0 (d, J_{F-C} = 247.4), 161.1, 156.1, 149.0, 148.6, 141.9, 130.7, 129.2 (d, J_{F-C} = 8.1), 126.6 (d, J_{F-C} = 3.0), 121.4, 120.5, 117.6, 113.9 (d, J_{F-C} = 21.4), 42.9, 39.1. HRMS calcd for $C_{17}H_{15}FN_4O$ 311.1303 $(M+H)^+$, found 311.1301.

N-((4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridine-2-yl)methyl)-N-methyl-formamide (17). Sodium hydride (4.8 g,120 mmol) was added to formamide **16** (31 g, 99 mmol) in THF (992 mL). After 30 min stirring, methyl iodide (21 g, 149 mmol) was added. The resulting suspension was stirred at rt for 18 h, quenched with 150 mL of saturated $NaHCO_3$ and extracted with DCM (3x400 mL). The combined organic extracts were dried over Na_2SO_4 and then filtered through a 1"x3" silica plug layered with 1" $MgSO_4$. The filtrate was concentrated under reduced pressure to give 29 g (90%) of the title compound as light yellow colored solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ~1:1 mixture of rotamers, 8.40/8.35 (two d's, 2H total, J = 5.3), 8.16 (s, 1H), 8.04 (s, 1H), 7.59 (d, 2H, J = 2.3), 7.34-7.39 (m, 4H total), 6.96-7.06 (m, 8H total), 4.54 (s, 2H), 4.39 (s, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 2.87 (s, 3H), 2.73 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ~1:1 mixture of rotamers, 163.8,163.7, 162.9, 162.5, 161.3, 156.1, 156.2, 149.8, 149.3, 148.5, 148.4, 142.0, 130.7, 130.6, 130.2, 130.1, 130.0, 128.9, 128.7, 121.5,

121.1, 120.3, 119.9, 117.6, 117.5, 115.5, 115.4, 115.3, 115.2, 54.9, 49.5, 39.7, 34.7, 29.8. HRMS calcd for $C_{18}H_{17}FN_4O$ 325.1459 $(M+H)^+$, found 325.1455.

1-(4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridine-2-yl)-N-methylmethanamine (4). *N*-Methylformamide **17 (**29g, 89 mmol) in 580 mL of 1:10 v/v of 12 N HCl : MeOH was refluxed for 30 min. The reaction was cooled to rt, concentrated under reduced pressure and triturated with MeOH (6x50 mL) to afford 26 g of light cream colored solid. Crystallization from boiling ISOPROPYL ALCOHOL/MeOH (4:1 ratio) afforded 18.5 g (56%) of the title compound as white cottony solid. ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.63 (d, J = 6.3, 1H), 8.53 (s, 1H), 8.26 (d, J = 1.8, 1H), 7.69 (dd, J = 6.3, 2.0, 1H), 7.51-7.56 (m, 2H), 7.19-7.26 (m, 2H), 4.60 (s, 2H), 4.05 (s, 3H), 2.88 (s, 3H). ^{13}C NMR (MeOH-d_4, 100 MHz) δ 162.2 (d, J_{F-C} = 247.6), 153.2, 152.1, 147.3, 144.5, 136.4, 130.1 (d, J_{F-C} = 8.7), 129.3 (d, J_{F-C} = 3.6), 126.7, 125.1, 115.0 (d, J_{F-C} = 22.3), 117.2, 50.3, 40.3, 34.6. HRMS calcd for $C_{17}H_{17}FN_4$ 297.151 $(M+H)^+$, found 297.1504.

Methyl 4-(3-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)picolinate (18) Nitrile **14** (330 mg, 1.18 mmol) andHCl/MeOH (1.25 M, 16 mL, 20.4 mmol) were refluxed for 20 h, then cooled to rt and concentrated under reduced pressure. The residue was dissolved in 100 mL DCM and basified using 20 mL of satd. $NaHCO_3$. The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure to recover 370 mg of crude product as semisolid. This was subjected to silica gel chromatography using 75-100% EtOAc/heptanes to afford 208 mg (55%) of the title compound as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.51 (d, J = 5.1, 1H), 7.99-8.01 (m, 1H), 7.64 (s, 1H), 7.34-7.39 (m, 2H), 7.21 (dd, J = 5.1, 1.8, 1H), 6.96-7.03 (m, 2H), 3.93 (s, 3H), 3.92 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 165.2, 162.3 (d, J_{F-C} = 248.1), 149.4,

148.2, 147.7, 141.9, 130.4, 129.7 (d, J_{F-C} = 8.1), 128.2 (d, J_{F-C} = 3.7), 124.8, 123.2, 116.7, 115.1 (d, J_{F-C} = 21.4), 52.4, 38.8. HRMS calcd for $C_{17}H_{14}FN_3O_2$ 312.1143 $(M+H)^+$, found 312.1148

(4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)methanol (3). To a solution of ester **18** (180 mg, 0.57 mmol) in THF (6 mL) at RT was added dropwise a THF solution ofLAH (1.0 M, 0.86 mL, 0.86 mmol). After 30 min stirring, the reaction was quenched with dropwise addition of water and diluted with THF (50 mL). After the addition of celite, the slurry was stirred for 30 min and then filtered through a pad of $MgSO_4$. The filtrate was concentrated under reduced pressure to recover 165 mg of desired alcohol as a pink colored gum. This was subjected to silica gel chromatography using 5:95:0.1 MeOH:EtOAc:NH_4OH (conc) to afford 41mg (25%) of the title compound as a colorless gum which solidified upon standing at RT. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.39 (d, J=5.1, 1H), 7.59 (s, 1H), 7.39-7.44 (m, 2H), 7.16 (s, 1H), 6.99-7.06 (m, 3H), 4.68 (s, 2H), 3.97 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4 (d, J_{F-C} = 247.4), 159.6, 148.6, 148.5, 141.8, 130.7, 128.3 (d, J_{F-C} = 8.1), 126.2 (d, J_{F-C} = 2.9), 121.3, 119.2, 117.9, 114.3 (d, J_{F-C} = 21.4), 64.2, 39.1. HRMS calcd for $C_{16}H_{14}FN_3O$ 284.1194 $(M+H)^+$, found 284.1188.

3-(4-Fluorophenyl)-1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-21. To a solution of *n*-BuLi (2.5M in hexanes, 500 mL, 1.25 mol) at -70° C was added slowly a solution of commercially available pyrazole **19** (160 g, 0.63 mol) inTHF (1.2L). The resulting solution was stirred at this temp for 1h. 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **20** (175 g, 0.94 mol) was then added slowly maintaining the temp below -60° C. After addition, the reaction was stirred at -70° C for 30 min and then quenched slowly by the addition of H_2O. The resulting slurry was filtered off and the filtrate was concentrated under reduced pressure. This was taken up in EtOAc (2L) and washed with (250 mL)and brine (100 mL), dried over

Na_2SO_4 and concentrated under reduced pressure to yield crude product which solidified upon cooling to 0° C. After triturating with 500 mL hexanes, 84 g (44 %) of the title compound was obtained as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91-7.97 (m, 2H), 7.72 (s, 1H), 7.03-7.09 (m, 2H), 3.91 (s, 3H), 1.31 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.8 (d, J_{F-C} = 245.9), 156.0, 139.8, 129.7 (d, J_{F-C} = 3.7), 127.7 (d, J_{F-C} = 8.1), 113.0 (d, J_{F-C} = 21.4), 99.7, 83.3, 38.6, 24.7. HRMS calcd for $C_{16}H_{20}BFN_2O_2$ 303.1678 $(M+H)^+$, found 303.1672

3-Fluoro-4-(3-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)picolinonitrile (23). Commercially available 3-fluoro-4-iodopicolinonitrile **22** (50g, 0.20 mol), 3-(4-boronate **21** (67 g, 0.22 mol), $Pd(OAc)_2$ (0.91 g, 4.0 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (Xphos) (3.8 g, 8.0 mmol) and Cs_2CO_3 (79 g, 0.24 mol) in ACN (350 mL) and H_2O (70 mL) were refluxed for 3 h under N_2. The reaction was cooled to RT, filtered and the filtrate was diluted with 300 mL EtOAc. The organic layer was washed with water (200 mL) and brine (150 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford crude product. This was washed with 1:1 EtOAc/hexanes (200 mL) to yield 48 g (80 %) of the title compound as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (d, J=4.9, 1H), 7.87 (d, J=2.9, 1H), 7.40-7.45 (m, 2H), 7.26-7.30 (m, 1H), 7.08-7.15 (m, 2H), 4.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8 (d, J_{F-C} = 248.9), 158.5 (d, J_{F-C} = 268.7), 149.9, 146.6 (d, J_{F-C} = 5.9), 132.7 (d, J_{F-C} = 7.4), 130.7 (d, J_{F-C} = 7.4), 130.2 (d, J_{F-C} = 7.4), 128.4 (d, J_{F-C} = 3.7), 127.4 (d, J_{F-C} = 2.2), 123.3 (d, J_{F-C} = 16.9), 115.9 (d, J_{F-C} = 22.1), 113.1 (d, J_{F-C} = 4.4), 108.7, 39.4. HRMS calcd for $C_{16}H_{10}F_2N_4$ 297.0946 $(M+H)^+$, found 297.0941

***tert*-Butyl(2-((2-cyano-4-(3-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-3-yl)oxy)ethyl)carbamate (25).** To **23** (1.00 g, 3.38 mmol) and *tert*-butyl (2-hydroxyethyl)carbamate **24** (777 mg, 4.82 mmol) in THF (17 mL) at -20 °C was added sodium

hydride (228 mg, 5.70 mmol). The mixture was stirred for 3.5 h keeping the bath temperature between -20 and -10 º C. The mixture was warmed to -5 °C for another 20 min, and was then quenched with satd. ammonium chloride (5 mL). Water (10 mL) was added and the mixture was extracted with 2 x 15 mL EtOAc. Extracts were washed with brine, dried with $MgSO_4$, filtered and concentrated to give a pale orange oil. The oil was diluted with 2 mL of ethyl acetate, and with stirring an equal volume of isooctane was added resulting in a thick slurry. This was stirred overnight and the title compound (1.03 g, 70%) was isolated by filtration as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.22 (d, J=4.9, 1H), 7.80 (s, 1H), 7.32-7.37 (m, 2H), 7.19 (d, J=4.9, 1H), 6.96-7.03 (m, 2H), 5.02 (br s, 1H), 3.99 (s, 3H), 3.96 (t, J=5.1, 2H), 3.35 (dd, J=5.3, 5.3, 2H), 1.38 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2 (d, J_{F-C} = 248.1), 155.5, 155.4, 148.6, 145.7, 135.6, 131.9, 129.2 (d, J_{F-C} = 8.1), 128.4, 128.2 (d, J_{F-C} = 2.9), 128.0, 115.3 (d, J_{F-C} = 22.1), 115.1, 110.9, 79.1, 73.4, 40.1, 38.9, 27.9. HRMS calcd for $C_{23}H_{24}FN_5O_3$ 438.1941 $(M+H)^+$, found 438.1936.

9-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)-3,4-dihydropyrido[2,3-f][1,4]oxazepin-5(2H)-one (27). To 7 mL of neat sulfuric acid was added with stirring carbamate **25** (2.00 g, 4.58 mmol). The mixture was heated to 110° C for 10 min, and then cooled to RT. The solution was then added slowly to ~30 mL of crushed ice and water, and cooled to -10° C. To the cold mixture was added slowly 5 N NaOH (45 mL) to bring the pH near neutral, followed by 1M pH 7 phosphate buffer to achieve a final pH of 7. The mixture was heated back to 110° C for 3 h, and then was cooled to RT and stirred for 12 h. The tan solids were collected via filtration and recrystallized from ethyl acetate/methanol (95:5) to yield 1.3 g (80%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.52 (t, J=6.0, 1H), 8.31 (d, J=4.7, 1H), 8.01 (s, 1H), 7.34-7.40 (m, 2H), 7.14-7.23 (m, 3H), 3.94 (s, 3H), 3.89 (t, J=5.3, 2H), 3.20 (dd, J=5.3,

10.9, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.86, 161.8 (d, J $_{F-C}$ = 244.4), 148.0, 147.6, 146.8, 144.9, 135.5, 133.1, 130.0 (d, J $_{F-C}$ = 3.7), 129.5 (d, J $_{F-C}$ = 8.1), 127.3, 115.6 (d, J $_{F-C}$ = 22.1), 112.4, 74.6, 39.0, 38.5. HRMS calcd for $C_{18}H_{15}FN_4O_2$ 339.1179 $(M+H)^+$, found 339.1251

9-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)-2,3,4,5-tetrahydropyrido[2,3-f][1,4]oxazepine (5). Oxazepinone **27** (300 mg, 0.89 mmol) was added in 50 mg increments over 10 min to a 50 ºC soln of LAH (1M in THF, 1.5 mL, 1.5 mmol) in THF (50 mL). The mixture was stirred at 50° C for 1 h. An additional 0.5 mL of the lithium aluminum hydride solution was added and the mixture stirred at 50° C for an additional hour. The mixture was cooled to RT and quenched by the sequential addition of 0.1 mL water, 0.1 mL of 15% NaOH, and 0.3 mL water. The mixture was stirred at RT for 4 days, and then was filtered through a bed of celite. The filter cake was rinsed with THF (20 mL) and EtOAc (20 mL). The combined filtrate was treated with decolorizing carbon, filtered, and concentrated to yield 234 mg of a pale yellow solid. This was dissolved in EtOAc (20 mL) and treated with *p*-toluenesulfonic acid (285 mg, 1.50 mmol) as a solution in 1 mL methanol. An additional 0.5 mL of methanol was added with gentle warming while a precipitate began to appear. The mixture was stirred at RT for 12 h at which point the solids were collected via filtration to provide 280 mg (42%) of the title compound as a pale yellow solid. ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.38 (d, J = 5.9, 1H), 8.35 (s, 1H), 7.63 (d, J = 6.3, 1H), 7.46-7.51 (m, 2H), 7.15-7.22 (m, 2H), 4.87 (s, 2H), 4.44-4.48 (m, 2H), 4.04 (s, 3H), 3.78-3.82 (m, 2H). ^{13}C NMR (MeOH-d_4, 100 MHz) δ 164.8(d, J $_{F-C}$ = 247.4), 156.2, 152.5, 145.5, 141.7, 138.9, 137.2, 135.5 (d, J $_{F-C}$ = 8.1), 129.3 (d, J $_{F-C}$ = 3.0), 128.4, 115.9 (d, J $_{F-C}$ = 22.1), 112.4, 70.5, 50.3, 48.0, 40.1. HRMS calcd for $C_{18}H_{17}FN_4O$ 325.1459 $(M+H)^+$, found 325.1454.

2-Bromo-4-(3-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridine (29). To a DMF (5 mL) solution of commercially available **28** (0.095 g, 0.34 mmol), boronate **21** (0.100 g, 0.335 mmol), and cesium fluoride (0.106 g, 0.67 mmol) under N_2 was added [1,1'-bis(diphenylphosphino)ferrocene]dichloro-palladium(II) (13 mg, 0.017 mmol). The mixture was heated to 140° C for 2 h and then cooled to RT. The reaction was diluted with EtOAc (100 mL) and filtered through a pad of celite and rinsed with another 10 mL of EtOAc. The filtrate was washed with H_2O (20 mL), brine (15 mL), dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to recover a residual oil. This was subjected to silica gel chromatography using 60% EtOAc/heptanes to recover 100 mg of solid. Crystallization from hot EtOAc/heptanes yielded 89 mg of the title compound as a white solid (80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.22 (d, J = 5.3, 1H), 7.62 (s, 1H), 7.41-7.45 (m, 2H), 7.39 (m, 1H), 7.06 - 7.10 (m, 3H), 3.99 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.8 (J_{F-C} = 247.4), 150.0, 148.7, 143.8, 142.6, 130.8, 130.2 (J_{F-C} = 8.1), 128.5 (J_{F-C} = 2.9), 126.2, 121.5, 116.6, 115.6 (J_{F-C}=21.4), 39.2. HRMS calcd for $C_{15}H_{11}BrFN_3$ 332.0193 $(M+H)^+$, found 332.0197

***tert*-Butyl (2-(2-(4-(3-(4-fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-oxoethoxy)ethyl)carbamate (31).** A suspension of **29** (10.0 g, 30.0 mmol) in toluene (300 mL) in a three neck flask fitted with a nitrogen inlet and a mechanical stirrer, was cooled to -78° C. *n*-BuLi (2.5 M in hexanes,12.0 mL, 30.0 mmol) was added at such a rate to maintain the internal temperature below -72° C. The reaction was then stirred at -78° C for 30 min and a solution of *tert*-butyl 3-oxomorpholine-4-carboxylate (6.1 g, 30.1 mmol) in toluene (50 mL) was added dropwise keeping the temperature below - 70° C. The reaction was stirred at -78° C for 1 h, warmed to RT and stirred for 2 h, quenched with a mixture of saturated NH_4Cl/EtOAc (1:5, 600

mL). The organic phase was dried ($MgSO_4$), evaporated to dryness in vacuo and the residue purified by flash chromatography using a 0 - 100% gradient of ethyl acetate in heptane to yield 3.0 g (22%) of title compound as an off-white foam ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.40 (d, J = 5.1, 1H), 7.93 (d, J = 1.6, 1H), 7.66 (s, 1H), 7.34-7.39 (m, 2H), 7.22 (dd, J = 1.9, 5.1, 1H), 6.98-7.03 (m, 2H), 5.26 (br s, 1H), 5.05 (s, 2H), 3.95 (s, 3H), 3.64 (dd, J = 4.7, 10.2, 2H), 3.36 (dd, J = 5.1, 10.2, 2H), 1.41 (s, 9H). ^{13}C NMR ($CDCl_3$,100 MHz,) δ 197.5, 171.4, 163.2 (J_{F-C} = 248.1), 156.3, 152.6, 149.4, 149.1, 142.6, 131.2, 130.5 (J_{F-C} = 8.1), 129.0 (J_{F-C} = 2.9), 126.4, 120.4, 117.6, 116.0 (J_{F-C} = 22.1), 74.0, 71.1, 40.7, 39.6, 28.7. LCMS m/z 399 (M+H-*t*Bu)

5-(4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-3,6-dihydro-2H-1,4-oxazine (32). A solution of carbamate **31** (3.0 g, 6.6 mmol) in ethanol was treated with 1.0 M HCl in ethanol (60.0 mL, 60.0 mmol) and the mixture was stirred at RT for 12 h and concentrated under high pressure to give title compound (3.0 g, >100%). This crude imine was used without further purification in next step. LCMS m/z 337 (M+H)

(-)-3-(4-(3-(4-Fluorophenyl)-1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)morpholine (6). A solution of **32** (2.7 g, 8.0 mmol) in methanol (20 mL) was cooled to - 40^o C, $NaBH_4$ (1.2 g, 32.0 mmol) was added in batches and the mixture stirred at RT for 30 min. The mixture was diluted with DCM (150 mL) and washed with water, brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography eluting with EtOAc, 10% EtOAc/EtOAc, 10% MeOH/DCM to yield 0.5 g (26%) of racemic title compound as colorless foam. ^{1}H NMR ($CDCl_3$,400 MHz) δ 8.37 (d, J = 5.1, 1H), 7.58 (s, 1H), 7.37-7.41 (m, 2H), 7.20-7.25 (m, 1H),), 6.98-7.04 (m, 3H), 3.96 (s, 3H), 3.93-4.00 (m, 2H), 3.86-3.89 (m, 1H), 3.51-3.58 (m, 1H), 3.39-3.45 (m, 1H), 3.04-3.11 (m, 1H), 2.95-3.0 (m, 1H). LCMS m/z 339 (M+H)

Chiral HPLC (Chiralcel OJ-H, 10/90 MeOH/CO_2 with 0.2% isopropylamine) was used to separate the enantiomers. Enantiomer 1 - title compound **6** $[\alpha]_D^{25}$: -29.5 (c = 1.0, MeOH) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.42 (d, J = 5.1, 1H), 7.60 (s, 1H), 7.37-7.45 (m , 2H), 7.22 (s ,1H), 6.99-7.07 (m, 3H), 3.95-4.02 (m, 2 H), 3.97 (s, 3H), 3.85 (dd, J = 1.5, 11.2, 1H), 3.53-3.61 (m, 1 H), 3.41-3.46 (m, 1H), 3.07-3.14 (m, 1H), 2.97-3.02 (m, 1 H), 2.2 (br s, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.4 (d, J_{F-C} = 247.4), 159.5, 149.5, 148.5, 141.5, 130.6, 130.1(J_{F-C} = 8.1), 128.9 (J_{F-C} = 3.7), 121.4, 120.3, 118.0, 115.5 (J_{F-C} = 21.4), 72.2, 67.4, 60.3, 45.7, 39.1. HRMS: Calculated for C_{19} H_{19} F N_4 O: 339.1616 (M+H): Found: 339.1618 (M+H). Enantiomer 2: $[\alpha]_D^{24}$: 34.7 (c = 1.0, MeOH)

Biology

Cell Free (Biochemical Assay): The CK1δ and CK1ε kinase assays were performed in a 20 µl final volume in buffer containing 50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 100 µg/mL BSA, 1 mM TCEP, 1.25% Glycerol, 0.0125% P104, 2.5 mM KCl, 10 µM ATP, and 42 µM peptide substrate PLSRTLpSVASLPGL (Flotow et al.,1990). The final enzyme concentraionts were 2 nM for CK1δ and 2.5 nM for Ck1ε. Assays were run in a panel format in the presence of 1 µl of compounds or 4% DMSO. The reactions were incubated for 60 min at 25°C for CK1δ and 85 min at 25°C for CK1ε, followed by detection with use of 20 µl of Kinase-Glo Plus Assay reagent according to the manufacturer's instructions. Luminescent output was measured on the Perkin-Elmer Envision plate reader.

Whole Cell (Translocation Assay): COS-7 cells were maintained at 37°C in 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. Log-phase cells were dislodged with 5 minute treatment of TrypLE Express and viable cell count was

determined with Cedex cell counter. Cells were diluted in DMEM medium to a density of 1.5E5 viable cells/mL in 2/3 of the final volume of final transfected cell mix. Cells were cotransfected with two plasmid DNAs, mPER3-GFP in pd2EGFP-N1 vector and human CK1 in pcDNA4/hisA vector (for CK1ε at a ratio of 1:5 respectively for CK1δ at a ratio of 1:11 respectively), using Lipofectamine 2000 reagent according to the manufacturer's recommendations. The transfection mix contained approximately 0.83 ug/mL of DNA and 6 uL/mL of Lipofectamine 2000, in a total of 1/3 of the final transfection volume in Opti-MEM medium. After 20 min at room temperature, the cell mix was combined with the DNA transfection mix, per manufacturer instructions. Transfected cell suspension (50 mL) was dispensed per well by multidrop dispenser into Greiner 384-well Cellcoat (PDL) plates. Compounds were diluted with Opti-MEM I to a 4X concentration before addition to plated cells. Inhibitors of CKIδ/ε were tested at a 11 concentrations to evaluate their ability to inhibit mPER3-GFP translocation to the nucleus. After overnight exposure at 37°C in a CO_2 incubator, cells were fixed by the addition of 12% paraformaldehyde in phosphate-buffered saline (PBS) with 20% sucrose to a final concentration of 4%, and then the cells were incubated for 30 min at room temperature. Fixative was removed, and cells were washed with PBS and then stained with 0.4 μg/ml Hoechst dye in blocking buffer containing 4% goat serum and 0.1% TritonX for 1 hour. In some experiments, GFP enhancing antibodies were used when needed for signal amplification. Cells were washed again with PBS and stored at 4°C in PBS or scanned immediately with the Cellomics ArrayScan VTI.

Circadian Timing methods follow those procedures published previously[7]**.** All procedures performed on these animals were in accordance with regulations and established guidelines and

were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process.

P38 in Vitro Activity Assay and Kinase Panel. Compounds were tested at 1 μM with use of Invitrogen SelectScreen Kinase Profiling. All kinases were tested at their K_m for ATP. P38 IC50 were also determined at Invitrogen for select compounds.

Pharmacokinetic Characterization. The single point concentration values for all compounds in plasma and whole brain were determined in male C57BL/6J mice after administration of a single subcutaneous dose. Plasma and whole brains were collected from mice while they were receiving isoflurane treatment at 0.5, 1, 2, 4, 8, and 24 h after dosing (n = 4 per time point). Plasma was obtained after centrifugation of whole blood. Whole brains were diluted in a 4× volume (w/v) 60% isopropyl alcohol and homogenized by use of a Mini-Beadbeater 96 (Biospec Products, Bartlesville, OK). Both plasma and brain homogenate samples were quantified with use of a liquid chromatography/mass spectrometry method following protein precipitation. Concentration and pharmacokinetic results are presented as mean ± S.E.M. AUC0-Tlast values were calculated with use of the linear trapezoidal rule.

Protein binding experiments were conducted with mouse plasma and brain homogenate with use of high-throughput 96-well equilibrium dialysis (Banker et al., 2003). Aliquots (n = 6 per matrix) of plasma and brain homogenate spiked with 1 μM compound were dialyzed against an equal volume of buffer for 6 h at 37°C. The dialysis membranes had a molecular cutoff of 12 to 14 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA). After incubation, donor and receiver samples were transferred to a 96-well block containing an equal volume of the opposite

matrix and internal standard. The diluted samples were then analyzed by liquid chromatography/mass spectrometry after protein precipitation. Free fraction in plasma was calculated as the ratio of instrument response between donor and receiver samples. Determination of undiluted brain-free fraction was calculated as described previously.

ASSOCIATED CONTENT

Accession Codes

All coordinates have been deposited in the PDB with accession codes 4KBA (3), 4KBB (4), 4KBH (5) and 4KBK (6).

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ABBREVIATIONS USED

CK1δ, Casien Kinase 1δ, CK1ε, Casien Kinase 1ε, CNS, Central Nervous System, MPO, multi parameter optimization, PDB, protein data bank, p38, p38 mitogen-activated kinase, hERG, human ether-a-go-go-related gene, ADME, absorption, distribution, metabolism and excretion.

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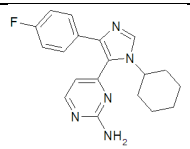
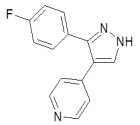
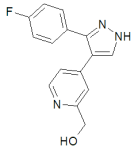
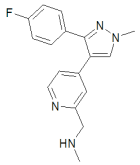
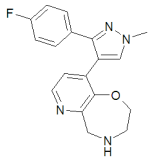
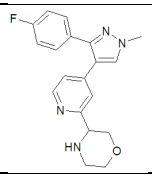
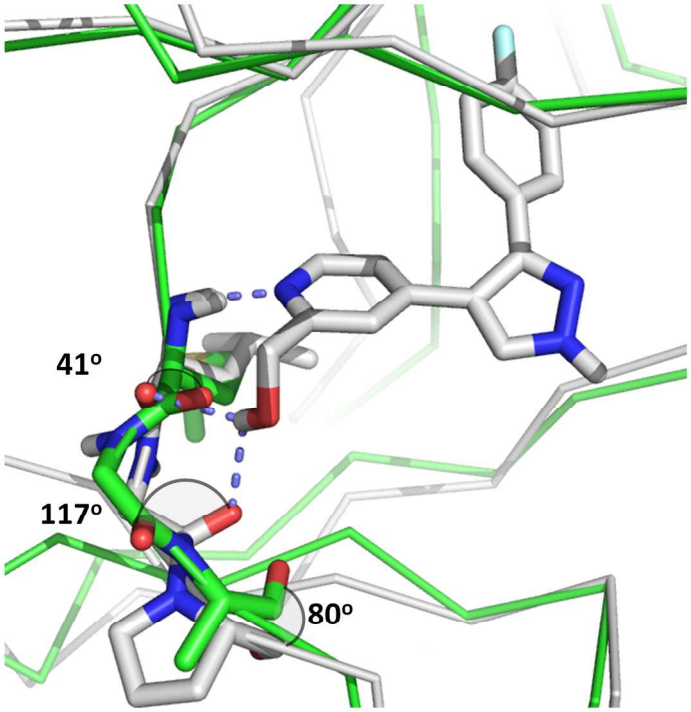
Table 1. Structure, pharmacology and exposure data.

			Kinase Potency[a] [nM]				Safety		Exposure[b] [uM]	
Cmpd	**Structure**	**ACD pKa**	**CK1δ**	**CK1ε**	**CK1δ WC**	**P38**	**Selectivity**	**Dof/herg [uM]**	**$C_{b,u}$**	**$C_{P,u}$**
1			7.8	29.0	80.5	39.2	6/34	27.7/ 27.0 (n = 1)	0.241[c]	0.752[c]
2			48.1	305	545	126	9/103	-- / >100 (n = 1)		
3			14.2 (n = 2)	81.6 (n = 2)	237 (n=2)	42%@1uM	1/36	6% @ 10uM/ --		
4		8.09	12.9	67.2	63.8	3040 (n=1)	0/43	38.2/3.99 (n = 1)	7.53	9.66
5		5.93	6.0	27.0	42.1	654	3/43	>79.6/25.7 (n = 1)	2.97	8.82
6		7.25	6.6	28.2	90.0 (n = 2)	>1000 (n = 1)	1/39	25.9/4.43 (n = 1)	3.70	1.95

[a] IC_{50} values reported as geometric means of n > 2 independent experiments, unless otherwise noted.
[b] Exposures are maximum concentrations at a 56 mg/kg dose, unless otherwise noted.
[c] 32 mg/kg dose.



TOC Graphic
190x142mm (300 x 300 DPI)

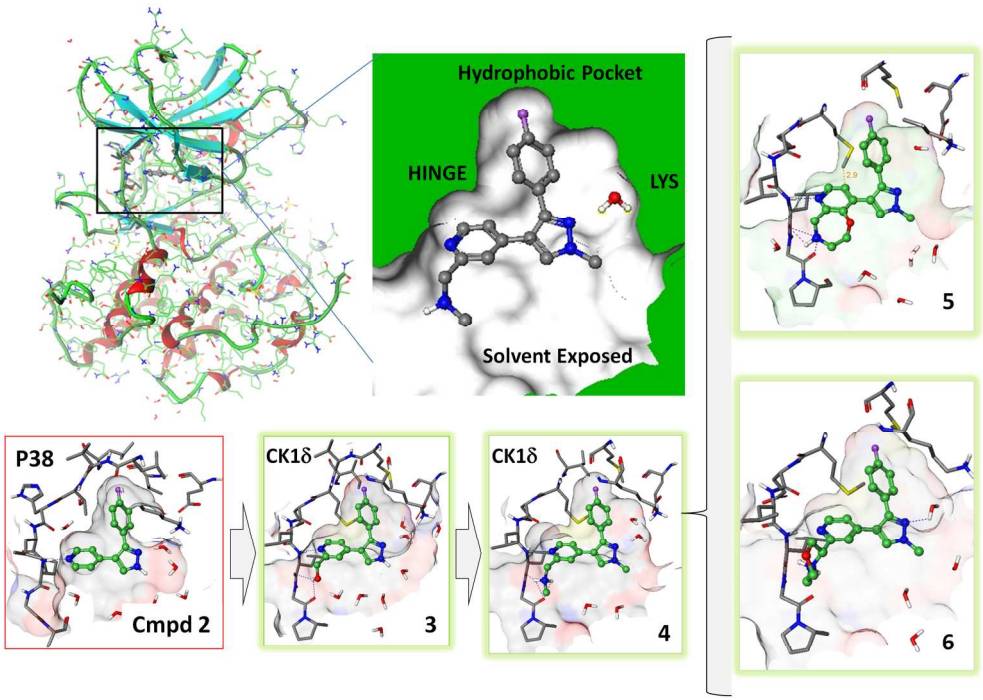


Figure 1
190x142mm (300 x 300 DPI)

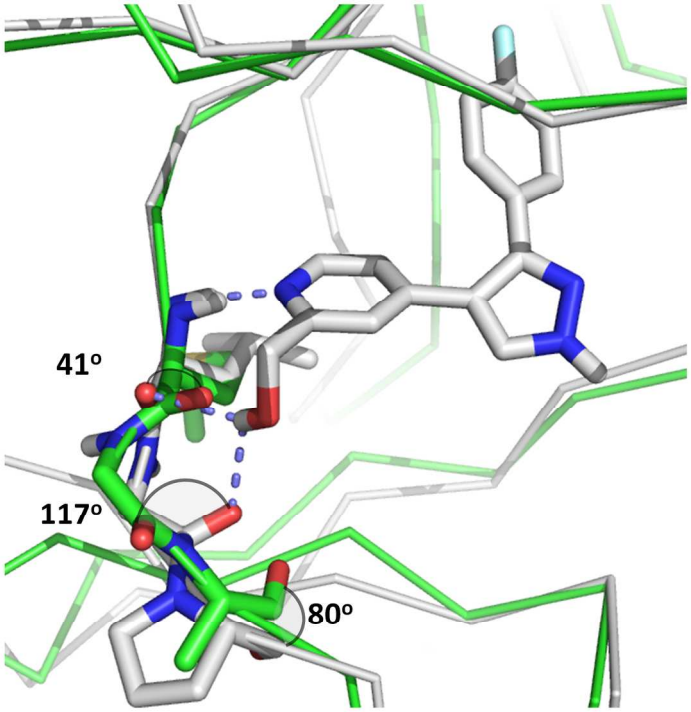


Figure 2
190x142mm (300 x 300 DPI)

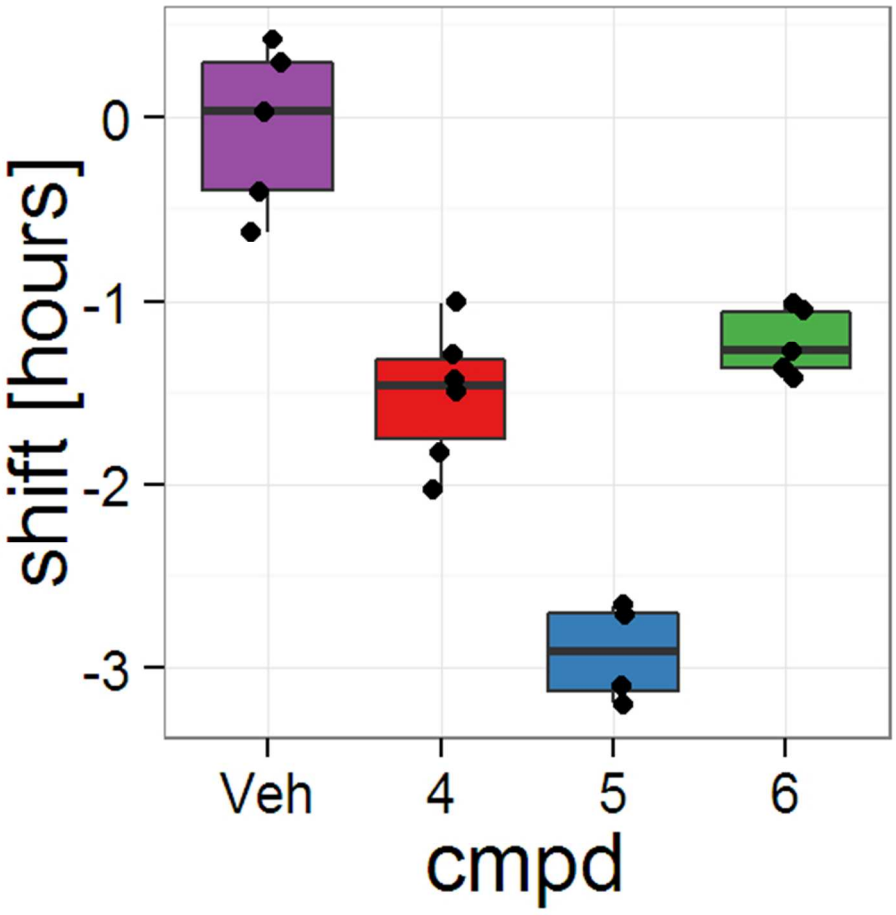


Figure 3
76x76mm (200 x 200 DPI)