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Discovery and SAR of 5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (CX-4945), the First Clinical Stage Inhibitor of Protein Kinase CK2 for the Treatment of Cancer

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Herein we chronicle the discovery of CX-4945 (**25n**), a first-in-class, orally bioavailable ATP-competitive inhibitor of protein kinase CK2 in clinical trials for cancer. CK2 has long been considered a prime cancer drug target because of the roles of deregulated and overexpressed CK2 in cancer-promoting prosurvival and antiapoptotic pathways. These biological properties as well as the suitability of CK2's small ATP binding site for the design of selective inhibitors, led us to fashion novel therapeutic agents for cancer. The optimization leading to **25n** (K_i = 0.38 nM) was guided by molecular modeling, suggesting a strong binding of **25n** resulting from a combination of hydrophobic interactions, an ionic bridge with Lys68, and hydrogen bonding with the hinge region. **25n** was found to be highly selective, orally bioavailable across species (20−51%) and efficacious in xenograft models. The discovery of **25n** will allow the therapeutic targeting of CK2 in humans for the first time.

Introduction

Protein kinase CK2 is a pleiotropic, highly conserved serine/threonine protein kinase ubiquitously distributed across the compartments of multiple cell types.[1−3] The protein is comprised of a heterotetrameric complex containing two catalytic isoforms (CK2α or CK2α′) and two regulatory β subunits in various combinations. Abundant evidence supports a multifunctional role for CK2 in a number of cellular events contributing to the cancer phenotype. CK2 regulates many antiapoptotic[4] and pro-proliferative signaling cascades, including PI3K/Akt[a] and Wnt signaling cascades, NF-κB transcription, and the DNA damage response (reviewed in refs 1 and 5). Additionally, CK2 has been characterized as a regulator of angiogenesis.[6]

CK2 is constitutively active and does not require phosphorylation by other kinases for activation. Unlike other signaling kinases, where genetic alterations lead to dysregulation of their pathways, no CK2 mutations have been found to date, yet its high expression and activity have been associated with disease states.[5,7] Overexpression of CK2 has been well documented in a number of cancers, including head and neck,[8] breast,[9] colorectal,[10] renal,[11] lung,[12] leukemias,[13] and prostate,[14,15] and has also been linked to poor prognosis and disease progression.[5,16]

These properties have defined CK2 as a prototypical "nononcogene target",[17] i.e., a nonmutated gene not directly transformative but which is overexpressed to maintain the cancer phenotype and protect cells from the increased cellular stress resulting from oncogenic activation.[18] The high dependency of cancer cells on nononcogenes for survival has made them an attractive yet underexploited new class of therapeutic targets for the treatment of cancer.

Among the possible strategies to modulate CK2, antisense nucleotides directed toward CK2α have scientifically validated CK2 as a target by inducing apoptosis in vivo in a xenograft model of prostate cancer.[19,20] A more practical therapy would involve the oral administration of a small molecule kinase inhibitor. The ATP binding site of CK2 is smaller than most of the other protein kinases due to the presence of unique bulky residues,[7] allowing for the design of very selective low molecular weight ATP-competitive inhibitors.[21,22]

Many ATP-competitive inhibitors of CK2 have already been reported in the literature,[7,23−33] but none of them have reached human clinical trials. Representative structural classes of CK2 inhibitory reagents are shown in Figure 1. The chlorinated nucleoside **1** (DRB) was one of the earliest described inhibitors of CK2 with an IC_{50} of 15 μM.[34] Flavonoids such as emodin **2** displayed greater potency (IC_{50} = 2 μM)[35] but generally lacked specificity. One of the most studied structural

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[a] Abbreviations: PI3K, phosphatidylinositol-3-kinase; Akt, v-akt murine thymoma viral oncogene homologue; NF-κB, nuclear factor κ light-chain enhancer of activated B cells; TBB, 4,5,6,7-tetrabromobenzotriazole; IQA, 2-(5-oxo-5,6-dihydroindolo[1,2-*a*]quinazolin-7-yl)acetic acid; PTEN, phosphatase and tensin homologue; NaHMDS, sodium bis(trimethylsilyl)-amide; PARP, poly(ADP-ribose) polymerase; NAD^+, nicotinamide adenine dinucleotide; PDK1, 3-phosphoinositide dependent protein kinase; mTOR, mammalian target of rapamycin; FLT3, fms-like tyrosine kinase receptor-3; PIM1, Proviral insertion site in Moloney murine leukemia 1; CDK1, cyclin-dependent kinase 1; NMP, *N*-methyl-2-pyrrolidone; THF, tetrahydrofuran; HOBt, hydroxybenzotriazole hydrate; DIEA, *N*,*N*-diisopropylethylamine; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; $PdCl_2$(dppf), 1,1′-bis(diphenylphosphino)ferrocene palladium(II) chloride complexed with dichloromethane; ES, electrospray; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; LCMS, liquid chromatography mass spectrometry; GCMS, gas chromatography mass spectrometry.

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classes of inhibitors include polyhalogenated-heteroaryls such as tetrabromo-benzimidazole **3** (TBB),[36−38] which has higher selectivity and potency than many of the other known agents.[21] These compounds have been widely used as molecular probes to elucidate the functional role of CK2. Although **3** has been reported to be well tolerated in mice,[7] the polyhalogenated aromatic nature of this molecule and its analogues raises serious concerns about its potential long-term toxicity in humans.[39]

The pharmaceutical industry's effort to develop CK2 inhibitors has been met with limited success. Vangrevelinghe et al. led the way by discovering the potent and selective CK2 inhibitor **6** (IQA) (Figure 2) (IC_{50} = 80 nM) by utilizing high throughput docking methodologies.[40] This molecule demonstrated inhibition of CK2 in cells, and the structural basis for its inhibition has been resolved by X-ray crystallography.[22] Compound **6** was reported to decompose slowly in aqueous media,[33] precluding its use as a drug. A novel series of pyrazolo-[1,5-*a*][1,3,5]triazines exemplified by analogue **4** (Figure 1) emerged as a potent class of CK2 inhibitors, with the K_i values of several analogues reported to be below 1 nM.[41,42] These molecules demonstrated cellular antiproliferative activity in the submicromolar range. However, no further data have been published in the literature and the current status of these noteworthy molecules remains undisclosed. A recent publication described the discovery and cellular characterization of a novel molecule **5**, which induced antiproliferative responses in cell culture, by inhibiting the phosphorylation of the CK2 substrate PTEN at Ser 370.[43] Additionally, a recent review by Prudent et al.[44,45] discussed an emerging class of inhibitors targeting structural elements located outside of the active site, providing exciting opportunities for the development of allosteric inhibitors.

Herein, we wish to report the discovery of the first-in-class orally available ATP-competitive inhibitor of CK2 **25n**, currently in clinical trials for the treatment of cancer. The discovery of **25n** is of considerable importance as it will allow for the evaluation of an entirely novel therapeutic strategy for cancer.

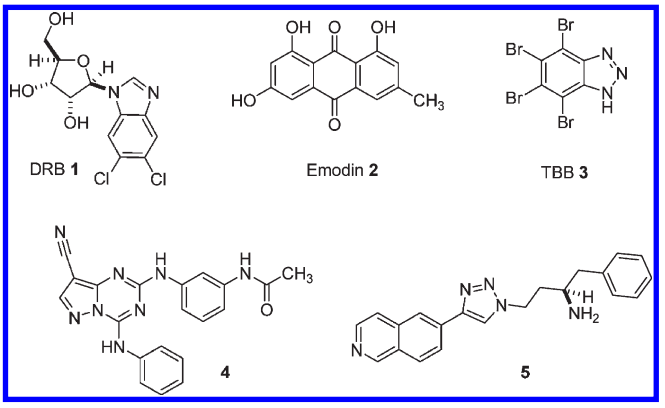


Figure 1. Representative structures of known ATP-competitive inhibitors of CK2.

Chemistry

Compounds **11a**−**b** were efficiently synthesized (Scheme 1) via a tandem Suzuki coupling/intramolecular amide formation between methyl 2-bromo-3-thiophene carboxylate **9** and the commercially available boronic acids **10a**−**b**.[46] Functional group interconversions afforded several analogues **11c**−**e** bearing various substituents on R2.

Lactam **11a** was alkylated with 3-bromo-1-propanol to afford a mixture of the *N*- and *O*-alkyl analogues **12a** and **12b**. After separation by chromatography on silica gel followed by ester hydrolysis, compounds **13a** and **13b** were isolated. Their respective structures were assigned on the basis of their proton NMR spectra. The loss of the carbonyl function in the *O*-alkylated isomer **13b** resulted in an upfield shift of the nearest thiophene proton, from 7.77 ppm in **13a** to 7.54 ppm in **13b**. Lactams **11a**−**b** were alternatively reacted with phosphorus oxychloride ($POCl_3$) to afford chloroquinolines **14a**−**b**. Intermediate **14a** was further derivatized into various substituted 4-aminothieno[3,2-*c*]quinoline-7-carboxylic acids **17a**−**p** in two steps. The first step involved nucleophilic displacement of the chlorine of **14a** with various substituted amines. The second step was performed by hydrolysis of the ester function in basic conditions. A tetrazole was introduced on R2 by reacting nitrile **16** with sodium azide. Alternatively, a nonacidic triazole was also prepared in one pot starting from carboxamide **18**, obtained by oxidation of the corresponding cyano **16**.

The chemistry described in Scheme 2 extended our previous chemistry to various six-membered ring systems. Various *ortho*-halogeno esters **22a**, **22e**−**g**, and **22i** were prepared from their commercially available carboxylic acids **21a**, **21e**−**g**, and **21i** using standard conditions. Under Suzuki conditions, *ortho*-halogeno esters **22a** and **22d**−**i** reacted with boronic acids **10a**−**c** to form lactams **23a**−**i** in one step. These materials were converted to various chloroquinolines **24a**−**i** using phosphorus oxychloride.

Compound **24a** was transformed (Scheme 3) into various substituted benzo[*c*][2,6]naphthyridine-8-carboxylate **25a**−**t** in two steps by reaction with the desired amines followed by ester hydrolysis. Functional group interconversions similar to Scheme 1 were used to prepare 3-chloroanilines **26a**−**g** bearing various substitutions on R2. Nucleophilic displacement of the chlorine by anilines in **24d**−**i** (Scheme 4) and ester hydrolysis provided carboxylates **27d**−**e**, **27h**−**i**, **28d**−**g**, and **28i**.

The chemistry described in Schemes 5 and 6 allowed variations on the position and nature of functional groups on the lower C-ring. Many ortho-substituted halogeno-anilines were prepared from commercially available chemicals (Scheme 5). The reaction of methyl 3-amino-2-methylbenzoate **29** with iodination reagent NIS produced a mixture of isomers, the

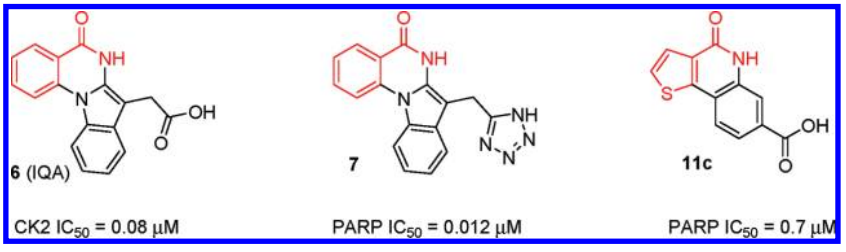
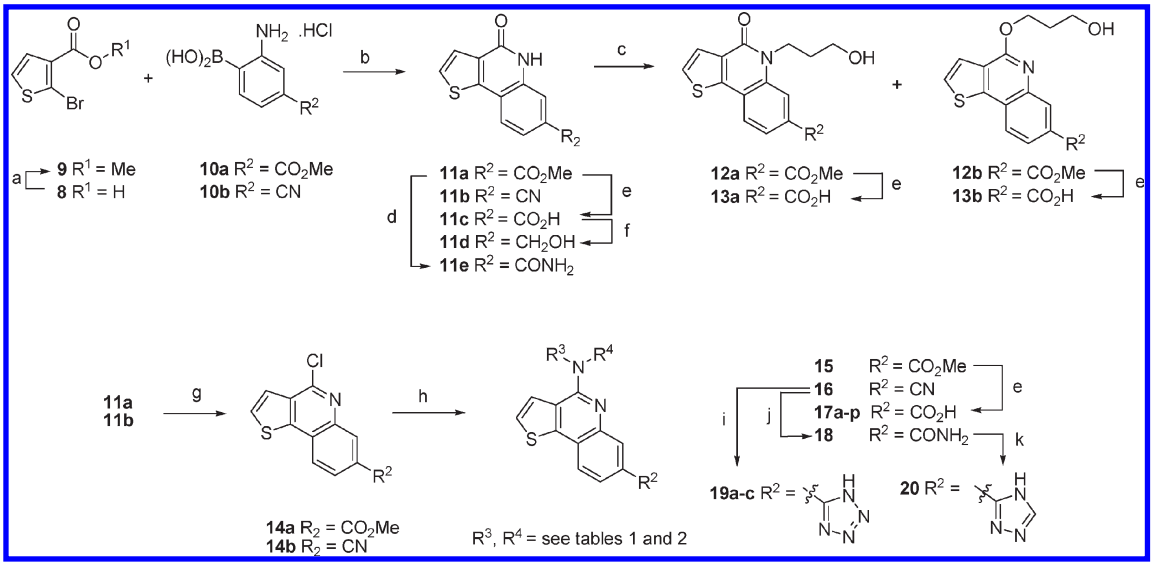


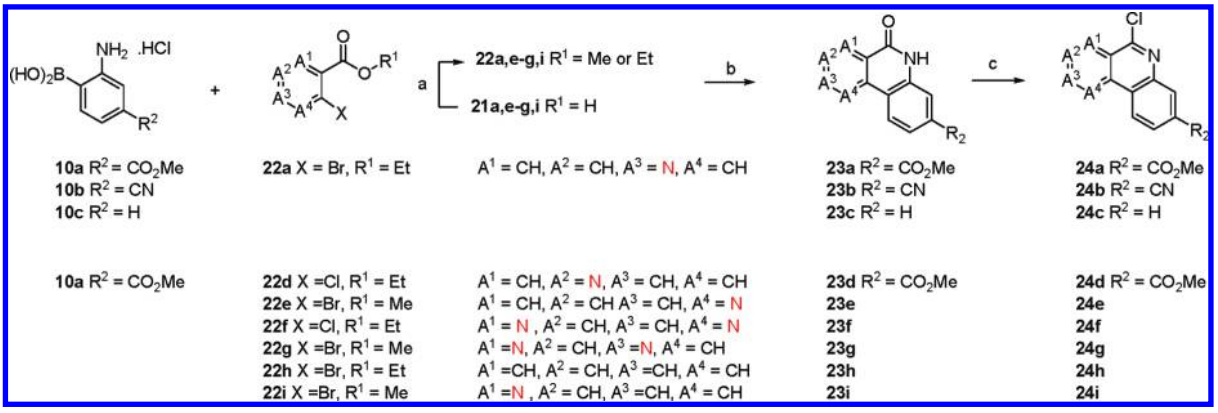
Figure 2. Structure of CK2 inhibitor **6**,[40] PARP inhibitor **7**,[49] and lead molecule **11c**.

Scheme 1[a]



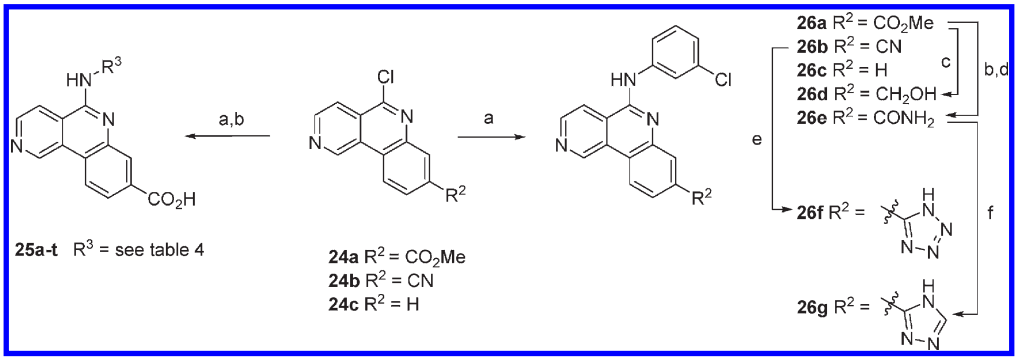
[a] Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, then MeOH; (b) NaOAc, $PdCl_2$(dppf), DMF, 120 °C; (c) K_2CO_3, 3-bromo-1-propanol, DMF, 100 °C; (d) NH_4OH, 100 °C; (e) LiOH, MeOH, THF, H_2O, rt; (f) $LiAlH_4$, dioxane, 100 °C; (g) $POCl_3$, $(i\text{-Pr})_2NEt$, toluene, reflux; (h) HNR^3R^4 (for structures of HNR^3R^4, see Tables 1 and 2), NMP, microwave 120 °C; (i) NaN_3, NH_4Cl, DMF, 120 °C; (j) H_2O_2, NaOH, NMP, 50 °C; (k) $(MeO)_2CHNMe_2$, 80 °C then AcOH, NH_2NH_2, 115 °C.

Scheme 2[a]



[a] Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, then R^1OH or H_2SO_4 (cat.), R^1OH, reflux; (b) NaOAc, $PdCl_2$(dppf), DMF, 120 °C or Cs_2CO_3, $PdCl_2$ (dppf), dioxane, 120 °C; (c) $POCl_3$ reflux.

Scheme 3[a]



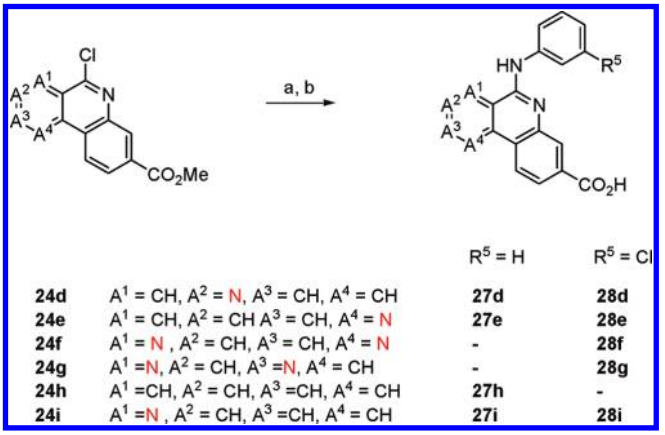
[a] Reagents and conditions: (a) R^3NH_2 (for structure of R^3NH_2, see Table 4) or 3-chloroaniline, NMP, microwave 120−160 °C or conventionnal heating at 80 °C; (b) NaOH, H_2O, EtOH, 50−80 °C; (c) $LiAlH_4$, THF, −40 °C to rt; (d) HOBt, EDCI, $(i\text{-Pr})_2NEt$, NH_4Cl, NMP, 70 °C; (e) NaN_3, NH_4Cl, DMF, 120 °C; (f) $(MeO)_2CHNMe_2$, 80 °C then AcOH, NH_2NH_2, 80 °C.

major (86%) component of the mixture being methyl 3-amino-6-iodo-2-methylbenzoate **30b** and the minor (14%) component being the desired methyl 3-amino-4-iodo-2-methylbenzoate **30a.** The desired minor product was isolated pure by flash chromatography on silica gel. The structure of this intermediate was unambiguously confirmed by its subsequent reactivity

leading to the synthesis of lactam **39a** (Scheme 6). The same chemistry tested on the major isomer **30b** led to a biaryl intermediate that failed to provide any lactam in the presence of NaHMDS. Methyl 5-amino-4-iodo-2-methylbenzoate **36** was the major isomer resulting from the iodination of methyl 5-amino-2-methylbenzoate **35** and could be easily isolated by chromatography. Compound **37** was commercially available, and the bromo aniline **32** was prepared in one step from the acid **31**.

Ortho-substituted halogeno-anilines **30a**, **32**, and **36**−**37** were coupled with boronic acid **38**[47,48] to yield 3-(*o*-aminophenyl)-pyridines intermediates (not shown) which was cyclized into **39a**−**d** in the presence of NaHMDS. Finally, conversions to molecules **41a**−**d** were carried out via chloroquinolines **40a**−**d** using the chemistry described above.

Scheme 4[a]

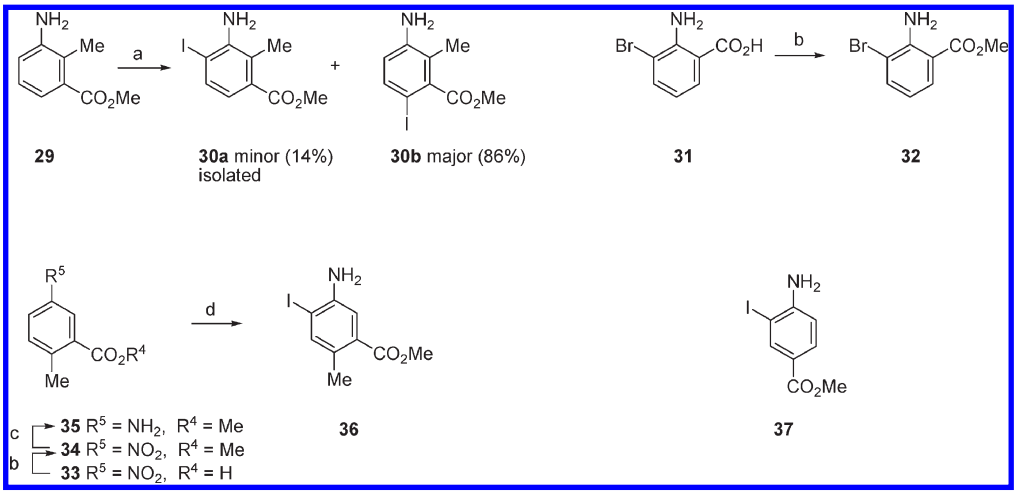


[a] Reagents and conditions: (a) H_2N-Ph-R^5, NMP, microwave 120−160 °C or conventionnal heating at 80 °C; (b) NaOH, H_2O, EtOH, 50−80 °C.

Results and Discussion

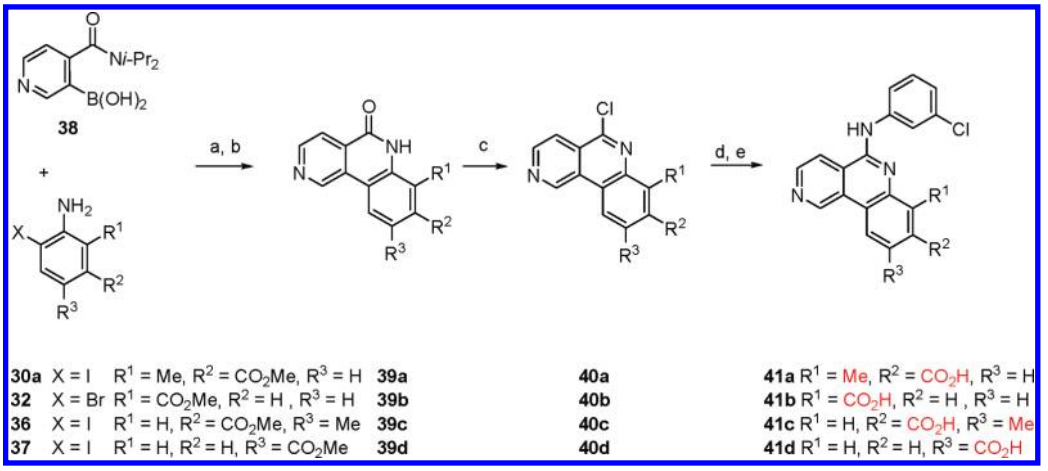
Structure-Based Design and Structure−Activity Relationship (SAR). During our initial structure-based design efforts to create a pharmaceutically suitable CK2 inhibitor, we noticed an obvious similarity between the CK2 inhibitor **6** (Figure 2)[22,40] and the poly(ADP-ribose) polymerase (PARP) inhibitor **7** developed by the same group and described elsewhere.[49] Examination of our own collection of PARP inhibitors as a potential source of novel CK2 inhibitors led us to

Scheme 5[a]



[a] Reagents and conditions: (a) NIS, AcOH, rt, minor isomer isolated by chromatography; (b) H_2SO_4 (cat.), MeOH, reflux; (c) H_2, Pd/C, MeOH; (d) NIS, AcOH, rt, major isomer isolated by chromatography.

Scheme 6[a]



[a] Reagents and conditions: (a) Cs_2CO_3, $PdCl_2$ (dppf), dioxane, 120 °C, workup; (b) NaHMDS, THF, rt; (c) $POCl_3$ reflux, (d) 3-chloroaniline, NMP, microwave 120 °C; (e) NaOH, H_2O, EtOH, 50−80 °C.

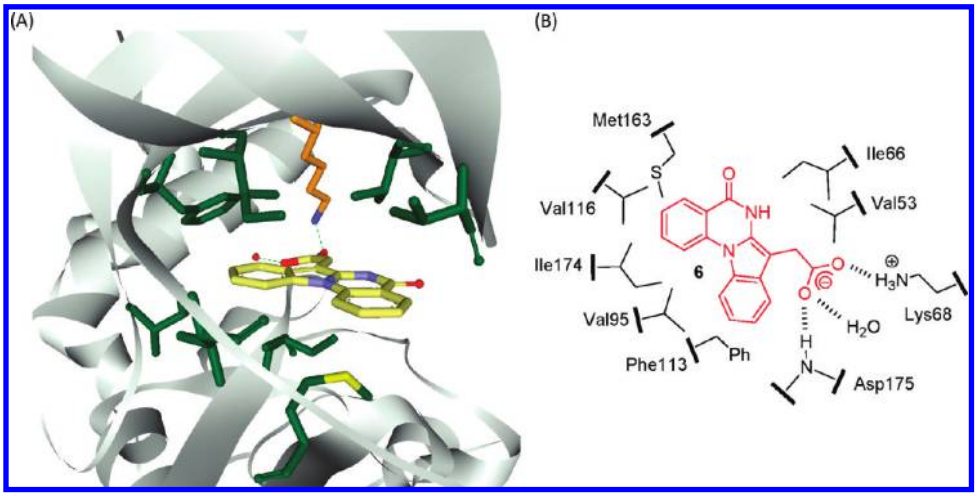
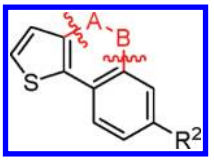


Figure 3. Crystal structure of **6** bound to maize CK2α (PDB ID 1MO1).[22] (A) 3D representation. Binding of **6** was largely determined by van der Waals contacts with several hydrophobic residues (in green) of the ATP-binding pocket (mainly Val45, Val53, Ile66, Val95, Phe113, Val116, Met163, and Ile174). The lactam −CONH− moiety of **6** was not directly involved in binding to the protein. (B) Schematic 2D view. The carboxylate, essential for activity, formed hydrogen bonds with Lys68, the backbone nitrogen of Asp175, and one water molecule.

Table 1. CK2 and PARP Inhibitory Activity of Thieno[3,2-*c*]quinoline Analogues **11c**−**e**, **13a**−**b**, and **17a**



Compds	A-B	R^2	PARP IC_{50}, μM	PARP % inh. at 1 μM	CK2 IC_{50}, μM
11c	-CO-NH-	CO_2H	0.70	58%	2.1
11e	-CO-NH-	$CONH_2$	0.06	-	>25
11d	-CO-NH-	CH_2OH	0.40	-	>25
13a	-CO-N($(CH_2)_3OH$)-	CO_2H	ND	21%	1.50
13b	-(C-O($(CH_2)_3OH$)=N-	CO_2H	ND	7%	0.99
17a	-(C-NH($(CH_2)_3OH$)=N-	CO_2H	ND	12%	0.75

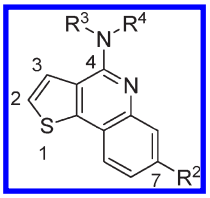
4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylic acid **11c** (Figure 2) because of its relatively close similarity with **6** and **7** and, in particular, because of its carboxylic acid group at a position roughly overlaying with that of **6**. Compound **11c** was tested in an enzymatic assay using recombinant CK2 along with eight other molecules from our library. Molecule **11c** was found to inhibit CK2 activity with an IC_{50} of 2.1 μM, quickly providing us with a unique lead amenable to further optimization.

The inhibitory activity of compounds bearing various substituents on R2, **11c**, **11e**, and **11d** (Table 1), suggested that modification of the carboxylic group on R2 offered little potential for increasing activity against CK2, a result consistent with the published SAR of **6**.[40] Replacement of the acid by a carboxamide in **11e** or an alcohol in **11d** suppressed CK2 activity while improving inhibition of PARP.

Most PARP inhibitors are designed to compete with the cofactor NAD^+, and therefore these generally display an essential aryl/heteroaryl substituted amide motif in their structure (highlighted in red in Figure 2), which strongly interacts with the PARP protein through three H-bonds.[49,50] A close examination of the published X-ray structure of **6** cocrystallized with CK2[22] (Figure 3) revealed that the lactam CONH motif of **6** did not form a H-bond with the CK2 protein. This led us to hypothesize that a chemical modification of the same lactam motif in **11c** could generate molecules with decreased PARP interaction and potentially provide improved selectivity for CK2. Compound **11a** (Scheme 1) was therefore subjected to chemical modifications to allow the preparation of analogues in which the lactam had been structurally modified.

The data summarized in Table 1 (compounds **11c**, **13a**−**b**, and **17a**) confirmed our hypothesis. O or N-substitution of the lactam decreased inhibition of PARP while preserving significant inhibition of CK2. Interestingly, substituted 4-aminothieno[3,2-*c*]quinoline-7-carboxylate **17a** displayed greater activity against CK2. The straightforward access to **17a** from the activated intermediate **14a** encouraged us to prepare more amino-substituted analogues **17b**−**p**, whose enzymatic activities are summarized in Table 2. Shorter hydroxy alkyl-substituted analogues **17b** showed moderate activity against CK2. Replacement of the hydroxy residue by a basic amine in

Table 2. CK2 Inhibitory Activity of Various C-4 and C-7 Substituted Thieno[3,2-*c*]quinoline Analogues



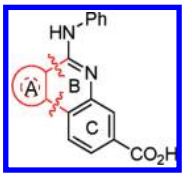
compd	$-NR^3R^4$	R^2	CK2 IC_{50}, μM
17b	$-NH-(CH_2)_2OH$	CO_2H	1.26
17c	$-NH-(CH_2)_2NMe_2$	CO_2H	0.102
17d	pyrrolidino	CO_2H	1.78
17e	−NH-phenyl	CO_2H	0.092
17f	−NMe-phenyl	CO_2H	1.07
17g	−NH-(2-Me-phenyl)	CO_2H	0.970
15	−NH-phenyl	CO_2Me	> 2.5
16	−NH-phenyl	CN	> 2.5
18	−NH-phenyl	$CONH_2$	> 1
19a	−NH-phenyl	*C*-(1*H*-tetrazol-5-yl)	0.096
20	−NH-phenyl	*C*-(4*H*-1,2,4-triazol-3-yl)	> 1
17h	$-NH-(CH_2)_2Ph$	CO_2H	0.516
17i	−NH-(4-F-phenyl)	CO_2H	0.219
17j	−NH-(3-F-phenyl)	CO_2H	0.068
17k	−NH-(4-Cl-phenyl)	CO_2H	0.178
17l	−NH-(3-Cl-phenyl)	CO_2H	0.032
17m	−NH-(3-MeO-phenyl)	CO_2H	0.077
17n	−NH-(3-acetylenyl-phenyl)	CO_2H	0.028
17o	−NH-(3-(PhO)-phenyl)	CO_2H	0.395
17p	−NH-(3-(CONHMe)-phenyl)	CO_2H	0.129
19b	−NH-(3-Cl-phenyl)	*C*-(1*H*-tetrazol-5-yl)	0.129
19c	−NH-(3-F-phenyl)	*C*-(1*H*-tetrazol-5-yl)	0.075

17c resulted in a 10-fold increase of inhibition. A similar boost was achieved with phenyl substituted compound **17e**, leading us into the potency range of **6**.[22,40] Introduction of a methyl group onto the aniline nitrogen (**17f**) or in the ortho position of the phenyl ring (**17g**) induced a notable loss of activity. This may be explained by a decreased planarity of the inhibitors, resulting in less favorable contact interactions within the small CK2 ATP binding site. This observation was also noticed on tertiary amine **17d**.

Various analogues lacking a carboxylate at C-7 (compounds **15**, **16**, **18**, and **20**) were inactive, revealing again the importance of an acidic function at that position. Tetrazole **19a** retained a similar activity, while its nonacidic triazole **20** counterpart was inactive, in accordance with the previous observation. Bulky moieties such as in **17h** and **17o** decreased activity, suggesting that C-4 substituents were interacting with a relatively small hydrophobic pocket. A number of substituted aniline analogues, bearing an acid (**17i**−**p**) or a tetrazole (**19b**−**c**) at C-7, were prepared (Table 2). Anilines substituted at the meta position by small hydrophobic groups showed the greatest inhibition of CK2, leading to the most potent analogues 3-chloroaniline **17l** (IC_{50} = 0.032 μM) and 3-acetylenylaniline **17n** (IC_{50} = 0.028 μM).

To improve potency, we considered replacing the thiophene ring with a six-membered ring, equivalent to the structural feature found in **6** (Figure 2). For that purpose, our chemistry was successfully extended to ethyl *ortho*-bromo benzoate **22h** (Scheme 2 and Scheme 4). CK2 assay on the resulting **27h** (Table 3) revealed a loss of activity when replacing the thiophene by a phenyl ring. On the basis of that observation, we began to explore analogues bearing nitrogen atoms embedded at various places in the A ring (compounds

Table 3. Effect of the A Ring on CK2 Inhibitory Activity



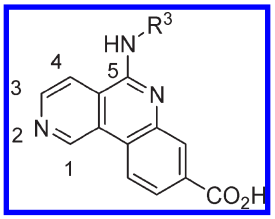
Compds	A ring	CK2 IC_{50}, μM
17e		0.092
27h		>1.0
27e		>0.50
25a		0.006
27d		>0.50
27i		>0.50

27e, **25a**, **27d**, and **27i**, Table 3), hypothesizing that we might pick up favorable H-bond interactions with the hinge region. This approach proved to be successful and resulted in the discovery of compound **25a**, which displayed a dramatic boost in activity (> 150-fold) over **27h** and a 15-fold increase in activity over thieno[3,2-*c*]quinoline-7-carboxylate **17e**.

Encouraged by these results and guided by the thieno[3,2-c]-quinoline-7-carboxylates SAR (Table 2), we prepared a focused collection of C-5 substituted analogues of **25a** whose activities are shown in Table 4 (compounds **25b**−**t**). All compounds were generally more potent inhibitors of CK2, and their IC_{50} values were 10- to 170-fold lower than their thiophene counterparts.

Compounds bearing small substituents on C-5 (**25d**) and various substituted aliphatic amino motifs (**25b**−**c** and **25e**−**f**) achieved good activity, with IC_{50} values ranging from 8 to 27 nM. Remarkably, this position of the molecule appeared to tolerate large extended phenyl substituted aliphatic amino groups (**25g**−**i**); the phenethylamino substituted analogue **25h** being the most potent of the series. Derivatives of anilines substituted by small hydrophobic groups in the meta position (**25j**−**l**, **25n**, and **25p**−**q**) again demonstrated the greatest inhibition of CK2, 3-chloroaniline **25n** being the most potent analogue of this novel class of CK2 inhibitors with an IC_{50} of 1 nM. Large substituents (**25r**, **25s**) or certain polar groups (**25t**) decreased the activity of the inhibitors by an order of magnitude. The 3-chloroaniline motif was conserved in subsequent molecules to complete the structure−activity relationship studies.

Table 4. CK2 Inhibitory Activity of C-5 Substituted Benzo[*c*]-[2,6]naphthyridine-8-carboxylate **25a**−**t**



compd	R^3	CK2 IC_{50}, μM
25a	−phenyl	0.006
25b	$-(CH_2)_2NMe_2$	0.025
25c	−cyclopentyl	0.027
25d	−OMe	0.008
25e	−cyclopropyl	0.016
25f	$-(CH_2)_2O$-*i*-Pr	0.011
25g	$-(CH_2)$phenyl	0.009
25h	$-(CH_2)_2$phenyl	0.003
25i	$-(CH_2)_3$phenyl	0.016
25j	−(3-MeO-phenyl)	0.004
25k	−(3-Cl, 4-F-phenyl)	0.004
25l	−(3-F-phenyl)	0.005
25m	−(2-Cl-phenyl)	0.008
25n	−(3-Cl-phenyl)	0.001
25o	−(4-Cl-phenyl)	0.007
25p	−(3-acetylenyl-phenyl)	0.003
25q	−(3-CN-phenyl)	0.004
25r	−(4-(PhO)-phenyl)	0.069
25s	−(3-(PhO)-phenyl)	0.019
25t	$-(3-(SO_2NH_2)$-phenyl)	0.043

The SAR of various 3-chloroanilines in Table 5 highlights the role of the A-ring and the C-ring substituents. Compounds **25n**, **26a**, and **26c**−**g** confirmed the essential role of the carboxylate on R2 for potent inhibition. Suppressing the carboxylate (**26c**) or replacing it by a small nonacidic group (**26g**−**26a**, **26d**−**e**) resulted in over 400-fold loss of activity. Acidic tetrazole **26f** retained activity but at a somewhat lower level.

Methyl groups adjacent to the carboxylate had a position dependent effect. On R1 (**41a**), a methyl group maintained activity at a potent level, while on R3 (**41c**), it suppressed activity, suggesting an unfavorable interaction with the protein at that position. Molecules **41d** and **41b**, in which the carboxylate was moved to adjacent carbons, were much less active, showing that its position on R2 was necessary for potent inhibition.

Molecules **28d**−**f** and **28i** confirmed the optimal position of the ring-A nitrogen atom on A3, implying a discrete interaction of the nitrogen with the protein. The potent activity of pyrimidine analogue **28g** further strengthened this observation; showing that an additional nitrogen atom within the ring did not negatively affect activity as far as a nitrogen atom was present in A3 for H-binding.

Binding Model. Compound **25n** was confirmed to be a potent, ATP-competitive inhibitor of CK2 with a $K_i = 0.38 \pm 0.02$ nM against the recombinant human holoenzyme ($\alpha\alpha\beta\beta$). Drawing on analysis of critical interactions and structural overlay with the numerous previously published and publically available structural studies,[22,51−55] we were able to build a binding model that was consistent with the SAR generated by the present CK2 program. No de novo docking or scoring was performed. Each molecule was subjected to in situ minimization using the CHARMm forcefield as implemented in Discovery Studio 2.5.1 from Accelrys, Inc., following manual placement of the compound. The model oriented the compounds within the ATP binding pocket of the kinase (the PDB structure 1JWH of human CK2α was used as the structural template[51]). Figure 4 shows the predicted binding of **25n** to CK2α. The binding model, which was continually revised to be consistent with information derived from recently published X-ray structures and the most recent in-house SAR data, was principally used to prioritize synthesis and rationalize CK2 inhibition data.

The structural similarity of these compounds to **6** suggested that the carboxylate group might bind in a similar orientation to **6**.[22] The SAR confirmed the importance of the carboxylate group for binding, so we predicted that the carboxylate of **25n** made analogous ionic interactions with Lys68.

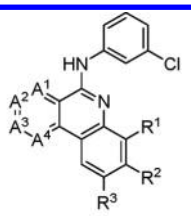
The mainly hydrophobic residues in the ATP binding site of CK2α create a small, flat envelope into which ATP and any inhibitor can bind, as outlined by previous works.[22,51−55] The model suggests that the flat tricyclic cores could make large areas of hydrophobic contacts with binding pocket residues. The flat, hydrophobic nature of the tricyclic core is likely to contribute strongly to the high degree of CK2 inhibition observed. The ring-A pyridine nitrogen of **25n** (Figure 4) is perfectly positioned to form a H-bond with the NH of Val116 in the hinge region, analogous to the interactions seen for ATP analogues. The presence of a hydrogen-bond acceptor in the preferred orientation can explain the increased activity of pyridine analogues. It became obvious from this model that displacement of the nitrogen or the carboxylate from their optimal positions resulted in a geometrically unfavorable configuration, translated experimentally by a major loss of activity in the CK2 assay (compounds **26c**, **41d**, **41b**, **28i**, and **28d**−**f** in Table 5). It is unclear why phenyl analogue **27h** is significantly less potent than thiophene **17e** (Table 3). We can speculate that it may be related to a greater affinity that sulfur-bearing thiophenes (and hence a reorientation to maximize the binding interaction) have for the sulfur group at Met163.

The three components of the interaction of **25n**, large surface hydrophobic interaction, ionic bridge with the Lys68 region, and H-bond with the hinge region, account for the very potent activity of the benzo[*c*][2,6]naphthyridine-8-carboxylate series. The 3-chloroaniline moiety has proved to maximize potency on both scaffolds, and the chloro group is likely to fit well within a small hydrophobic region, possibly in the glycine rich loop. Interestingly, the 3D conformation of the anilino moiety of our molecules overlay well with anilino substituents of some pyrazolo[1,5-*a*][1,3,5]triazines inhibitors, previously cocrystallized with CK2 and described by Nie et al.[41]

Finally, our model explains the loss of activity of R3-methyl substituted analogue **41c** (Table 5) by suggesting that the methyl group would clash with the gatekeeper residue phe113.

Kinase Selectivity Profile. Compound **25n** was screened at 0.5 μM against a panel of 238 kinases (Millipore) to assess its selectivity profile. At 0.5 μM, **25n** showed no major inhibition of the vast majority of tested kinases, including all isoforms of PI3K, Akt, PDK1, and mTOR. IC_{50} values were measured against the seven kinases for which it exhibited greater than 90% inhibition at 0.5 μM (Table 6). As anticipated, the molecule displayed an excellent selectivity profile, which is even more remarkable when considering the

Table 5. CK2 Inhibitory Activity of Several Chloroanilines Derivatives



Compds	A^1	A^2	A^3	A^4	R^1	R^2	R^3	CK2 IC_{50}, μM
25n	CH	CH	N	CH	H	CO_2H	H	0.001
26f	CH	CH	N	CH	H	*C*-(1*H*-tetrazol-5-yl)	H	0.045
26g	CH	CH	N	CH	H	*C*-(4*H*-1,2,4-triazol-3-yl)	H	>0.5
26a	CH	CH	N	CH	H	CO_2Me	H	>0.5
26d	CH	CH	N	CH	H	CH_2OH	H	>0.5
26e	CH	CH	N	CH	H	$CONH_2$	H	0.417
26c	CH	CH	N	CH	H	H	H	>0.5
41a	CH	CH	N	CH	Me	CO_2H	H	0.006
41c	CH	CH	N	CH	H	CO_2H	Me	>0.5
41d	CH	CH	N	CH	H	H	CO_2H	0.350
41b	CH	CH	N	CH	CO_2H	H	H	>0.5
28i	N	CH	CH	CH	H	CO_2H	H	>0.5
28d	CH	N	CH	CH	H	CO_2H	H	>0.5
28e	CH	CH	CH	N	H	CO_2H	H	>0.5
28f	N	CH	CH	N	H	CO_2H	H	0.220
28g	N	CH	N	CH	H	CO_2H	H	0.007

low molecular weight (349.8 g/mol) of the molecule. In addition, **25n** was evaluated at a concentration of 10 μM in relevant cell-based assays for FLT3, PIM1, and CDK1 and was found to be functionally inactive against these kinases.[56]

Cellular Characterization. Several of the most potent CK2 inhibitors were tested for their inhibition of the proliferation of various cancer cell lines (Table 7). The molecules were active against a broad range of cell types, including breast, lung, and prostate cancers in which CK2 has been previously shown to be overexpressed. The cellular biology of the potent inhibitor **25n** was further investigated. Compound **25n** was found to potently inhibit endogenous intracellular CK2 activity with an IC_{50} of 0.1 μM in Jurkat cells (Figure 5). The differences between IC_{50} values of **25n** in the kinase enzyme assay relative to the cell proliferation assay may be due to multiple factors. However, the greatest contributing factor is likely that the ATP concentration in cells (1−10 mM) is far higher than the ATP concentration (15 μM) used in molecular screening assays. Thus, one would expect that higher concentration of ATP-competitive **25n** is needed to reach the same level of CK2 inhibition in cells.

Additional data demonstrating a CK2-inhibition based antiproliferative and antiangiogenic effect of **25n** will be fully discussed elsewhere.[56] Briefly, in various cell lines, CK2 inhibition was confirmed by measuring the phosphorylation level of the CK2 specific phosphorylation site on Akt(S129). **25n** induced dephosphorylation of Akt(S129)[57] and a rapid dephosphorylation of the Akt substrate p21(T145). Compound **25n** induced apoptosis in multiple cancer cell lines as measured by concentration-dependent induction of caspase 3/7 activity. Normal cells failed to show a detectable change in caspase 3/7 activation within the concentration range tested, indicating that cancer cells might be more vulnerable to **25n** than normal cells.[56]

Pharmacokinetics and Safety. The pharmacokinetic properties of **25n** were evaluated in multiple species including mouse, rat, and dog (Table 8). The drug exhibited low to moderate clearance and a wide range of volume of distribution across species. Terminal half-life ranged between 5 and 12 h across species, and oral bioavailability was moderate in all species ranging between 20 and 51%. Furthermore, **25n** showed minimal inhibition of five of the principal cytochrome P450 isoforms (CYP1A2, CYP2C9, CY2C19, CYP2D6, and CYP3A4).[58] Also, there was no significant inhibition of the hERG channel by this compound (< 10% inhibition at 0.1, 1.0, and 10.0 μM) in the patch clamp assay,[59] and the molecule was considered nonmutagenic based on the Ames assay and also deemed nongenotoxic in the mouse micronucleus test.

Efficacy in Tumor Xenograft Studies. Compound **25n** was selected for in vivo efficacy in established human prostate PC3 xenograft model in athymic mice (Figure 6). Mice bearing subcutaneous PC3 tumors were treated with **25n** (25 mg/kg, 50 mg/kg, and 75 mg/kg, po, bid). **25n** demonstrated tumor growth inhibition (TGI = 19%, 40%, and

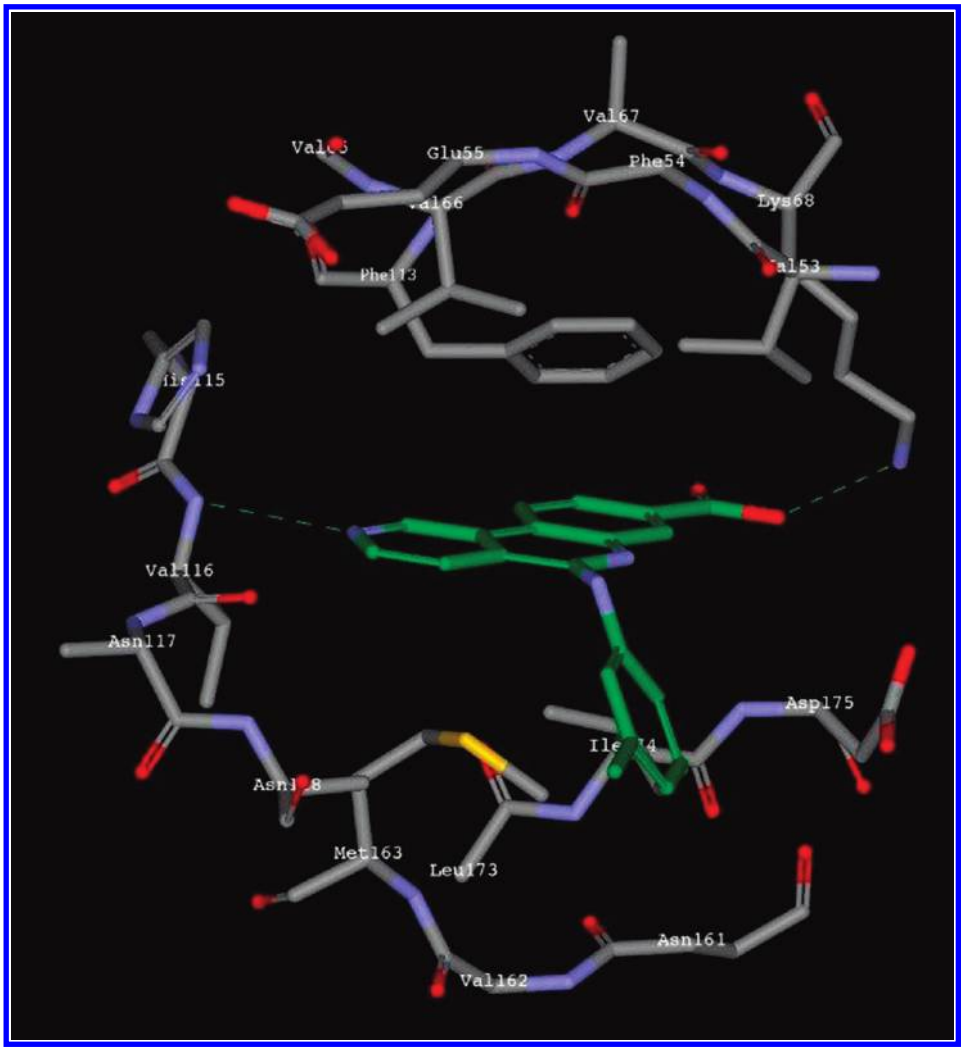


Figure 4. Binding model of **25n** (in green) in the ATP-pocket of CK2.

Table 6. IC_{50} Determination for **25n** against Several Kinases

kinase	CK2α	CK2α′	DAPK3	FLT3	TBK1	CLK3	HIPK3	PIM1	CDK1/cyclinB
IC_{50}, nM	1	1	17	35	35	41	45	46	56

Table 7. Cell Antiproliferative Activity of Derivatives for Selected Benzo[*c*][2,6]naphthyridine-8-carboxylate Analogues

		cell viability IC_{50}, μM												
		prostate		pancreatic		lung		leukemia		breast			colorectal	melanoma
compd	CK2 IC_{50}, μM	PC3	LNCaP	BxPC3	MIA PaCa-2	A549	H1299	Jurkat	K-562	MCF7	Hs578T	MDA-MB-231	HCT-116	A375
25n	0.001	2.1	4.7	4.4	1.1	3.0	2.4	2.5	5.3	8.9	13.1	6.4	2.2	3.9
25p	0.003	16.8	17.1	3.7	3.9	30.2	3.7	2.8	3.3	1.4	4.0	11.8	10.8	7.4
25h	0.003	23.6	8.6	ND	5.2	47.0	21.0	11.1	ND	14.7	16.5	ND	13.2	ND
25q	0.004	26.4	15.5	3.6	> 10	> 50	ND	22.0	9.2	41.0	35.6	28.7	> 10	ND
25k	0.004	4.9	4.6	7.7	3.6	6.8	3.1	2.7	5.0	8.2	3.3	6.1	6.0	3.2
25j	0.004	17.4	ND	ND	8.5	ND	10.7	6.5	5.9	ND	12.9	ND	9.5	ND
25l	0.005	9.2	ND	ND	6.0	16.7	8.3	3.2	> 10	ND	5.9	ND	7.6	ND

86%, respectively) compared to vehicle treated control, and a dose responsive efficacy was observed (Figure 6). Last, **25n** was well tolerated in mice as assessed by minimal changes in body weight during the course of treatment compared to vehicle control. Other efficacy studies in various models further confirmed the tolerability of the drug and will be reported elsewhere.[56]

Conclusion

We have discovered a novel family of ATP-competitive inhibitors of CK2 using both structure- and chemistry-based optimization. This work culminated in the discovery of **25n**, a potent and selective inhibitor of CK2 that is efficacious in animal models of cancer. The SAR information acquired

during the lead optimization of this new class of drugs has been used to identify key structural elements necessary to achieve potent CK2 inhibition. As a result, we have established a binding model that rationalizes the multiple components of the strong interaction of **25n** with the ATP binding pocket, providing valuable information for the design of future classes of small molecule CK2 inhibitors.

While being beneficial for selectivity, the small size of the cofactor site of CK2 also predisposes for the discovery of hydrophobic inhibitors with poor drug-like properties. Remarkably, **25n** attained a favorable balance between potency and pharmacokinetics, a challenging achievement that we believe to be atypical with small molecule CK2 inhibitors.

The complexity of the regulation of CK2, its pleiotropy, and the wide distribution of the protein has raised the question of whether CK2 inhibitors can selectively kill cancer cells while sparing normal cells. We have shown that **25n** was efficacious in xenograft models of cancer and remained very well tolerated. Compound **25n** (CX-4945) demonstrated an acceptable pharmaceutical profile and is currently being evaluated clinically as a first-in-class CK2 inhibitor for the treatment of cancer.[60]

Beyond cancer, CK2 plays multiple roles in inflammation disorders, neurodegenerative diseases, and viral or parasitic infections.[5] The discovery of **25n** and the human pharmacology resulting from the modulation of CK2 may open the door to the discovery of new treatments for diseases in which this protein is dysregulated.

Experimental Section

Chemistry. General Methods. All solvents and reagents, unless otherwise stated, were reagent grade, commercially available, and used without further purification. All experiments using moisture sensitive reagents were carried out under a dry nitrogen atmosphere. Microwave irradiation was carried out in a CEM Discover series microwave reactor with the Explorer autosampler. Thin layer chromatography was performed Watman Partisil K6F 60 Å silica gel plates (250 μm for analytical or 20 cm × 20 cm, 1000 μm for preparative) with fluorescent indicator and visualized with 254 nm UV light. Flash column chromatography was conducted either under medium pressure using Watman 60 Å, 230−400 mesh ASTM bulk silica gel or on prepacked silica gel cartridges (Biotage). Reverse phase HPLC purification was conducted utilizing mass directed fraction collection employing a Waters Micromass ZQ mass spectrometer with an electrospray probe. Fraction collection was achieved with a Waters 2767 fraction collector driven by a Waters 2525 binary pump. Fractions were collected by mass, UV signal (Waters 2487 dual wave) or a combination of both. The stationary phase is a Phenomenex Luna 5 μm C18 100 Å Axia 21.2 mm × 50 mm column with a mobile phase gradient of 25−95% CH_3CN or MeOH in H_2O with 0.1% TFA. Compounds were generally isolated after preparative HPLC by evaporation of the solvents using a Genevac DD-4 evaporator with a fixed rotor. NMR spectra were recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 on a Varian Mercury 400 with an AutoX DB PFG probe and Protune accessory operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Trimethyl silane (TMS) was used as internal reference. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) in hertz (Hz). The following abbreviations are used for multiplicities: s = singlet, br = broad, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, q = quadruplet, qi = quintuplet, sext = sextuplet. Unless otherwise specified, all final compounds were homogeneous with a purity of ≥95%, as determined by LCMS analysis conducted on a Waters 2695 HPLC/MS system with Micromass ZQ and 2487 UV detectors using a gradient of 10−90% 0.05% TFA in ACN and 0.1% TFA in DI water.

5-Bromopyrimidine-4-carboxylic acid **21g** was prepared according to a procedure previously described in a patent.[61] 4-(Diisopropylcarbamoyl)pyridin-3-ylboronic acid **38** was prepared using a published procedure.[47,48] Compounds **8**, **10a−c**, **21a**, **21e−g**, **21i**, **22d**, **22h**, **29**, **31**, **33**, **37**, and the various amines

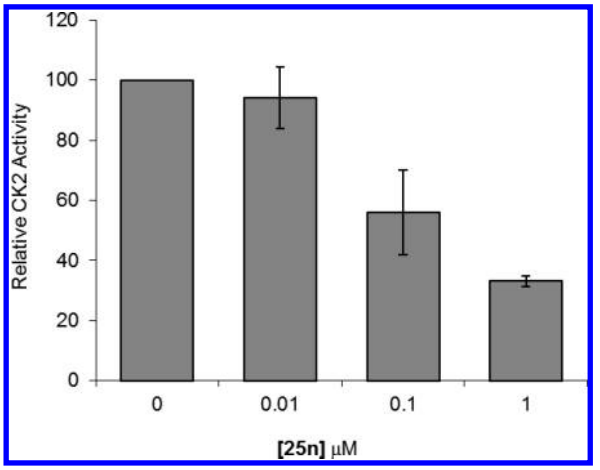


Figure 5. Endogenous CK2 activity of **25n** in Jurkat cells.

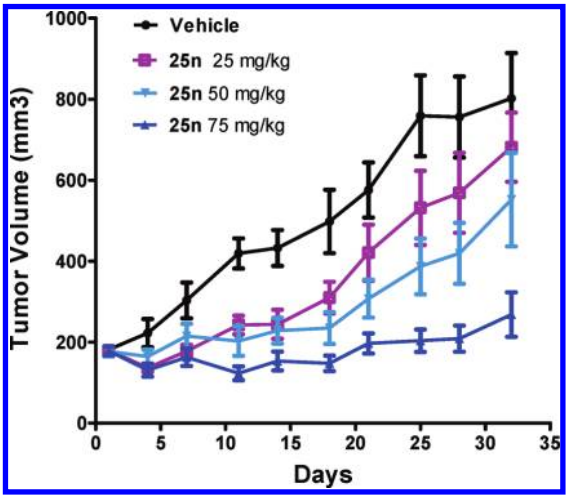


Figure 6. In vivo growth inhibitory effect of **25n** on human prostate cancer PC3 xenografts in nude mice. Compound **25n** dosed bid at 25 mg/kg (tumor growth inhibition TGI = 19%), 50 mg/kg (TGI = 40%), and 75 mg/kg (TGI = 86%).

Table 8. Pharmacokinetic Parameters for Compound **25n**

species (n = 3)	iv mg/kg	po mg/kg	Cls[a] L/h/kg	Vss L/kg	$T_{1/2}$ Hr	po C_{max} ng/mL	po AUC ng·h/mL	%F
mouse[b]	5[c]	25[c]	2.5	18.1	5.0	311	2009	20
rat	5.2[c]	10.5[c]	0.1	2.0	11.6	7369	42343	48
dog	1.9[c]	8.2[d]	0.7	8.9	8.3	2178	5646	51

[a] Abbreviations: Cls, clearance; Vss, volume of distribution at steady state; $T_{1/2}$, elimination half-life; C_{max}, maximum concentration; AUC, area under the curve; %F, bioavailability. [b] (n = 4). [c] The sodium salt of **25n** was administered in a 25 mM sodium phosphate buffer. [d] Dogs received PO dose via **25n** sodium salt filled capsules.

used to prepare analogues described in Tables 1−5 are commercially available.

Methyl 2-Bromothiophene-3-carboxylate (9). 2-Bromo-3-thiophene carboxylic acid **8** (1.0 equiv, 12.56 g, 60.66 mmol) was suspended in CH_2Cl_2 (200 mL). Oxalyl chloride (1.1 equiv, 5.9 mL, 67.16 mmol) and 5 drops of DMF were added, inducing formation of gas. The mixture was stirred overnight at room temperature, and the volatiles were removed in vacuo. The resulting solid was suspended in dry methanol (150 mL) and the mixture heated to reflux. Evaporation of the solvents afforded methyl 2-bromo-3-thiophene carboxylate **9** (13.16 g, 98%) as a crude brown oil. LCMS (ES): >95% pure, *m*/*z* not detected. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H), 7.23 (d, J = 5.6, 1H), 7.56 (d, J = 5.6, 1H) ppm.

Methyl 4-Oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate (11a). In a microwave vial, methyl 2-bromo-3-thiophene carboxylate **9** (1.0 equiv, 260 mg, 1.18 mmol), 2-amino-4-(methoxycarbonyl)phenylboronic acid hydrochloride **10a** (1.1 equiv, 300 mg, 1.30 mmol), sodium acetate (3.0 equiv, 292 mg, 3.56 mmol), and $PdCl_2$ (dppf) (0.05 equiv, 31 mg, 0.059 mmol) were mixed together in anhydrous DMF (2 mL). The mixture was heated in a microwave oven at 120 °C for 10 min. Water was added and the solid filtered and dried. The material was suspended in dichloromethane, filtered, and dried to afford methyl 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **11a** as a yellow solid (152 mg, 50%). LCMS (ES): 95% pure, *m*/*z* 260 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3. 99 (s, 3H), 7.54 (d, J = 5.2, 1H), 7.79 (d, J = 4.8, 1H), 7.86 (d, J = 8.4, 1H), 7.91 (dd, J = 8.4, J = 1.6, 1H), 8.03 (d, J = 1.2, 1H) ppm.

4-Oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylic Acid (11c). Methyl 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **11a** (1.0 equiv, 618 mg, 2.38 mmol) was suspended in 10 mL of a mixture of methanol, THF, and water (1:1:1, v:v:v). LiOH (2.0 equiv, 114 mg, 4.76 mmol) was added, and the mixture was stirred at room temperature for 2 h. An additional amount of LiOH (114 mg) was added, and the mixture was stirred for an hour. LiOH (50 mg) was added and the mixture stirred for an additional 2 h. Water was added and the solution filtered through a pad of Celite. The pad of Celite was thoroughly washed with aqueous 1 N NaOH. The solution was acidified with 6 N aqueous hydrochloric acid to induce precipitation of the expected material. Filtration and drying afforded **11c** as a yellow solid (562 mg, 96%). LCMS (ES): 95% pure, *m*/*z* 246 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.61 (d, J = 5.2, 1H), 7.73 (dd, J = 1.6, J = 8.0, 1H), 7.88 (d, J = 5.6, 1H), 7.92 (d, J = 8.4, 1H), 8.02 (d, J = 1.6, 1H), 11.92 (s, 1H), 13.21 (br s, 1H) ppm.

7-(Hydroxymethyl)thieno[3,2-*c*]quinolin-4(5*H*)-one (11d). 4-Oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylic acid **11c** (1.0 equiv, 38 mg, 0.155 mmol) was suspended in dioxane (1 mL). $LiAlH_4$ (7.0 equiv, 40 mg, 1.05 mmol) was added and the mixture stirred at 100 °C for 45 min. Water was added, then methanol and CH_2Cl_2. The solid salts were filtered off and washed with methanol and CH_2Cl_2. After evaporation of the volatiles in vacuo, the material was dissolved in a mixture of NMP, methanol and water and was purified by preparative HPLC. Genevac evaporation afforded 7-(hydroxymethyl)thieno[3,2-*c*]quinolin-4(5*H*)-one **11d** as an off-white solid (12 mg, 34%). LCMS (ES): 95% pure, *m*/*z* 232 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.56 (s, 2H), 7.15 (d, J = 7.6, 1H), 7.39 (br s, 1H), 7.55 (d, J = 5.2, 1H), 7.73 (d, J = 5.2, 1H), 7.76 (d, J = 8.0, 1H), 11.73 (s, 1H) ppm.

4-Oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxamide (11e). In a closed vessel, methyl 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **11a** (44 mg, 0.170 mmol) was suspended in concentrated aqueous ammonia (1 mL). The mixture was stirred at 100 °C overnight. Aqueous 1N NaOH was added and the mixture stirred at room temperature for 2 h. The solid was filtered and dried to provide 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxamide **11e** as a brown solid (13 mg, 32%). LCMS (ES): 95% pure, *m*/*z* 245 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.51 (br s, 1H), 7.62 (d, J = 4.8, 1H), 7.70 (d, J = 8.4, 1H), 7.87 (d, J = 5.2, 1H), 7.92 (m, 2H), 8.11 (br s, 1H), 11.89 (br s, 1H) ppm.

4-Oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carbonitrile (11b). In a microwave vial, methyl 2-bromo-3-thiophene carboxylate **9** (1.0 equiv, 250 mg, 1.13 mmol), 2-amino-3-cyanophenyl boronic acid hydrochloric acid **10b** (1.1 equiv, 250 mg, 1.24 mmol), sodium acetate (3.0 equiv, 278 mg, 3.39 mmol), and $PdCl_2$ (dppf) (0.007 equiv, 4.3 mg, 0.0082 mmol) were mixed together in anhydrous DMF (2.5 mL). The mixture was heated in a microwave oven at 120 °C for 10 min. Water was added and the material extracted with CH_2Cl_2. The organic extracts were washed with brine, dried over Na_2SO_4, and the solvents removed in vacuo. The resulting solid was sonicated in EtOAc, filtered, and dried to afford 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carbonitrile **11b** as a tan solid (121 mg, 48%). LCMS (ES): 95% pure, *m*/*z* 227 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.65 (m, 2H), 7.75 (br s, 1H), 7.97 (d, J = 5.2, 1H), 8.06 (d, J = 8.4, 1H), 12.03 (br s, 1H) ppm.

Methyl 4-(3-Hydroxypropoxy)thieno[3,2-*c*]quinoline-7-carboxylate (12b) and Methyl 5-(3-Hydroxypropyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate (12a). Methyl 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **11a** (1.0 equiv, 18 mg, 0.069 mmol) was dissolved in anhydrous DMF (0.4 mL). K_2CO_3 (7.0 equiv, 70 mg, 0.506 mmol) and 3-bromo-1-propanol (16 equiv, 100 μL, 1.144 mmol) were added and the mixture stirred at 100 °C for 1.5 h. After adding water, the mixture was extracted with CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and the solvents removed in vacuo. Compounds **12a** and **12b** were separated by preparative TLC on silica gel (eluted twice with 30% EtOAc in hexanes, then once with 50% EtOAc in hexanes). The less polar compound was identified as methyl 4-(3-hydroxypropoxy)thieno[3,2-*c*]quinoline-7-carboxylate **12b** (12 mg). TLC (50% EtOAc/hexanes) R_f = 0.5. LCMS (ES): 80% pure, *m*/*z* 318 $[M + H]^+$. The more polar compound was identified as methyl 5-(3-hydroxypropyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **12a** (19 mg). TLC (50% EtOAc/hexanes) R_f = 0.2. LCMS (ES): 80% pure, *m*/*z* 318 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.08 (qi, J = 6.0, 2H), 3.61 (t, J = 5.2, 2H), 4.62 (t, J = 6.0, 2H), 7.53 (d, J = 5.2, 1H), 7.77 (d, J = 5.2, 1H), 7.93 (d, J = 8.0, 1H), 7.99 (dd, J = 1.2, J = 8.4, 1H), 8.26 (d, J = 0.8, 1H) ppm. Structural assignement was based on NMR spectra of their respective carboxylic acids. The two compounds were used for the following step without any further purification.

5-(3-Hydroxypropyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylic Acid (13a). Methyl 5-(3-hydroxypropyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **12a** (1.0 equiv, 19 mg, 0.060 mmol) was dissolved in a 1:1:1 mixture of THF, methanol, and water (0.5 mL). LiOH (40 mg) was added and the resulting mixture stirred at room temperature for 1.5 h. Water, methanol, and hydrochloric acid were added and the solution purified by preparative HPLC. Genevac evaporation afforded 5-(3-hydroxypropyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylic acid **13a** as a white solid (4 mg, 22%). LCMS (ES): 95% pure, *m*/*z* 304 $[M + H]^+$. ^{1}H NMR ($CDCl_3$/CD_3OD, 9:1, 400 MHz): δ 2.08 (qi, J = 6.0, 2H), 3.61 (t, J = 5.2, 2H), 4.62 (t, J = 6.0, 2H), 7.53 (d, J = 5.2, 1H), 7.77 (d, J = 5.2, 1H), 7.93 (d, J = 8.0, 1H), 7.99 (dd, J = 1.2, J = 8.4, 1H), 8.26 (d, J = 0.8, 1H) ppm.

4-(3-Hydroxypropoxy)thieno[3,2-*c*]quinoline-7-carboxylic Acid (13b). The title compound was prepared according to the procedure used to prepare compound **13a** starting from methyl 4-(3-hydroxypropoxy)thieno[3,2-*c*]quinoline-7-carboxylate **12b**. The title compound **13b** was isolated as a solid (3 mg, 26%). LCMS (ES): 95% pure, *m*/*z* 304 $[M + H]^+$. ^{1}H NMR ($CDCl_3$/CD_3OD, 9:1, 400 MHz): δ 2.04 (qi, J = 6.0, 2H), 3.68 (t, J = 6.0, 2H), 4.69 (t, J = 6.4, 2H), 7.52 (d, J = 4.8, 1H), 7.54 (dd, J = 5.2, J = 1.6, 1H), 7.93 (d, J = 8.4, 1H), 7.97 (dd, J = 7.6, J = 0.8, 1H), 8.49 (d, J = 0.8, 1H) ppm.

4-Chlorothieno[3,2-*c*]quinoline-7-carboxylate (14a). Methyl 4-oxo-4,5-dihydrothieno[3,2-*c*]quinoline-7-carboxylate **11a** (1.0 equiv, 1.50 g, 5.79 mmol) was suspended in dry toluene (15 mL). Phosphorus oxychloride (1.2 equiv, 0.64 mL, 6.99 mmol) and

DIEA (0.8 equiv, 0.81 mL, 4.65 mmol) were added and the mixture vigorously stirred at 120 °C for 3 h under nitrogen atmosphere. The mixture was hydrolyzed by addition of ice and water. The compound was extracted with CH_2Cl_2 (4×). The combined extracts were dried over Na_2SO_4 and the black solution filtered through a pad of Celite. After evaporation of the volatiles in vacuo, the resulting solid was triturated in a mixture of EtOAc and hexanes. Filtration and drying provided methyl 4-chlorothieno[3,2-*c*]quinoline-7-carboxylate **14a** as a yellow fluffy solid (1.14 g, 71%). LCMS (ES): 95% pure, *m*/*z* 278 $[M + H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ 4.01 (s, 3H), 7.72 (d, J = 4.8, 1H), 7.74 (d, J = 5.2, 1H), 8.14 (d, J = 8.4, 1H), 8.25 (d, J = 8.4, 1H), 8.85 (d, J = 1.6, 1H) ppm.

4-Chlorothieno[3,2-*c*]quinoline-7-carbonitrile (14b). The title compound was prepared according to the procedure used to prepare **14a** starting from 4-chlorothieno[3,2-*c*]quinoline-7-carbonitrile **11b** (1.00 g, 4.42 mmol). The title compound **14b** was isolated as a brown solid (833 mg, 77%). LCMS (ES): 95% pure, *m*/*z* 245 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.80 (d, J = 4.8, 1H), 8.04 (br d, J = 7.2, 1H), 8.27 (d, J = 5.2, 1H), 8.44 (d, J = 8.4, 1H), 8.63 (br s, 1H) ppm.

Methyl 4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carboxylate (15). Methyl 4-chlorothieno[3,2-*c*]quinoline-7-carboxylate **14a** (1.0 equiv, 60 mg, 0.216 mmol) was mixed with aniline (10.1 equiv, 0.2 mL, 2.19 mmol) and NMP (0.2 mL). The mixture was reacted in a microwave oven at 120 °C for 10 min. Water was added, and the product was extracted with dichloromethane. The organic phase was dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was triturated with dichloromethane, EtOAc, and hexanes to give a solid. After filtration and drying, the title compound **15** was isolated as a brown solid. (56 mg, 81%). LCMS (ES): 95% pure, *m*/*z* 335 $[M + H]^+$.

4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carbonitrile (16). 4-Chlorothieno[3,2-*c*]quinoline-7-carbonitrile **14b** (1.0 equiv, 130 mg, 0.533 mmol), aniline (0.2 mL), and NMP (0.2 mL) were mixed in a microwave vessel. The mixture was heated in a microwave oven at 120 °C for 10 min. Water was added, and the resulting solid was filtered and dried. 4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carbonitrile **16** was isolated as a gray solid (132 mg, 83%). LCMS (ES): 95% pure, *m*/*z* 302 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.07 (t, J = 7.2, 1H), 7.38 (td, J = 7.6, J = 2.0, 2H), 7.66 (dd, J = 8.0, J = 1.6, 1H), 8.08 (dd, J = 8.8, J = 1.2, 2H), 8.11 (d, J = 4.8, 1H), 8.14 (d, J = 8.4, 1H), 8.19 (d, J = 1.6, 1H), 8.26 (d, J = 5.2, 1H), 9.40 (s, 1H) ppm.

4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17e). Methyl 4-(phenylamino)thieno[3,2-*c*]quinoline-7-carboxylate **15** (15 mg, 0.045 mmol) was reacted in a mixture of THF (0.5 mL), methanol (0.5 mL), water (0.5 mL), and lithium hydroxide (60 mg) at room temperature overnight. Water was added and the mixture acidified by addition of hydrochloric acid. The volatiles solvents were removed in vacuo, and the resulting solid was filtered and dried. The title compound **17e** was isolated as an off-white solid (6 mg, 42%). LCMS (ES): >95% pure, *m*/*z* 321 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.28 (br s, 1H), 7.51 (t, J = 6.8, 2H), 7.87 (br s, 2H), 7.93 (d, J = 8.8, 1H), 8.16 (m, 2H), 8.35 (d, J = 5.2, 1H), 8.47 (br s, 1H) ppm.

General Procedure for the Synthesis of Compounds 17a−d and 17f−p. Synthesis of 4-(3-Hydroxypropylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17a). Methyl 4-chlorothieno[3,2-*c*]-quinoline-7-carboxylate **14a** (7 mg, 0.025 mmol) was mixed with 3-amino-1-propanol (0.1 mL) and NMP (0.1 mL). The mixture was reacted in a microwave oven at 100 °C for 5 min. The mixture was diluted with NMP and purified by preparative HPLC. After evaporation of the solvents in vacuo, the resulting ester was reacted with lithium hydroxide (40 mg, 1.67 mmol) in a 1:1:1 mixture of THF/methanol/water (0.5 mL) at room temperature for 2 h. The resulting mixture was diluted and the compound purified by preparative HPLC. After evaporation of the solvents in vacuo, the title compound **17a** was isolated as a white solid (4.6 mg, 61%). Alternatively, materials could be isolated in a pure form after dilution of the mixture, acidification by hydrochloric acid and filtration of the resulting precipitate. LCMS (ES): >95% pure, *m*/*z* 303 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 1.89 (qi, J = 6.4, 2H), 3.56 (t, J = 6.4, 2H), 3.71 (q, J = 6.0, 2H), 7.86 (br s, 1H), 8.06 (br s, 3H), 8.40 (br s, 1H) ppm.

4-(2-Hydroxyethylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17b). Yield, 21%. LCMS (ES): >95% pure, *m*/*z* 289 $[M + H]^+$.

4-(2-(Dimethylamino)ethylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid, Trifluoroacetate Salt (17c). After preparative HPLC, the material was isolated as a TFA salt. Yield, 57%. LCMS (ES): >95% pure, *m*/*z* 316 $[M + H]^+$, 271 $[M + H - NHMe_2]^+$.

4-(Pyrrolidin-1-yl)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17d). Yield, 47%. LCMS (ES): >95% pure, *m*/*z* 299 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 2.11 (br s, 4H), 3.95 (br s, 2H), 4.20 (br s, 2H), 7.95 (br s, 1H), 8.18 (br s, 3H), 8.82 (br s, 1H), 12.0 (br s, 1H) ppm.

4-(Methyl(phenyl)amino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17f). Yield, 75%. LCMS (ES): >95% pure, *m*/*z* 335 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 3.68 (s, 3H), 6.12 (d, J = 5, 1H), 7.38 (m, 3H), 7.49 (m, 2H), 7.73 (d, J = 5.2, 1H), 7.95 (d, J = 8.8, 1H), 8.15 (d, J = 8.8, 1H), 8.55 (br s, 1H) ppm.

4-(*o*-Tolylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17g). Yield, 90%. LCMS (ES): >95% pure, *m*/*z* 335 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 2.31 (s, 3H), 7.44 (br s, 2H), 7.50 (br s, 1H), 7.55 (m, 1H), 7.98 (br m, 1H), 8.24 (br m, 2H), 8.40 (br s, 1H), 8.56 (br s, 1H) ppm.

4-(Phenethylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17h). Yield, 56%. LCMS (ES): >95% pure, *m*/*z* 349 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 3.12 (t, J = 7.6, 2H), 4.01 (br s, 2H) 7.23 (t, J = 6.8, 1H), 7.33 (t, J = 7.6, 2H), 7.39 (d, J = 7.6, 2H), 7.98 (br m, 1H), 8.18 (br m, 2H), 8.34 (br s, 1H), 8.78 (br s, 1H), 10.22 (br s, 1H), 12.83 (br s, 1H), 13.45 (br s, 1H) ppm.

4-(4-Fluorophenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17i). Yield, 75%. LCMS (ES): >95% pure, *m*/*z* 339 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.38 (br t, J = 7.4, 2H), 7.95 (m, 3H), 8.18 (m, 2H), 8.36 (br d, J = 5.9, 1H), 8.48 (br s, 1H) ppm.

4-(3-Fluorophenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17j). Yield, 75%. LCMS (ES): >95% pure, *m*/*z* 339 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.06 (br t, J = 8.0, 1H), 7.51 (q, J = 6, 1H), 7.77 (br d, J = 9.6, 1H), 7.96 (dd, J = 8.0, J = 1.6, 1H), 8.04 (br d, J = 12.8, 1H), 8.16 (s, 1H), 8.17 (d, J = 4.0, 1H), 8.47 (m, 2H) ppm.

4-(4-Chlorophenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17k). Yield, 100%. LCMS (ES): 95% pure, *m*/*z* 355 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.50 (d, J = 8.0, 2H), 7.92 (d, J = 8.4, 1H), 8.13 (m, 3H), 8.28 (d, J = 5.6, 1H), 8.41(s, 1H), 9.80 (br s, 1H) ppm.

4-(3-Chlorophenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17l). Yield, 49%. LCMS (ES): >95% pure, *m*/*z* 355 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.20 (br d, J = 8, 1H), 7.47 (t, J = 8, 1H), 7.93 (d, J = 8.4, 1H), 8.05 (d, J = 8, 1H), 8.14 (m, 2H), 8.32 (m, 2H), 8.40 (m, 1H), 9.95 (br s, 1H) ppm.

4-(3-Methoxyphenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17m). Yield, 87%. LCMS (ES): >95% pure, *m*/*z* 351 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 3.83 (s, 3H), 6.88 (br s, 1H), 7.42 (m, 2H), 7.62 (br, 1H), 7.96 (br d, J = 9, 2H), 8.19 (br d, J = 8, 2H), 8.38 (br d, J = 4.1, 1H), 8.50 (br s, 1H) ppm.

4-(3-Ethynylphenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17n). Yield, 59%. LCMS (ES): >95% pure, *m*/*z* 345 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 4.23 (s, 1H), 7.26 (br s, 1H), 7.47 (t, J = 7.6, 1H), 7.93 (d, J = 8.4, 1H), 8.13 (m, 3H), 8.22 (s, 1H), 8.31 (d, J = 5.2, 1H), 8.39 (s, 1H), 9.78 (br, 1H) ppm.

4-(3-Phenoxyphenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17o). Yield, 80%. LCMS (ES): 95% pure, *m*/*z* 413 $[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 6.91 (br m, 1H), 7.19 (m, 3H), 7.46 (m, 3H), 7.64 (br d, J = 8, 1H), 7.82 (br s, 1H), 7.93 (d, J = 8.8, 1H), 8.13 (d, J = 6, 2H), 8.36 (br s, 1H), 8.48 (br d, J = 4.4, 1H) ppm.

4-(3-(Methylcarbamoyl)phenylamino)thieno[3,2-*c*]quinoline-7-carboxylic Acid (17p). Yield, 49%. LCMS (ES): 95% pure, *m*/*z* 378

$[M + H]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 2.83 (d, $J = 4$, 3H), 7.58 (br t, 1H), 7.71 (br s, 1H), 7.95 (d, $J = 8$, 1H), 8.17 (m, 2H), 8.33 (br s, 1H), 8.46 (br m, 2H), 8.55 (br s,1H) ppm.

4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carboxamide (18). 4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carbonitrile **16** (34 mg, 0.113 mmol) was dissolved in NMP (0.3 mL). Aqueous H_2O_2 (30%, 0.2 mL) was added, followed by addition of 6N NaOH (50 μL). The mixture was stirred at 50 °C for 2 h. An extra amount of 30% aqueous H_2O_2 (0.3 mL) and 6N NaOH (100 μL) were added to reach 70% conversion in 30 min. Water was added and the solid filtered and dried. The material was further reacted under the same conditions in order to achieve a complete transformation. 4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carboxamide **18** was isolated as a solid (30 mg, 83%). LCMS (ES): 95% pure, m/z 320 $[M + H]^+$.

***N*-Phenyl-7-(4*H*-1,2,4-triazol-3-yl)thieno[3,2-*c*]quinolin-4-amine (20).** 4-(Phenylamino)thieno[3,2-*c*]quinoline-7-carboxamide **18** (28 mg, 0.088 mmol) was suspended in *N*,*N*-dimethylformamide dimethylacetal and the mixture stirred at 80 °C under nitrogen atmosphere for 2 h. The volatiles were removed in vacuo. Acetic acid (0.5 mL) and anhydrous hydrazine (0.1 mL) were added, and the mixture was stirred at 115 °C for 1 h. Water and brine were added and the solid was filtered. The material was purified by preparative HPLC. Genevac evaporation and trituration in EtOAc/hexanes afforded the title compound **20** as an off-white solid (9 mg, 30%). LCMS (ES): 94% pure, m/z 344 $[M + H]^+$. ^1H NMR ($CDCl_3/CD_3OD$, 9:1, 400 MHz) δ 6.57 (d, $J = 4.8$, 1H), 7.42−7.44 (m, 3H), 7.47−7.52 (m, 3H), 8.10 (d, $J = 8.4$, 1H), 8.14 (s, 1H), 8.30 (d, $J = 7.2$, 1H), 8.39 (s, 1H) ppm.

General Procedure for the Synthesis of Tetrazole Analogues 19a−c. *N*-Phenyl-7-(1*H*-tetrazol-5-yl)thieno[3,2-*c*]quinolin-4-amine (19a). 4-Chlorothieno[3,2-*c*]quinoline-7-carbonitrile **14b** (1.0 equiv, 23 mg, 0.094 mmol), aniline (0.1 mL), and NMP (0.1 mL) were mixed in a microwave vial. The mixture was heated in a microwave oven at 120 °C for 10 min. Water was added and the resulting intermediate 4-(phenylamino)thieno[3,2-*c*]quinoline-7-carbonitrile **16** was filtered and dried. This crude material was mixed in a vial with DMF (0.5 mL), NH_4Cl (50 mg), and NaN_3 (50 mg). The mixture was stirred at 120 °C for 3 h. After addition of water and filtration, *N*-phenyl-7-(1*H*-tetrazol-5-yl)thieno[3,2-*c*]quinolin-4-amine **19a** was isolated as a beige solid (13 mg, 41%). LCMS (ES): >95% pure, m/z 345 $[M + H]^+$, 317 $[M + H - N_2]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.07 (t, $J = 7.2$, 1H), 7.40 (t, $J = 7.6$, 2H), 8.00 (dd, $J = 1.6$, $J = 8.4$, 1H), 8.04 (d, $J = 5.2$, 1H), 8.10 (dd, $J = 1.2$, $J = 8.8$, 2H), 8.19 (d, $J = 8.0$, 1H), 8.25 (d, $J = 5.6$, 1H), 8.43 (d, $J = 1.6$, 1H), 9.34 (s, 1H) ppm.

***N*-(3-Chlorophenyl)-7-(1*H*-tetrazol-5-yl)thieno[3,2-*c*]quinolin-4-amine (19b).** Yield, 13%. LCMS (ES): >95% pure, m/z 379 $[M + H]^+$, 351 $[M + H - N_2]^+$.

***N*-(3-Fluorophenyl)-7-(1*H*-tetrazol-5-yl)thieno[3,2-*c*]quinolin-4-amine (19c).** Yield, 47%. LCMS (ES): > 95% pure, m/z 363 $[M + H]^+$, 335 $[M + H - N_2]^+$. ^1H NMR (DMSO-d_6, 400 MHz): δ 6.89 (td, $J = 8.4$, $J = 2.4$, 1H), 7.43 (q, $J = 7.9$, 1H), 7.85 (dd, $J = 1.2$, $J = 8.0$, 1H), 8.06−8.09 (m, 2H), 8.23 (d, $J = 8.0$, 1H), 8.27 (d, $J = 5.6$, 1H), 8.33 (dt, $J = 2.0$, $J = 12.4$, 1H), 8.51 (d, $J = 1.2$, 1H), 9.53 (s, 1H) ppm.

Ethyl 3-Bromoisonicotinate (22a). 3-Bromoisonicotinic acid **21a** (3.0 g, 14.9 mmol) in ethanol (100 mL) was treated with concentrated sulfuric acid (5 mL). The mixture was brought to reflux, at which time everything went into solution. After 12 h at reflux, LCMS indicated that the reaction was complete. The reaction mixture was cooled to room temperature and concentrated on a rotary evaporator to a third of its original volume. The mixture was then diluted with 250 mL of ethyl acetate and washed twice with saturated aqueous sodium bicarbonate. Concentration on a rotary evaporator yielded ethyl 3-bromoisonicotinate **22a** (3.25 g, 95%) as a yellowish oil which was sufficiently pure for subsequent chemical transformations. LCMS (ES): >95% pure, m/z 230 $[M + H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (t, $J = 8.0$, 3H), 4.39 (q, $J = 7.2$, 2H), 7.57 (d, $J = 5.2$, 1H), 8.57 (d, $J = 4.8$, 1H), 8.04 (s, 1H) ppm.

Methyl 5-Bromopyrimidine-4-carboxylate (22g). Crude 5-bromopyrimidine-4-carboxylic acid **21g** (prepared according to the procedure described in literature[61]) (1.0 equiv, 6.14 g, 30.2 mmol) was suspended in CH_2Cl_2 (100 mL). Oxalylchloride (1.1 equiv, 2.9 mL, 33.0 mmol) was added followed by 2 drops of DMF. The mixture was stirred at room temperature overnight, and the volatiles were removed in vacuo. The residue was dissolved in methanol (50 mL) and heated. After evaporation of methanol in vacuo, the compound was dissolved in CH_2Cl_2 and poured on a prepacked silica gel column. The material was eluted using 20% of ethyl acetate in hexanes. Evaporation of the solvent provided the title compound **22g** as a light-orange crystalline solid (2.54 g, 39%). LCMS (ES): 95% pure, m/z 217 $[M]^+$, 219 $[M + 2]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ 4.04 (s, 3H), 9.02 (s, 1H), 9.21 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.73, 118.31, 155.49, 156.78, 161.52, 163.85 ppm.

Methyl 3-Bromopicolinate (22i). 3-Bromopicolinic acid **21i** (1.0 equiv, 5.67 g, 28.08 mmol) was reacted with oxalyl chloride (1.2 equiv, 3.0 mL, 34.39 mmol) in dichloromethane (75 mL). DMF (0.2 mL) was added, and the mixture was stirred for 2 h. The mixture was cooled to 0 °C, and methanol (10 mL) was added. The mixture was diluted with dichloromethane (200 mL). The organic phase was washed with a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (100 mL). The solution was dried over Na_2SO_4 and the volatiles removed in vacuo to give methyl 3-bromopicolinate **22i** as a brownish oil (5.82 g, 96%). ^1H NMR ($CDCl_3$, 400 MHz) δ 4.00 (s, 3H), 7.30 (dd, $J = 8.1$, $J = 4.0$, 1H), 8.00 (dd, $J = 8.1$, $J = 1.3$, 1H), 8.61 (dd, $J = 4.0$, $J = 1.3$, 1H) ppm.

Methyl 2-Bromonicotinate (22e). 2-Bromopicolinic acid **21e** (1.0 equiv, 2.51 g, 12.45 mmol) was reacted with oxalyl chloride (1.3 equiv, 1.4 mL, 15.81 mmol) in dichloromethane (40 mL). DMF (0.1 mL) was added, and the mixture was stirred for 2 h. An additional amount of oxalyl chloride (1.4 mL) was added and the reaction stirred for an additional hour. The mixture was cooled to 0 °C, and methanol (5 mL) was added. The mixture was diluted with dichloromethane (200 mL). The organic phase was washed with a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (100 mL). The solution was dried over Na_2SO_4 and the volatiles removed in vacuo to give methyl 2-bromopicolinate **22e** as an oil (1.97 g, 73%). ^1H NMR ($CDCl_3$, 400 MHz) δ 3.95 (s, 3H), 7.32 (dd, $J = 7.7$, $J = 5.1$, 1H), 8.17 (dd, $J = 7.7$, $J = 2.2$, 1H), 8.60 (dd, $J = 5.1$, $J = 2.0$, 1H) ppm.

Ethyl 3-Chloropyrazine-2-carboxylate (22f). 3-Chloropyrazine-2-carboxylic acid **21f** (1.0 equiv, 2.08 g, 13.11 mmol) was reacted with oxalyl chloride (1.6 equiv, 1.8 mL, 20.63 mmol) in dichloromethane (75 mL). DMF (0.4 mL) was added, and the mixture was stirred at room temperature overnight. The mixture was concentrated and dissolved in dichloromethane (50 mL). Ethanol (5 mL) was added at 0 °C, and the mixture was stirred for one hour at room temperature. The mixture was diluted with dichloromethane (200 mL). The organic phase was washed with a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (100 mL). The solution was dried over Na_2SO_4 and the volatiles removed in vacuo. The solution was concentrated and filtered through a plug of silica gel. Evaporation of the solvents gave the title compound **22f** as an oil (1.65 g, 67%). One spot TLC. LCMS (ES): >80% pure, m/z 187 $[M + H]^+$. The material was used for next step without any further purification.

Methyl 5-Oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carboxylate (23a). Ethyl 3-bromoisonicotinate **22a** (1.15 g, 5.0 mmol), 2-amino-4-methoxycarbonyl-phenylboronic acid hydrochloride **10a** (1.04 g, 4.5 mmol), sodium acetate (1.64 g, 20 mmol), $PdCl_2$ (dppf) (182 mg, 0.25 mmol), and dimethylformamide (7.5 mL) were combined in a flask. The flask was evacuated and filled with nitrogen twice and heated to 125 °C with stirring for 12 h or until LCMS indicated the absence of any starting material. The mixture was cooled to room temperature and water (100 mL) was added to form a brown precipitate. The

precipitate was filtered to yield 637 mg (56%) of methyl 5-oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carboxylate **23a**. LCMS (ES): 95% pure, *m*/*z* 255 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.87 (s, 3H), 7.69 (d, J = 8.0, 1H), 7.93 (s, 1H), 8.08 (d, J = 5.2, 1H), 8.60 (d, J = 8.4, 1H), 8.79 (d, J = 5.2, 1H), 9.85 (s, 1H) ppm.

5-Oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carbonitrile (23b). Sodium acetate (410 mg, 5 mmol) and $PdCl_2$(dppf) (36 mg, 0.05 mmol) were added to a mixture of ethyl 3-bromoisonicotinate **22a** (230 mg, 1.0 mmol) and 2-amino-4-cyanophenylboronic acid hydrochloric acid salt **10b** (179 mg, 0.9 mmol). The mixture was connected to an exit bubbler and heated to 120 °C for 18 h, at which time LCMS analysis indicated that the reaction was done based on the disappearance of starting material. After cooling to room temperature, water was added and the dark solids were filtered and washed with dichloromethane to give 5-oxo-5,6-dihydrobenzo[*c*]-[2,6]naphthyridine-8-carbonitrile **23b** (156 mg, 46%) as a gray solid. LCMS (ES): >90% pure, *m*/*z* 222 $[M + H]^+$. ^{1}HNMR (400 MHz, DMSO-d_6): δ 12.2 (1H, s), 9.96 (1H, s), 8.90 (1H, d, J = 5.1), 8.77 (1H, d, J = 8.2), 8.13 (1H, d, J = 5.1), 7.73 (1H, dd J = 8.2, J = 1.6), 7.70 (1H, d, J = 1.6) ppm.

Benzo[*c*][2,6]naphthyridin-5(6*H*)-one (23c). Ethyl 3-bromoisonicotinate **22a** (1.0 equiv, 1.76 g, 7.65 mmol), 2-aminophenylboronic acid hydrochloride **10c** (1.0 equiv, 1.33 g, 7.67 mmol), and cesium carbonate (2.0 equiv, 4.99 g, 15.31 mmol) were suspended in dioxane (15 mL). The mixture was degassed by bubbling nitrogen for 10 min. $PdCl_2$ (dppf) (0.05 equiv, 280 mg, 0.383 mmol) was added, and the mixture was stirred at reflux for 2 h. The resulting solid was filtered, washed with methanol, water, and methanol, and dried. Benzo[*c*][2,6]naphthyridin-5(6*H*)-one **23c** was isolated as an off-white solid (823 mg, 55%). LCMS (ES): 95% pure, *m*/*z* 197 $[M + H]^+$.

Methyl 6-Oxo-5,6-dihydrophenanthridine-3-carboxylate (23h). Ethyl 2-bromobenzoate **22h** (1.3 equiv, 300 mg, 1.3 mmol) was combined with 2-amino-4-methoxycarbonyl-phenylboronic acid hydrochloride **10a** (1.0 equiv, 195 mg, 1.0 mmol), sodium acetate (3.9 equiv, 226 mg, 3.9 mmol), $PdCl_2$ (dppf) (0.025 equiv, 25 mg, 0.025 mmol), and dimethylformamide (2.5 mL). The resulting mixture was reacted in a microwave oven at 120 °C for 10 min. Water was added, and the material was extracted with EtOAc (2×). The organic phase was dried over Na_2SO_4 and filtered through a plug of silica gel. Trituration with dichloromethane and hexanes provided methyl 6-oxo-5,6-dihydrophenanthridine-3-carboxylate **23h** as a solid (125 mg, 49%). The crude solid was used for next step without any further characterization and purification.

General Procedure for the Synthesis of Compounds 23d−g and 23i. Synthesis of Methyl 5-Oxo-5,6-dihydropyrimido[4,5-*c*]-quinoline-8-carboxylate (23g). Methyl 5-bromopyrimidine-4-carboxylate **22g** (1.0 equiv, 10.0 g, 46.08 mmol) was mixed in a flask with 2-amino-4-methoxycarbonyl-phenylboronic acid hydrochloride **10a** (1.1 equiv, 11.70 g, 50.56 mmol), cesium carbonate (3.0 equiv, 45.0 g, 138.11 mmol), and $PdCl_2$ (dppf) (0.015 equiv, 505 mg, 0.69 mmol) in anhydrous dioxane (200 mL). The reaction mixture was degassed by bubbling nitrogen through the suspension of reagents for about 10 min. The reaction was stirred under reflux for 3 h, at which time LCMS monitoring indicated the presence of starting materials. The conversion was completed after adding an extra amount of palladium catalyst (505 mg) and water (200 μL) and further reacting at reflux overnight. After adding water and then brine, the precipitate was filtered and washed with water (200 mL) and methanol (200 mL). The solid was stirred in ethyl acetate for 2 h, filtered, and dried. The title compound **23g** was isolated as a tan solid (9.39 g, 80%). LCMS (ES): 95% pure, *m*/*z* 256 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.79 (s, 3H), 7.81 (d, J = 8.0, 1H), 8.68 (d, J = 8.8, 1H), 9.49 (s, 1H), 10.19 (s, 1H), 12.37 (s, 1H) ppm.

Methyl 5-Oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carboxylate (23e). Yield, 21%. LCMS (ES): >95% pure, *m*/*z* 255 $[M + H]^+$.

Methyl 5-Oxo-5,6-dihydrobenzo[*c*][2,7]naphthyridine-8-carboxylate (23d). Yield, 90%. LCMS (ES): >95% pure, *m*/*z* 255 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.91 (s, 3H), 7.80 (d, J = 8.8, 1H), 7.99 (s, 1H), 8.45 (d, J = 5.6, 1H), 8.58 (d, J = 8.0, 1H), 8.96 (d, J = 5.6, 1H), 9.45 (br s, 1H), 12.07 (s, 1H) ppm.

Methyl 5-Oxo-5,6-dihydropyrazino[2,3-*c*]quinoline-8-carboxylate (23f). Yield, 34%. LCMS (ES): >95% pure, *m*/*z* 256 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.92 (s, 3H), 7.57 (d, J = 8.4, 1H), 8.01 (s, 1H), 8.65 (d, J = 8.4, 1H), 9.03 (d, J = 2.0, 1H), 9.13 (d, J = 1.6, 1H), 12.05 (s, 1H) ppm.

Methyl 5-Oxo-5,6-dihydrobenzo[*f*][1,7]naphthyridine-8-carboxylate (23i). Yield, 29%. The crude solid was used for next step without any further purification and characterization.

Methyl 2-Amino-3-bromobenzoate (32). Commercially available 2-amino-3-bromobenzoic acid **31** (1.00 g, 4.62 mmol) was mixed with methanol (10 mL) and concentrated sulfuric acid (1 mL). The mixture was stirred at reflux for 31 h. The solvent were evaporated, and saturated aqueous sodium bicarbonate was carefully added. The solid was extracted with CH_2Cl_2 (3×). The combined extracts were dried over Na_2SO_4 and the solvents removed in vacuo to afford methyl 2-amino-3-bromobenzoate **32** as a semicrystalline solid (976 mg, 91%). LCMS (ES): >85% pure, *m*/*z* 230 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.88 (s, 3H), 6.34 (br s, 2H), 6.53 (t, J = 8.0, 1H), 7.57 (dd, J = 1.6, J = 7.6, 1H), 7.85 (dd, J = 1.6, J = 8.4, 1H) ppm.

Methyl 3-Amino-4-iodo-2-methylbenzoate (30a). Commercially available methyl 3-amino-2-methylbenzoate **29** (1.0 equiv, 11.62 g, 70.4 mmol) was suspended in acetic acid (100 mL). *N*-Iodosuccinimide (1.03 equiv, 16.32 g, 72.0 mmol) was added in several portions. The reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated and the residue diluted with EtOAc (150 mL). The organic phase was washed with a solution of potassium carbonate (100 mL), water (3 × 40 mL), and brine (3 × 40 mL). After drying over Na_2SO_4, the solvent was removed by rotary evaporation. The reaction produced a mixture of compounds, the major (86%) component of the mixture being methyl 3-amino-6-iodo-2-methylbenzoate **30b** (R_f = 0.25, SiO_2, 25% ethyl acetate in hexanes, GCMS *m*/*z* = 291) and the minor (14%) component being the desired methyl 3-amino-4-iodo-2-methylbenzoate **30a** (R_f = 0.5, SiO_2, 25% ethyl acetate in hexanes, GCMS *m*/*z* = 291). The desired minor product was isolated pure by flash chromatography on silica gel (EtOAc 15% to 25% gradient in hexanes) to afford the title compound **30a** as a yellow solid (1.23 g, 6%). GCMS: >95% pure, *m*/*z* 291. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.08 (s, 3H), 3.60 (br s, 2H), 3.94 (s, 3H), 6.45 (d, J = 8.8, 1H), 7.39 (d, J = 8.0, 1H) ppm.

Methyl 2-Methyl-5-nitrobenzoate (34). Commercially available 2-methyl-5-nitrobenzoic acid **33** (24 g, 132.5 mmol) was dissolved in a mixture of methanol (240 mL) and concentrated sulfuric acid (8 mL). The mixture was stirred at reflux overnight. Upon cooling, the ester crystallized out. The material was isolated by filtration to afford methyl 2-methyl-5-nitrobenzoate **34** as a white solid (19.0 g, 74%). A second crop of material (4.92 g, 19%) was isolated upon concentration and addition of water to the mother liquor. The crude solid was used for next step without any further characterization and purification.

Methyl 5-Amino-2-methylbenzoate (35). Methyl 2-methyl-5-nitrobenzoate **34** (5.06 g, 25.95 mmol) was suspended in methanol (100 mL). The mixture was degassed by bubbling nitrogen for 15 min. Pd/C (10% wet Degussa type E101 NE/WW, 260 mg) was added and the mixture stirred under hydrogen atmosphere (balloon) overnight. The suspension was filtered and the solvents evaporated to afford the title compound **35** as an orange oil (4.18 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H), 3.45 (br s, 2H), 3.85 (s, 3H), 6.74 (dd, J = 7.4, J = 2.3, 1H), 7.02 (d, J = 7.4, 1H), 7.25 (d, J = 2.5, 1H) ppm.

Methyl 5-Amino-4-iodo-2-methylbenzoate (36). Methyl 5-amino-2-methylbenzoate **35** (1.0 equiv, 3.75 g, 22.7 mmol) was dissolved in acetic acid (70 mL). *N*-Iodosuccinimide

(1.03 equiv, 5.27 g, 23.4 mmol) was added in small portions over 60 min. The mixture was stirred at room temperature for 30 min. Acetic acid was evaporated. The residue was diluted with ethyl acetate (80 mL) and neutralized with saturated sodium carbonate (80 mL). The organic layer was washed with 1 M sodium thiosulfate (2 × 40 mL) and then water (2 × 40 mL) and brine (2 × 40 mL). The material was purified by flash chromatography on silica gel (gradient 10−30% ethyl acetate in hexanes) to provide methyl 5-amino-4-iodo-2-methylbenzoate **36** as a yellow−orange solid (3.19 g, 49%). GCMS >95% pure, *m*/*z* 291. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H), 3.78 (s, 3H), 5.27 (br s, 2H), 7.24 (s, 1H), 7.54 (s, 1H) ppm.

General Procedure for the Synthesis of Compounds 39a−d. Synthesis of Methyl 5-Oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-7-carboxylate (39b). Methyl 2-amino-3-bromobenzoate **32** (1.0 equiv, 652 mg, 2.61 mmol) and 4-(diisopropylcarbamoyl)pyridin-3-ylboronic acid **38** (prepared according to a procedure described in literature),[47,48] (1.0 equiv, 600 mg, 2.61 mmol) were combined with cesium carbonate (2.0 equiv, 1.699 g, 5.21 mmol) in dioxane containing 5% of water (6 mL). The mixture was degassed by bubbling nitrogen for 10 min. $PdCl_2$ (dppf) (0.05 equiv, 95 mg) was added and the reaction stirred at reflux for 2 h. Dioxane was evaporated, water was added, and the material extracted with CH_2Cl_2 (3×). The combined extracts were dried over Na_2SO_4 and the solvents removed in vacuo. The material was purified by flash chromatography on silica gel (eluent 0.5% methanol in CH_2Cl_2) to afford the intermediate methyl 2-amino-3-(4-(diisopropylcarbamoyl)pyridin-3-yl)benzoate as a greenish foam. (LCMS (ES): >95% pure, *m*/*z* 356 [M + H]$^+$). This material was dissolved under nitrogen atmosphere in anhydrous THF (1.5 mL). A NaHMDS solution (1.0 M in THF, 2.0 equiv, 1.4 mL, 1.4 mmol) was added dropwise from a syringe. The resulting suspension was stirred at room temperature for 1 h. The reaction was quenched by addition of a saturated aqueous solution of ammonium chloride. The solid that formed was filtered and dried. After trituration in methanol and filtration, the title compound was isolated as a gray fluffy solid (93 mg, 14% over two steps). LCMS (ES): >95% pure, *m*/*z* 255 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.97 (s, 3H), 7.44 (t, J = 7.6, 1H), 8.11 (dd, J = 4,8, J = 0.8, 1H), 8.20 (dd, J = 7.6, J = 1.2, 1H), 8.87 (dd, J = 5.2, 1H), 8.91 (d, J = 7.2, 1H), 9.94 (s, 1H), 11.77 (br s, 1H) ppm.

Methyl 7-Methyl-5-oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carboxylate (39a). Yield, 52%. LCMS (ES): >95% pure, *m*/*z* 269 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.88 (s, 3H), 7.60 (d, J = 7, 1H), 8.16 (d, J = 5, 1H), 8.56 (d, J = 7, 1H), 8.88 (d, J = 5, 1H), 9.94 (s, 1H), 11.13 (br s, 1H) ppm.

Methyl 9-Methyl-5-oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-8-carboxylate (39c). Yield, 41%. LCMS (ES): 89% pure, *m*/*z* 269 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.62 (s, 3H), 3.88 (s, 3H), 7.89 (s, 1H), 8.13 (d, J = 4.8, 1H), 8.58 (s, 1H), 8.87 (d, J = 5.2, 1H), 9.95 (s, 1H), 12.07 (br s, 1H) ppm.

Methyl 5-Oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-9-carboxylate (39d). Yield, 41%. LCMS (ES): 90% pure, *m*/*z* 255 [M + H]$^+$.

General Procedure for the Synthesis of Compounds 24a−b, 24e−f, and 24h−i. Methyl 5-Chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate (24a). Methyl 5-oxo-5,6-dihydrobenzo[*c*][2,6]-naphthyridine-8-carboxylate **23a** (200 mg, 0.787 mmol) was combined with phosphorus oxychloride (1 mL) and heated to reflux. After 2 h, LCMS indicated the absence of any starting material. The volatiles were removed under reduced pressure. The residue was taken up in dichloromethane (50 mL) and washed twice with saturated aqueous sodium bicarbonate. The organic phase was dried over sodium sulfate and concentrated on a rotary evaporator to give methyl 5-chlorobenzo[*c*][2,6]-naphthyridine-8-carboxylate **24a** (140 mg, 65%) as a grayish solid. An alternate workup consisted in adding acetonitrile after evaporation of phosphorus oxychloride. The material was precipitated by addition of ice and water. Reasonably pure material was obtained after drying in vacuo. LCMS (ES): 95% pure, *m*/*z* 273 [M + H]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.03 (s, 3H), 8.25 (dd, J = 6.0, J = 1.2, 1H), 8.40 (dd, J = 8.4, J = 1.6, 1H), 8.73 (d, J = 8.8, 1H), 8.82 (d, J = 2.0, 1H), 9.03 (d, J = 5.6, 1H), 10.08 (s, 1H) ppm.

5-Chlorobenzo[*c*][2,6]naphthyridine-8-carbonitrile (24b). Yield, 77%. LCMS (ES): 95% pure, *m*/*z* 240 [M + H]$^+$.

Methyl 6-Chlorophenanthridine-3-carboxylate (24h). Yield, 97%. The crude solid was used for next step without any further characterization and purification.

Methyl 5-Chlorobenzo[*h*][1,6]naphthyridine-8-carboxylate (24e). Yield, 57%. LCMS (ES): >95% pure, *m*/*z* 273 [M + H]$^+$.

Methyl 5-Chloropyrazino[2,3-*c*]quinoline-8-carboxylate (24f). Yield, 97%. LCMS (ES): >90% pure, *m*/*z* 274 [M + H]$^+$.

Methyl 5-Chlorobenzo[*f*][1,7]naphthyridine-8-carboxylate (24i). Yield, 80%. LCMS (ES): >85% pure, *m*/*z* 273 [M + H]$^+$. The material was used for next step without any further purification.

Methyl 5-Chlorobenzo[*c*][2,7]naphthyridine-8-carboxylate (24d). A solution of methyl 5-oxo-5,6-dihydrobenzo[*c*][2,7]naphthyridine-8-carboxylate **23d** (650 mg, 2.56 mmol) in phosphorus oxychloride (4.0 mL) was heated at 120 °C for 2.5 h. The reaction was concentrated under reduced pressure and diluted with ACN (20 mL) and H_2O (40 mL). The solution was neutralized with NaOH (3N), and the resulting precipitate was collected by filtration to give the title compound **24d** (600 mg, 86%). LCMS (ES): >95% pure, *m*/*z* 273 [M + H]$^+$.

Methyl 5-Chloropyrimido[4,5-*c*]quinoline-8-carboxylate (24g). In a vial, methyl 5-oxo-5,6-dihydropyrimido[4,5-*c*]quinoline-8-carboxylate **23g** (1.0 equiv, 151 mg, 0.59 mmol) was mixed in toluene (1 mL) with DIEA (1.5 equiv, 155 μL, 0.89 mmol) and phosphorus oxychloride (5 equiv, 270 μL, 3.0 mmol). The mixture was stirred at 120 °C for 1 h and cooled down to room temperature. After adding ice and water, the compound was extracted with CH_2Cl_2 (4×). The solution was filtered over Na_2SO_4 and filtered through a pad of Celite. After evaporation of the volatiles, the material was triturated in a mixture of ethyl acetate and hexanes, filtered, and dried to afford methyl 5-chloropyrimido[4,5-*c*]quinoline-8-carboxylate **24g** as a light-brown fluffy solid (115 mg, 71%). LCMS (ES): 95% pure, *m*/*z* 274 [M + H]$^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.96 (s, 3H), 8.37 (dd, J = 1.6, J = 8.4, 1H), 8.60 (d, J = 1.6, 1H), 9.15 (d, J = 8.8, 1H), 9.74 (s, 1H), 10.61 (s, 1H) ppm.

5-Chlorobenzo[*c*][2,6]naphthyridine (24c). Benzo[*c*][2,6]naphthyridin-5(6*H*)-one **23c** (1.0 equiv, 813 mg, 4.15 mmol) was mixed with phosphorus oxychloride (5.0 equiv, 2 mL, 21.84 mmol) and acetonitrile (10 mL). The mixture was stirred at reflux for 5 h. The mixture was poured on ice and the resulting solid filtered and dried. 5-Chlorobenzo[*c*][2,6]naphthyridine **24c** was isolated as a gray solid (459 mg, 52%). LCMS (ES): 95% pure, *m*/*z* 215 [M + H]$^+$.

General Procedure for the Synthesis of Compounds 40a−d. Synthesis of Methyl 5-Chlorobenzo[*c*][2,6]naphthyridine-7-carboxylate (40b). Methyl 5-oxo-5,6-dihydrobenzo[*c*][2,6]naphthyridine-7-carboxylate **39b** (1.0 equiv, 85 mg, 0.334 mmol) was stirred in phosphorus oxychloride (2 mL) at 120 °C for 2 h. The solvent was removed in vacuo. Ice and water were added. The resulting solid was filtered and dried to afford the title compound **40b** as a solid (84 mg, 92%). LCMS (ES): >95% pure, *m*/*z* 273 [M + H]$^+$.

Methyl 5-Chloro-7-methylbenzo[*c*][2,6]naphthyridine-8-carboxylate (40a). Yield, 85%. LCMS (ES): >95% pure, *m*/*z* 287 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.95 (s, 3H), 3.94 (s, 3H), 8.14 (d, J = 7.8, 1H), 8.28 (d, J = 5.7, 1H), 8.98 (d, J = 9, 1H), 9.08 (d, J = 6, 1H), 10.37 (s, 1H) ppm.

Methyl 5-Chloro-9-methylbenzo[*c*][2,6]naphthyridine-8-carboxylate (40c). Yield, 76%. LCMS (ES): >95% pure, *m*/*z* 287 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.79 (s, 3H), 3.94 (s, 3H), 8.25 (d, J = 5, 1H), 8.45 (s, 1H), 9.02 (s, 1H), 9.07 (d, J = 5, 1H), 10.35 (s 1H) ppm.

Methyl 5-Chlorobenzo[*c*][2,6]naphthyridine-9-carboxylate (40d). Yield, 50%. LCMS (ES): >95% pure, *m*/*z* 273 [M + H]$^+$.

Methyl 5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylate (26a). Methyl 5-chlorobenzo[*c*][2,6]naphthyridine-8-carboxylate **24a** (1.0 equiv, 529 mg, 1.94 mmol) was combined with 3-chloroaniline (1.5 equiv, 370 mg, 2.92 mmol) in NMP (4 mL) in a flask, and the mixture was heated to 80 °C for 2 h. The mixture was dissolved in CH_2Cl_2, washed with saturated aqueous sodium bicarbonate, and dried over Na_2SO_4. The material was purified by flash chromatography (SiO_2, 1:1 to 9:1 gradient of EtOAc/hexanes) to obtain the title compound **26a**. LCMS (ES): >95% pure, *m*/*z* 364 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.91 (s, 3H), 7.16 (dd, J = 1.2, J = 8.0, 1H), 7.44 (t, J = 8.0, 1H), 7.94 (dd, J = 8.4, J = 2.0, 1H), 8.06 (dd, J = 8.4, J = 1.2, 1H), 8.20−8.22 (m, 2H), 8.82−8.86 (m, 2H), 9.04 (d, J = 5.6, 1H), 10.05 (br s, 1H), 10.21 (s, 1H) ppm.

***N*-(3-Chlorophenyl)benzo[*c*][2,6]naphthyridin-5-amine (26c).** 5-Chlorobenzo[*c*][2,6]naphthyridine **24c** (1.0 equiv, 20 mg, 0.093 mmol) was mixed with 3-chloroaniline (15 equiv, 150 μL, 1.42 mmol) and NMP (0.8 mL). The mixture was stirred at 60 °C overnight. Water was added and the material extracted with dichloromethane. The organic phase was extracted with water (2×) and the solvents evaporated. The material was purified by preparative TLC (SiO_2, 5% methanol in dichloromethane). The title compound **26c** was isolated as a solid (19 mg, 68%). LCMS (ES): >95% pure, *m*/*z* 306 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.12 (br d, J = 8.4, 1H), 7.42 (t, J = 8.0, 1H), 7.52 (t, J = 7.2, 1H), 7.70 (t, J = 7.2, 1H), 7.77 (d, J = 7.2, 1H), 8.07 (d, J = 8.0, 1H), 8.34 (s, 1H), 8.58 (d, J = 5.2, 1H), 8.79 (d, J = 8.0, 1H), 8.97 (br s, 1H), 9.62 (s, 1H), 10.18 (br s, 1H) ppm.

(5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridin-8-yl)methanol (26d). Methyl 5-(3-chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylate **26a** (50 mg, 0.137 mmol) was dissolved in THF (4 mL) and the solution cooled to −40 °C. A solution of $LiAlH_4$ (1.0 M in diethylether, 270 μL, 0.27 mmol) was added, and the reaction mixture was allowed to warm to room temperature. After aqueous workup, the product was purified by flash chromatography on silica gel (ethyl acetate/hexanes gradient from 1:1 to 9:1). The title compound **26d** was isolated as a solid (46 mg, 100%). LCMS (ES): >95% pure, *m*/*z* 336 $[M + H]^+$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.72 (s, 2H), 7.16 (dd, J = 8, J = 1.2, 1H), 7.43−7.49 (m, 2H), 7.75 (s, 1H), 8.05 (br d, J = 8.4, 1H), 8.32 (s, 1H), 8.69 (d, J = 5.6, 1H), 8.74 (d, J = 8.8, 1H), 8.96 (d, J = 6.0, 1H), 9.82 (br s, 1H), 10.18 (s, 1H) ppm.

General Procedure for the Synthesis of Compounds 25a−t, 27d−e, 27h−i, 28d−g, 28i, and 41a−d. 6-(Phenylamino)phenanthridine-3-carboxylic Acid (27h). Methyl 6-chlorophenanthridine-3-carboxylate **24h** (1.0 equiv, 53 mg, 0.20 mmol) was combined with aniline (3 equiv, 57 mg, 0.59 mmol) and *N*-methyl pyrrolidinone (0.2 mL) in a microwave tube, and the mixture was heated to 120 °C for 10 min, at which time LCMS indicated that the reaction was complete as indicated by the absence of any starting material. 6N NaOH (0.5 mL) was added, and the mixture was stirred at 50 °C for 30 min. The mixture was neutralized with 1N hydrochloric acid. The material was filtered. Purification by preparative HPLC and Genevac evaporation afforded the title compound **27h** as a solid (28 mg, 45%). LCMS (ES): >95% pure, *m*/*z* 315 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.56−7.70 (m, 5H), 7.99 (t, J = 8.4, 1H), 8.17 (td, J = 8, J = 1.6, 1H), 8.19 (br t, J = 6.8, 1H), 8.49 (d, J = 1.6, 1H), 8.70 (d, J = 8.2, 1H), 8.73 (d, J = 7.2, 1H), 8.86 (d, J = 8, 1H) ppm.

The following compounds were prepared using a similar two-step procedure. The nucleophilic substitutions between various chloro compounds **24a**, **24d−i**, and **40a−d** and various amines was achieved in NMP at temperatures ranging from 80 to 120 °C using microwave (10−15 min) or conventional heating (2 h). The ester hydrolyses were carried out in one pot by adding aqueous (3N or 6N) NaOH to the precedent mixture and heating the solution at 50−80 °C for up to 2 h. Alternatively, the hydrolysis reaction could be accomplished after isolation of the ester by precipitation in water and purification by flash chromatography. The final carboxylic acid materials could be isolated by filtration as pure solids after adding water and neutralizing the solution with an aqueous solution of hydrochloric acid or could be isolated by filtration as sodium carboxylate salts when precipitation occurred during the reaction. An alternate isolation method consisted in diluting the reaction mixture with a mixture of acetonitrile, water, and trifluoroacetic acid. The resulting solutions were submitted to preparative HPLC, providing the pure carboxylic acids after Genevac evaporation. Compounds bearing a basic moiety were isolated as trifluoroacetate salts.

5-(Phenylamino)benzo[*h*][1,6]naphthyridine-8-carboxylic Acid (27e). Yield, 88%. LCMS (ES): >95% pure, *m*/*z* 316 $[M + H]^+$. ^{1}HNMR (400 MHz, DMSO-d_6): δ 7.10 (t, J = 7.2, 1H), 7.43 (t, J = 7.6, 2H), 7.89 (dd, J = 4.0, J = 8.0, 1H), 7.95 (dd, J = 2, J = 8.4, 1H), 8.10 (d, J = 1.6, 2H), 8.25 (d, J = 1.6, 1H), 8.90 (d, J = 8.4, 1H), 9.14 (dd, J = 1.6, J = 8.4, 1H), 9.21 (dd, J = 1.2, J = 4.4, 1H), 9.54 (s, 1H), 13.16 (s, 1H) ppm.

5-(Phenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid, Trifluoroacetate Salt (25a). Yield, 23%. LCMS (ES): >95% pure, *m*/*z* 316 $[M + H]^+$. ^{1}HNMR (400 MHz, CD_3OD):δ 10.17 (1H, s), 9.67 (1H, br), 8.99 (1H, d, J = 5.9), 8.83 (1H, d, J = 8.6), 8.62 (1H, d, J = 5.9), 8.24 (1H, d, J = 1.6), 8.04 (1H, s), 8.02 (1H, s), 7.93 (1H, dd, J = 8.2, J = 1.6), 7.43 (1H, d, J = 7.4), 7.41 (1H, d, J = 7.4), 7.10 (1H, m) ppm.

5-(Phenylamino)benzo[*c*][2,7]naphthyridine-8-carboxylic Acid (27d). Yield, 72%. LCMS (ES): 82% pure, *m*/*z* 316 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.10 (t, J = 7.6, 1H), 7.43 (t, J = 7.6, 2H), 7.93 (dd, J = 8.4, J = 1.6, 1H), 8.09 (d, J = 7.6, 2H), 8.22 (d, J = 1.6, 1H), 8.67 (d, J = 6.0, 1H), 8.71 (d, J = 8.4, 1H), 8.99 (d, J = 5.6, 1H), 9.69 (s, 1H), 10.01 (s, 1H), 13.20 (br s, 1H) ppm.

Sodium 5-(Phenylamino)benzo[*f*][1,7]naphthyridine-8-carboxylate (27i). Yield, 10%. LCMS (ES): >95% pure, *m*/*z* 316 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.05 (t, J = 6.8, 1H), 7.42 (t, J = 8, 2H), 7.95 (m, 2H), 8.26 (s, 1H), 8.32 (d, J = 8.0, 2H), 8.46 (d, J = 8.4, 1H), 9.02 (d, J = 4.4, 1H), 9.17 (d, J = 8.4, 1H), 9.73 (s, 1H) ppm.

2-(8-Carboxybenzo[*c*][2,6]naphthyridin-5-ylamino)-*N*,*N*-dimethylethanaminium Trifluoroacetate (25b). Yield, 45%. LCMS (ES): >95% pure, *m*/*z* 311 $[M + H]^+$, 266 $[M + H - NHMe_2]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.90 (s, 6H), 3.47 (br t, 2H), 3.95 (br s, 2H), 7.88 (dd, J = 8.4, J = 2.0, 1H), 8.24 (m, 2H), 8.41 (br s, 1H), 8.79 (d, J = 8.8, 1H), 8.94 (d, J = 5.2, 1H), 9.49 (br, 1H), 10.12 (s, 1H) ppm.

5-(Cyclopentylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25c). Yield, 39%. LCMS (ES): 94% pure, *m*/*z* 308 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62−1.77 (m, 6H), 2.12 (m, 2H), 4.64 (sext, J = 6.8, 1H), 7.70 (br, 1H), 7.84 (m, 1H), 8.16 (br s, 1H), 8.42 (br s, 1H), 8.74 (d, J = 8.0, 1H), 8.89 (m, 1H), 10.07 (s, 1H), 13.0 (br s, 1H) ppm.

5-(Methoxyamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25d). Yield, 26%. LCMS (ES): 94% pure, *m*/*z* 270 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.92 (s, 3H), 7.55 (d, J = 8.0, 1H), 7.83 (d, J = 5.2, 1H), 8.09 (s, 1H), 8.35 (d, J = 8.4, 1H), 8.58 (d, J = 5.6, 1H), 9.56 (s, 1H), 10.30 (s, 1H), 12.24 (br s, 1H) ppm.

5-(Cyclopropylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25e). Yield, 49%. LCMS (ES): 85% pure, *m*/*z* 280 $[M + H]^+$.

5-(2-Isopropoxyethylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25f). Yield, 30%. LCMS (ES): 91% pure, *m*/*z* 326 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (d, J = 6.0, 6H), 3.61−3.76 (m, 5H), 7.84 (d, J = 8.5, 1H), 8.06 (br, 1H), 8.16 (br s, 1H), 8.30 (d, J = 6.4, 1H), 8.75 (d, J = 8.5, 1H), 8.90 (d, J = 6.4, 1H), 10.08 (s, 1H), 13.10 (br s, 1H) ppm.

5-(Benzylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25g). Yield, 69%. LCMS (ES): >95% pure, *m*/*z* 330 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.87 (s, 2H), 7.23 (t, J = 6.8, 1H), 7.33 (m, 2H), 7.47 (m, 2H), 7.83 (d,

J = 8.4, 1H), 8.13 (s, 1H), 8.37 (d, J = 4.4, 1H), 8.73 (d, J = 8.0, 1H), 8.91 (J = 4.8, 1H), 10.08 (s, 1H), 12.20 (br s, 1H) ppm.

5-(Phenethylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25h). Yield, 33%. LCMS (ES): >95% pure, m/z 344 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 3.21 (t, J = 6.8, 2H), 4.11 (t, J = 7.2, 2H), 7.21 (m, 1H), 7.31 (m, 2H), 7.37 (m, 2H), 8.20 (dd, J = 8, J = 1.2, 1H), 8.39 (br d, J = 6, 1H), 8.58 (d, J = 1.6, 1H), 8.80 (d, J = 8.4, 1H), 9.02 (d, J = 6, 1H), 10.10 (s, 1H) ppm.

5-(3-Phenylpropylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25i). Yield, 50%. LCMS (ES): >95% pure, m/z 358 $[M + H]^+$. ^{1}HNMR (400 MHz, DMSO-d_6): δ 2.09 (qi, J = 7.2, 2H), 2.77 (t, J = 7.2, 2H), 3.72 (br q, J = 4.8, 2H), 7.18 (m, 1H), 7.28 (m, 4H), 7.91 (d, J = 7.6, 1H), 8.40 (br s, 1H), 8.44 (d, J = 4.0, 1H), 8.78 (d, J = 8.4, 1H), 8.97 (d, J = 5.2, 1H), 10.09 (s, 1H) ppm.

5-(3-Methoxyphenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25j). Yield, 48%. LCMS (ES): >95% pure, m/z 346 $[M + H]^+$. ^{1}HNMR (400 MHz, CD_3OD): δ 3.89 (s, 3H), 7.05 (d, J = 8, 1H), 7.28 (d, J = 7.6, 1H), 7.38 (br s, 1H), 7.52 (t, J = 8, 1H), 8.20 (d, J = 8.8, 1H), 8.51 (s, 1H), 8.56 (d, J = 5.6, 1H), 8.82 (d, J = 8.4, 1H), 9.05 (d, J = 5.6, 1H), 10.16 (s, 1H) ppm.

5-(3-Chloro-4-fluorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25k). Yield, 40%. LCMS (ES): 95% pure, m/z 368 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.34 (t, J = 9.2, 1H), 7.84−7.88 (m, 1H), 8.11 (dd, J = 8.0, J = 1.2, 1H), 8.20 (dd, J = 6.8, J = 2.8, 1H), 8.42 (d, J = 1.6, 1H), 8.48 (d, J = 5.6, 1H), 8.75 (d, J = 8.0, 1H), 8.94 (d, J = 6.0, 1H), 10.11 (s, 1H) ppm.

5-(3-Fluorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25l). Yield, 22%. LCMS (ES): >95% pure, m/z 334 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.90 (td, J = 8.4, J = 2.8, 1H), 7.43 (q, J = 8.4, 1H), 7.87 (d, J = 8.4, 1H), 7.96 (dd, J = 8.4, J = 2.0, 1H), 8.14 (br d, J = 12, 1H), 8.60 (d, J = 5.6, 1H), 8.86 (d, J = 8.4, 1H), 9.00 (d, J = 6,0, 1H), 9.72 (br s, 1H), 10.18 (s, 1H) ppm.

5-(2-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25m). Yield, 22%. LCMS (ES): 94% pure, m/z 350 $[M + H]^+$.

Sodium 5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylate (25n). Yield, 86%. LCMS (ES): >95% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.10 (dd, J = 7.6, J = 2.0, 1H), 7.43 (t, J = 8.0, 1H), 8.04 (dd, J = 8.4, J = 2.0, 1H), 8.13 (dd, J = 8.0, J = 1.6, 1H), 8.27 (d, J = 1.6, 1H), 8.46 (t, J = 2.0, 1H), 8.65 (d, J = 7.6, 1H), 8.67 (d, J = 8.4, 1H), 8.90 (d, J = 6.0, 1H), 9.75 (s, 1H), 10.2 (s, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 116.0, 116.43, 118.64, 119.19, 119.61, 120.70, 121.53, 125.67, 127.0, 127.56, 129.96, 132.74, 142.12, 142.44, 143.18, 146.38, 147.37, 149.3, 169.25 ppm.

5-(4-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25o). Yield, 62%. LCMS (ES): 95% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.48 (d, J = 8.0, 2H), 7.96 (d, J = 7.0, 1H), 8.17 (d, J = 6.4, 2H), 8.27 (s, 1H), 8.61 (d, J = 5.9, 1H), 8.86 (d, J = 8.8, 1H), 9.00 (d, J = 5.9, 1H), 9.69 (s, 1H), 10.19 (s, 1H), 13.20 (br s, 1H) ppm.

5-(3-Ethynylphenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25p). Yield, 61%. LCMS (ES): 95% pure, m/z 340 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.21 (s, 1H), 7.21 (d, J = 7.6, 1H), 7.98 (dd, J = 8.4, J = 2.0, 1H), 8.22 (br d, J = 8, 1H), 8.26 (br s, 1H), 8.28 (br s, 1H), 8.63 (d, J = 6, 1H), 8.86 (d, J = 8.8, 1H), 9.00 (d, J = 6, 1H), 9.66 (s, 1H), 10.19 (s, 1H) ppm.

5-(3-Cyanophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid Trifluoroacetate Salt (25q). Yield, 30%. LCMS (ES): 95% pure, m/z 341 $[M + H]^+$.

5-(4-Phenoxyphenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25r). Yield, 54%. LCMS (ES): 95% pure, m/z 408 $[M + H]^+$.

5-(3-Phenoxyphenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25s). Yield, 70%. LCMS (ES): 90% pure, m/z 408 $[M + H]^+$.

5-(3-Sulfamoylphenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylic Acid (25t). Yield, 26%. LCMS (ES): >95% pure, m/z 395 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.43 (s, 2H), 7.56 (d, J = 8.0, 1H), 7.64 (t, J = 7.6, 1H), 7.99 (dd, J = 8.4, J = 1.6, 1H), 8.32 (d, J = 1.6, 1H), 8.40 (d, J = 8.0, 1H), 8.68 (m, 2H), 8.88 (d, J = 8.4, 1H), 9.02 (d, J = 5.6, 1H), 9.89 (s, 1H), 10.20 (s, 1H), 13.20 (br s, 1H) ppm.

Sodium 5-(3-Chlorophenylamino)-7-methylbenzo[*c*][2,6]naphthyridine-8-carboxylate (41a). Yield, 46%. LCMS (ES): >95% pure, m/z 364 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.76 (s, 3H), 7.08 (dd, J = 8, J = 2, 1H), 7.41 (t, J = 8.4, 1H), 7.47 (d, J = 8.4, 1H), 8.00 (dd, J = 8.4, J = 2, 1H), 8.45 (d, J = 8.4, 1H), 8.55 (d, J = 5.6, 1H), 8.61 (d, J = 2, 1H), 8.86 (d, J = 2, 1H), 9.66 (s, 1H), 10.08 (s, 1H) ppm.

5-(3-Chlorophenylamino)-9-methylbenzo[*c*][2,6]naphthyridine-8-carboxylic Acid (41c). Yield, 16%. LCMS (ES): >95% pure, m/z 364 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.73 (s, 3H), 7.15 (d, J = 8.0, 1H), 7.45 (t, J = 8.0, 1H), 8.08 (d, J = 6, 1H), 8.20 (s, 1H), 8.29 (br s, 1H), 8.65 (m, 1H), 8.73 (s, 1H), 9.01 (d, J = 6.0, 1H), 9.74 (br s, 1H), 10.22 (br s, 1H) ppm.

5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-9-carboxylic Acid (41d). Yield, 21%. LCMS (ES): 91% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (d, J = 8.4, 1H), 7.47 (t, J = 8.0, 1H), 7.83 (d, J = 8.8, 1H), 8.09 (d, J = 8.4, 1H), 8.21 (d, J = 8.8, 1H), 8.34 (br s, 1H), 8.69 (d, J = 5.6, 1H), 9.03 (br d, J = 5.6, 1H), 9.29 (s, 1H), 9.90 (br s, 1H), 10.23 (br s, 1H) ppm.

5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-7-carboxylic Acid (41b). Yield, 9%. LCMS (ES): 95% pure, m/z 350 $[M + H]^+$.

5-(3-Chlorophenylamino)benzo[*f*][1,7]naphthyridine-8-carboxylic Acid (28i). Yield, 10%. LCMS (ES): >95% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.12 (dd, J = 7, J = 2, 1H), 7.43 (t, J = 7, 1H), 7.97 (dd, J = 7, J = 2, 1H), 8.05 (dd, J = 7, J = 4, 1H), 8.22 (dd, J = 7, J = 2, 1H), 8.32 (d, J = 1.6, 1H), 8.62 (t, J = 2, 1H), 8.72 (d, J = 7, 1H), 9.12 (dd, J = 5, J = 2, 1H), 9.28 (d, J = 7, 1H), 10.12 (s, 1H), 12.25 (br s, 1H) ppm.

5-(3-Chlorophenylamino)benzo[*c*][2,7]naphthyridine-8-carboxylic Acid (28d). Yield, 40%. LCMS (ES): 89% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.15 (dd, J = 8, J = 2, 1H), 7.45 (t, J = 8, 1H), 7.96 (d, J = 8, 1H), 8.08 (d, J = 8, 1H), 8.25 (d, J = 2, 1H), 8.31 (t, J = 2, 1H), 8.70 (d, J = 5.6, 1H), 8.74 (d, J = 8.4, 1H), 9.01 (d, J = 6, 1H), 9.82 (s, 1H), 10.00 (s, 1H), 13.3 (br s, 1H) ppm.

5-(3-Chlorophenylamino)benzo[*h*][1,6]naphthyridine-8-carboxylic Acid (28e). Yield, 18%. LCMS (ES): 95% pure, m/z 350 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.11 (d, J = 7.2, 1H),7.42 (t, J = 7.6, 1H), 7.84 (br m, 1H), 7.95 (d, J = 8, 1H), 8.05 (d, J = 7.6, 1H), 8.22 (s, 1H), 8.30 (s, 1H), 8.86 (d, J = 8.4, 1H), 9.06 (d, J = 8, 1H), 9.16 (s, 1H), 9.61 (s, 1H), 13.18 (br s, 1H) ppm.

5-(3-Chlorophenylamino)pyrazino[2,3-*c*]quinoline-8-carboxylic Acid (28f). Yield, 83%. LCMS (ES): >95% pure, m/z 351 $[M + H]^+$. ^{1}HNMR (400 MHz, DMSO-d_6): δ 7.14 (dd, J = 8.4, J = 1.6, 1H), 7.44 (t, J = 8.4, 1H), 8.02 (dd, J = 8.4, J = 1.6, 1H), 8.26 (br d, J = 7.6, 1H), 8.32 (br s, 1H), 8.56 (m, 1H), 8.84 (d, J = 8.4, 1H), 9.17 (d, J = 2.4, 1H), 9.33 (d, J = 2.0, 1H), 10.16 (s, 1H), 13.3 (br s, 1H) ppm.

5-(3-Chlorophenylamino)pyrimido[4,5-*c*]quinoline-8-carboxylic Acid (28g). Yield, 83%. LCMS (ES): >95% pure, m/z 351 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14 (dd, J = 7.6, J = 2.0, 1H), 7.44 (t, J = 8.0, 1H), 8.02 (dd, J = 8.4, J = 1.6, 1H), 8.26 (d, J = 8.0, 1H), 8.32 (d, J = 1.6, 1H), 8.56 (t, J = 2.4, 1H), 8.86 (d, J = 8.4, 1H), 9.66 (s, 1H), 10.18 (s, 1H), 10.44 (s, 1H), 13.33 (br s, 1H) ppm.

***N*-(3-Chlorophenyl)-8-(1*H*-tetrazol-5-yl)benzo[*c*][2,6]naphthyridin-5-amine Trifluoroacetate Salt (26f).** A mixture of the 5-chlorobenzo[*c*][2,6]naphthyridine-8-carbonitrile **24b** (25 mg, 0.104 mmol), 3-chloroaniline (50 mg, 0.394 mmol), and NMP (0.2 mL) was heated to 100 °C in a microwave reactor for 20 min. LCMS indicated the absence of starting material. Sodium azide (100 mg, 1.53 mmol), ammonium chloride (100 mg, 1.89 mmol), and DMF (0.2 mL) were added to the crude mixture. The

mixture was heated for 18 h at 125 °C, at which time LCMS analysis indicated the absence of any starting material. The mixture was diluted with water and purified by preparative HPLC to give the title compound **26f** (12 mg, 24%). LCMS (ES): >95% pure, m/z 374 $[M + H]^+$, 346 $[M + H - N_2]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.18 (dd, J = 7.5, J = 1.6, 1H), 7.47 (t, J = 8, 1H), 8.08 (d, J = 7.5, 1H), 8.15 (dd, J = 8.4, J = 1.6, 1H), 8.31 (br s, 1H), 8.43 (d, J = 1.6, 1H), 8.68 (d, J = 6, 1H), 9.00 (d, J = 8.8, 1H), 9.03 (d, J = 6, 1H), 9.83 (br s, 1H), 10.24 (s, 1H) ppm.

5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxamide (26e). Sodium 5-(3-chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxylate **25n** (1.0 equiv, 58 mg, 0.16 mmol) was reacted with HOBt (2.0 equiv, 42 mg, 0.31 mmol), ammonium chloride (7.7 equiv, 65 mg, 1.21 mmol), DIEA (4.0 equiv, 109 μL, 0.63 mmol), and EDCI (2.0 equiv, 60 mg, 0.31 mmol) in NMP (0.5 mL) at 70 °C for 30 min. Water was added, and the resulting precipitate was filtered and washed with water. The crude material was suspended in a mixture of EtOAc and hexanes, filtered, and dried. The title compound **26e** was isolated as a tan solid (27 mg, 50%). LCMS (ES): >95% pure, m/z 349 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.15 (br d, J = 9.2, 1H), 7.45 (t, J = 8.4, 1H), 7.54 (br s, 1H), 7.97 (dd, J = 1.6, J = 8.4, 1H), 8.11 (dd, J = 1.2, J = 8, 1H), 8.279 (br s, 1H), 8.284 (s, 1H), 8.31 (t, J = 2, 1H), 8.59 (d, J = 6, 1H), 8.85 (d, J = 8.4, 1H), 8.99 (d, J = 6, 1H), 9.67 (s, 1H), 10.20 (s, 1H) ppm.

***N*-(3-Chlorophenyl)-8-(4*H*-1,2,4-triazol-3-yl)benzo[*c*][2,6]naphthyridin-5-amine (26g).** 5-(3-Chlorophenylamino)benzo[*c*][2,6]naphthyridine-8-carboxamide **26e** (1.0 equiv, 36 mg) was stirred in *N*,*N*-dimethylformamide dimethyl acetal (2 mL) at 80 °C for 4 h. The volatiles were removed in vacuo. Acetic acid (0.5 mL) and hydrazine hydrate (0.1 mL) were added. The mixture was stirred at 80 °C for 1 h. Water was added and the solid filtered and dried. After trituration in a mixture of CH_2Cl_2 and hexanes, the title compound **26g** was isolated as a solid (22 mg, 67%). LCMS (ES): 95% pure, m/z 373 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.15 (br d, J = 7.4, 1H), 7.46 (t, J = 8.4, 1H), 8.10 (d, J = 7.5, 1H), 8.15 (d, J = 7.5, 1H), 8.36 (s, 1H), 8.39 (s, 1H), 8.59 (d, J = 5.6, 1H), 8.89 (d, J = 5.6, 1H), 8.97 (d, J = 5.6, 1H), 9.68 (s, 1H), 10.18 (s, 1H) ppm.

CK2 Kinase Assay. Test compounds in aqueous solution were added at a volume of 10 μL to a reaction mixture comprising 10 μL of assay dilution buffer (ADB; 20 mM MOPS, pH 7.2, 25 mM β-glycerolphosphate, 5 mM EGTA, 1 mM sodium orthovanadate, and 1 mM dithiothreitol), 10 μL of substrate peptide (RRRDDDSDDD, dissolved in ADB at a concentration of 1 mM), 10 μL of recombinant human CK2 ($\alpha\alpha\beta\beta$-holoenzyme, 25 ng dissolved in ADB; Millipore). Reactions were initiated by the addition of 10 μL of ATP solution (90% 75 mM $MgCl_2$, 75 μM ATP (final ATP concentration = 15 μM) dissolved in ADB; 10% [γ-33P]ATP (stock 1 mCi/100 μL; 3000 Ci/mmol (Perkin-Elmer) and maintained for 10 min at 30 °C. The reactions were quenched with 100 μL of 0.75% phosphoric acid and then transferred to and filtered through a phosphocellulose filter plate (Millipore). After washing each well five times with 0.75% phosphoric acid, the plate was dried under vacuum for 5 min and, following the addition of 15 μL of scintillation fluid to each well, the residual radioactivity was measured using a Wallac luminescence counter. The IC_{50} values were derived from eight concentrations of test inhibitors.

25n K_i Measurement. The K_i value (inhibition constant) for **25n** against recombinant human CK2 ($\alpha\alpha\beta\beta$-holoenzyme) was determined by plotting the IC_{50} values of **25n** determined in the presence of various concentrations of ATP against the concentration of ATP (performed at Millipore). The K_i value is equivalent to the Y-intercept according to the Cheng−Prussoff equation[62] ($K_i = IC_{50}/(1 + [ATP]/K_m)$, where K_i is the inhibition constant and K_m is the Michaelis constant.

PARP1 Assay. PARP assays were conducted using a chemiluminescent PARP1 assay kit (Trevigen). Briefly, reactions were performed in histone-coated strip wells, by adding 10 μL of test compound dissolved in 1× PARP buffer (prepared by mixing 20× PARP buffer diluted with high-purity water) and 15 μL of diluted PARP-HSA enzyme (diluted in 1× PARP buffer, 0.1 unit per well) to 25 μL of PARP1 cocktail (prepared from 10× PARP cocktail and 10× activated DNA, both 2.5 μL per well and 20 μL per well of 1× PARP buffer). The reactions were incubated at ambient temperature for 60 min, and then the liquid was removed. After washing the wells four times with PBS (200 μL), 50 μL of STREP-HRP (horseradish peroxidase) solution (diluted 500-fold in 1× Strep-Diluent) was added, and the reactions were allowed to incubate for 30 min at ambient temperature. The liquid was removed and, after washing the wells four times with PBS (200 μL), 50 μL each of PeroxyGlo A and B (chemiluminescent horseradish peroxidase substrates) were added and the resulting chemiluminescence quantified on the SpectraMax M5 plate reader.

Kinase Selectivity Panel. The 238 kinase selectivity panel was conducted using the Kinase Profiler service offered by Millipore (Billerica, MA), which utilizes a radiometric filter-binding assay. The % inhibition of each kinase was estimated using 0.5 μM of **25n** at ATP concentrations equivalent to the K_m value for ATP for each respective human recombinant kinase. The determination of IC_{50} values was conducted at similar concentrations of ATP (equivalent to the K_m for ATP) using nine concentrations of **25n** over a range of 0.0001−1 μM.

Cell Viability Assay. Various cell lines were seeded at a density of 3000 cells per well 24 h prior to treatment, in appropriate media, and then treated with indicated concentrations of CK2 inhibitors. Suspension cells were seeded and treated on the same day. Following 4 days of incubation, 20 μL of Alamar Blue (10% of volume/well) was added and the cells were further incubated at 37 °C for 4−5 h. Fluorescence with excitation wavelength at 530−560 nm and emission wavelength at 590 nm was measured.

Endogenous CK2 Assay. The human leukemia Jurkat T-cell line were cultured in RPMI 1640 (Cambrex) supplemented with 10% fetal calf serum and 50 ng/mL gentamycin. Before treatment, cells were washed, resuspended at a density of ~106 cells/mL in medium containing 1% fetal calf serum, and incubated in the presence of **25n** at 0.01, 0.1, and 1.0 μM for 2 h. Cells were recovered by centrifugation, lysed using a hypotonic buffer (20 mMTris/HCl pH 7.4; 2 mM EDTA; 5 mM EGTA; 10 mM mercaptoethanol; 10 mM NaF; 1 μM okadaic acid; 10% v/v glycerol; 0.05% NP-40; 1% protease inhibitor cocktail) and protein from the cleared lysate was diluted to 1 μg per μL in assay dilution buffer (ADB; 20 mM MOPS, pH 7.2, 25 mM β-glycerolphosphate, 5 mM EGTA, 1 mM sodium orthovanadate, and 1 mM dithiothreitol). To 20 μL of diluted protein was added 10 μL of substrate peptide (RRRDDDSDDD, dissolved in ADB at a concentration of 1 mM) and 10 μL of PKA inhibitor cocktail (Upstate/Millipore). Reactions were initiated by the addition of 10 μL of ATP solution (90% 75 mM $MgCl_2$, 500 μL ATP dissolved in ADB; 10% [γ-^{32}P]ATP: stock 1 mCi/100 μL; 3000 Ci/mmol (Perkin-Elmer) and maintained for 15 min at 32 °C. The reactions were quenched with 100 μL of 0.75% phosphoric acid and then transferred to and filtered through a phosphocellulose filter plate (Millipore). After washing each well 5 times with 0.75% phosphoric acid, the residual radioactivity was measured using a Wallac luminescence counter.

In Vivo Pharmacokinetics. The pharmacokinetic profile of **25n** was investigated in overnight fasted ICR mice, SD rats, and beagle dogs following a single dose given intravenously (iv) and orally (po). The compound was a sodium salt formulated in phosphate buffer for the mice and rats and as a sodium salt dry powder filled capsule for the dogs. Eight mice (four for each leg), six rats (three for each leg), and six dogs (three for each leg) were treated with **25n**. Blood samples of each mouse were collected at 0.08, 0.25, 0.50, 1, 2, 4, 6, 8, and 24 h following iv dosing and at 0.25, 0.50, 1, 2, 4, 6, 8, and 24 h following oral dosing. Blood samples of each rat were collected at 0.08, 0.25, 0.50, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h following iv dosing and at 0.25, 0.50, 1, 2, 4,

6, 8, 12, 24, 36, and 48 h following oral dosing. Blood samples of each dog were collected at 0.08, 0.25, 0.50, 1, 2, 4, 6, 8, 12, and 24 h following iv dosing and at 0.25, 0.50, 1, 2, 4, 6, 8, 12, and 24 h following oral dosing. Samples were centrifuged at 8000 rpm for 5 min and the plasma collected and stored at −80 °C until analysis. Samples were analyzed by LC-MS/MS technique. The pharmacokinetic parameters were derived by noncompartmental analysis.

In Vivo Antitumor Efficacy in PC3 Xenograft. Tumors were initiated by subcutaneous injection of PC3 tumor cell suspensions into the right hind flank region of each mouse. When tumors reached a designated volume of 150−200 mm^3 mice were randomized and divided into groups of 10 mice per group. The sodium salt of **25n** was administered as a solution in 25 mM NaH_2PO_4 buffer, by oral gavage twice daily at 25 mg/kg, 50 mg/kg and 75 mg/kg. Tumor volumes and body weights were measured twice weekly. The length and width of the tumor were measured with calipers and the volume calculated using the following formula: tumor volume = (length × $width^2$)/2. Percent tumor growth inhibition (TGI) values were calculated on the final day of the study for **25n**-treated compared to vehicle-treated mice and were calculated as 100 × {1 − [(TreatedFinal Day − TreatedDay 1)/(ControlFinal Day − ControlDay 1)]}. The significance of the differences between the treated versus vehicle groups were determined using one-way ANOVA (Graphpad Prism).

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References

(1) Duncan, J. S.; Litchfield, D. W. Too much of a good thing: the role of protein kinase CK2 in tumorigenesis and prospects for therapeutic inhibition of CK2. *Biochim. Biophys. Acta* **2007**, *1784* (1), 33–47.
(2) Meggio, F.; Pinna, L. A. One-thousand-and-one substrates of protein kinase CK2? *FASEB J.* **2003**, *17* (3), 349–368.
(3) Pagano, M. A.; Cesaro, L.; Meggio, F.; Pinna, L. A. Protein kinase CK2: a newcomer in the "druggable kinome". *Biochem. Soc. Trans.* **2006**, *34*, 1303–1306.
(4) Ahmad, K. A.; Wang, G.; Unger, G.; Slaton, J.; Ahmed, K. Protein kinase CK2—a key suppressor of apoptosis. *Adv. Enzyme Regul.* **2008**, *48*, 179–187.
(5) Guerra, B.; Issinger, O. G. Protein kinase CK2 in human diseases. *Curr. Med. Chem.* **2008**, *15* (19), 1870–1886.
(6) Kramerov, A. A.; Saghizadeh, M.; Caballero, S.; Shaw, L. C.; Li Calzi, S.; Bretner, M.; Montenarh, M.; Pinna, L. A.; Grant, M. B.; Ljubimov, A. V. Inhibition of protein kinase CK2 suppresses angiogenesis and hematopoietic stem cell recruitment to retinal neovascularization sites. *Mol. Cell. Biochem.* **2008**, *316* (1−2), 177–186.
(7) Sarno, S.; Pinna, L. A. Protein kinase CK2 as a druggable target. *Mol. Biosyst.* **2008**, *4* (9), 889–894.
(8) Faust, R. A.; Tawfic, S.; Davis, A. T.; Bubash, L. A.; Ahmed, K. Antisense oligonucleotides against protein kinase CK2-alpha inhibit growth of squamous cell carcinoma of the head and neck in vitro. *Head Neck* **2000**, *22* (4), 341–346.
(9) Landesman-Bollag, E.; Romieu-Mourez, R.; Song, D. H.; Sonenshein, G. E.; Cardiff, R. D.; Seldin, D. C. Protein kinase CK2 in mammary gland tumorigenesis. *Oncogene* **2001**, *20* (25), 3247–3257.
(10) Pistorius, K.; Seitz, G.; Remberger, K.; Issinger, O. G. Differential CKII Activities in Human Colorectal Mucosa, Adenomas and Carcinomas. *Onkologie* **1991**, *14* (3), 256–260.
(11) Stalter, G.; Siemer, S.; Becht, E.; Ziegler, M.; Remberger, K.; Issinger, O. G. Asymmetric expression of protein kinase CK2 subunits in human kidney tumors. *Biochem. Biophys. Res. Commun.* **1994**, *202* (1), 141–147.
(12) O-charoenrat, P.; Rusch, V.; Talbot, S. G.; Sarkaria, I.; Viale, A.; Socci, N.; Ngai, I.; Rao, P.; Singh, B. Casein kinase II alpha subunit and C1-inhibitor are independent predictors of outcome in patients with squamous cell carcinoma of the lung. *Clin. Cancer Res.* **2004**, *10* (17), 5792–5803.
(13) Kim, J. S.; Eom, J. I.; Cheong, J. W.; Choi, A. J.; Lee, J. K.; Yang, W. I.; Min, Y. H. Protein kinase CK2alpha as an unfavorable prognostic marker and novel therapeutic target in acute myeloid leukemia. *Clin. Cancer Res.* **2007**, *13* (3), 1019–1028.
(14) Yenice, S.; Davis, A. T.; Goueli, S. A.; Akdas, A.; Limas, C.; Ahmed, K. Nuclear casein kinase 2 (CK-2) activity in human normal, benign hyperplastic, and cancerous prostate. *Prostate* **1994**, *24* (1), 11–16.
(15) Laramas, M.; Pasquier, D.; Filhol, O.; Ringeisen, F.; Descotes, J. L.; Cochet, C. Nuclear localization of protein kinase CK2 catalytic subunit (CK2alpha) is associated with poor prognostic factors in human prostate cancer. *Eur. J. Cancer* **2007**, *43* (5), 928–934.
(16) Tawfic, S.; Yu, S.; Wang, H.; Faust, R.; Davis, A.; Ahmed, K. Protein kinase CK2 signal in neoplasia. *Histol. Histopathol.* **2001**, *16* (2), 573–582.
(17) Ruzzene, M.; Pinna, L. A. Addiction to protein kinase CK2: a common denominator of diverse cancer cells? *Biochim. Biophys. Acta* **2010**, *1804* (3), 499–504.
(18) Luo, J.; Solimini, N. L.; Elledge, S. J. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* **2009**, *136* (5), 823–837.
(19) Slaton, J. W.; Unger, G. M.; Sloper, D. T.; Davis, A. T.; Ahmed, K. Induction of apoptosis by antisense CK2 in human prostate cancer xenograft model. *Mol. Cancer Res.* **2004**, *2* (12), 712–721.
(20) Trembley, J. H.; Chen, Z.; Unger, G.; Slaton, J.; Kren, B. T.; Van Waes, C.; Ahmed, K. Emergence of protein kinase CK2 as a key target in cancer therapy. *Biofactors* **2010**, *36* (3), 187–195.
(21) Pagano, M. A.; Bain, J.; Kazimierczuk, Z.; Sarno, S.; Ruzzene, M.; Di Maira, G.; Elliott, M.; Orzeszko, A.; Cozza, G.; Meggio, F.; Pinna, L. A. The selectivity of inhibitors of protein kinase CK2. An update. *Biochem. J.* **2008**, *415* (3), 353–365.
(22) Sarno, S.; de Moliner, E.; Ruzzene, M.; Pagano, M. A.; Battistutta, R.; Bain, J.; Fabbro, D.; Schoepfer, J.; Elliott, M.; Furet, P.; Meggio, F.; Zanotti, G.; Pinna, L. A. Biochemical and three-dimensional-structural study of the specific inhibition of protein kinase CK2 by [5-oxo-5,6-dihydroindolo-(1,2-*a*)quinazolin-7-yl]-acetic acid (IQA). *Biochem. J.* **2003**, *374* (3), 639–646.
(23) Lopez-Ramos, M.; Prudent, R.; Moucadel, V.; Sautel, C. F.; Barette, C.; Lafanechere, L.; Mouawad, L.; Grierson, D.; Schmidt, F.; Florent, J. C.; Filippakopoulos, P.; Bullock, A. N.; Knapp, S.; Reiser, J. B.; Cochet, C. New potent dual inhibitors of CK2 and Pim kinases: discovery and structural insights. *FASEB J.* **2010**, *24*, 3171–3185.
(24) Sandholt, I. S.; Olsen, B. B.; Guerra, B.; Issinger, O. G. Resorufin: a lead for a new protein kinase CK2 inhibitor. *Anticancer Drugs* **2009**, *20* (4), 238–248.
(25) Nakaniwa, T.; Kinoshita, T.; Sekiguchi, Y.; Tada, T.; Nakanishi, I.; Kitaura, K.; Suzuki, Y.; Ohno, H.; Hirasawa, A.; Tsujimoto, G. Structure of human protein kinase CK2alpha2 with a potent indazole-derivative inhibitor. *Acta Crystallogr., Sect F: Struct. Biol. Cryst. Commun.* **2009**, *65* (2), 75–79.
(26) Najda-Bernatowicz, A.; Lebska, M.; Orzeszko, A.; Kopanska, K.; Krzywinska, E.; Muszynska, G.; Bretner, M. Synthesis of new analogs of benzotriazole, benzimidazole and phthalimide-potential inhibitors of human protein kinase CK2. *Bioorg. Med. Chem.* **2009**, *17* (4), 1573–1578.
(27) Hung, M. S.; Xu, Z.; Lin, Y. C.; Mao, J. H.; Yang, C. T.; Chang, P. J.; Jablons, D. M.; You, L. Identification of hematein as a novel inhibitor of protein kinase CK2 from a natural product library. *BMC Cancer* **2009**, *9* (1), 135.
(28) Gianoncelli, A.; Cozza, G.; Orzeszko, A.; Meggio, F.; Kazimierczuk, Z.; Pinna, L. A. Tetraiodobenzimidazoles are potent inhibitors of protein kinase CK2. *Bioorg. Med. Chem.* **2009**, *17* (20), 7281–7289.
(29) Cozza, G.; Mazzorana, M.; Papinutto, E.; Bain, J.; Elliott, M.; Di Maira, G.; Gianoncelli, A.; Pagano, M. A.; Sarno, S.; Ruzzene, M.; Battistutta, R.; Meggio, F.; Moro, S.; Zagotto, G.; Pinna, L. A. Quinalizarin as a potent, selective and cell permeable inhibitor of protein kinase CK2. *Biochem. J.* **2009**, *421* (3), 387–395.
(30) Suzuki, Y.; Cluzeau, J.; Hara, T.; Hirasawa, A.; Tsujimoto, G.; Oishi, S.; Ohno, H.; Fujii, N. Structure−activity relationships of pyrazine-based CK2 inhibitors: synthesis and evaluation of 2,6-disubstituted pyrazines and 4,6-disubstituted pyrimidines. *Arch. Pharm. (Weinheim, Ger.)* **2008**, *341* (9), 554–561.
(31) Prudent, R.; Lopez-Ramos, M.; Moucadel, V.; Barette, C.; Grierson, D.; Mouawad, L.; Florent, J. C.; Lafanechere, L.; Schmidt, F.; Cochet, C. Salicylaldehyde derivatives as new protein kinase CK2 inhibitors. *Biochim. Biophys. Acta* **2008**, *1780* (12), 1412–1420.
(32) Cozza, G.; Bortolato, A.; Moro, S. How druggable is protein kinase CK2? *Med. Res. Rev.* **2009**, *30* (3), 419–462.
(33) Sarno, S.; Ruzzene, M.; Frascella, P.; Pagano, M. A.; Meggio, F.; Zambon, A.; Mazzorana, M.; Di Maira, G.; Lucchini, V.; Pinna, L. A. Development and exploitation of CK2 inhibitors. *Mol. Cell. Biochem.* **2005**, *274* (1−2), 69–76.

(34) Zandomeni, R. Z.; Zandomeni, M. C.; Shugar, D.; Weinmann, R. Casein kinase type II is involved in the inhibition by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole of specific RNA polymerase II transcription. *J. Biol. Chem.* **1986**, *261*, 3414–3419.
(35) Yim, H. L.; Lee, Y. H.; Lee, C. H.; Lee, S. K. Emodin, an anthraquinone derivative isolated from the rhizomes of *Rheum palmatum*, selectively inhibits the activity of casein kinase II as a competitive inhibitor. *Planta Med.* **1999**, *65*, 9–13.
(36) Pagano, M. A.; Andrzejewska, M.; Ruzzene, M.; Sarno, S.; Cesaro, L.; Bain, J.; Elliott, M.; Meggio, F.; Kazimierczuk, Z.; Pinna, L. A. Optimization of protein kinase CK2 inhibitors derived from 4,5,6,7-tetrabromobenzimidazole. *J. Med. Chem.* **2004**, *47* (25), 6239–6247.
(37) Ruzzene, M.; Penzo, D.; Pinna, L. A. Protein kinase CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) induces apoptosis and caspase-dependent degradation of haematopoietic lineage cell-specific protein 1 (HS1) in Jurkat cells. *Biochem. J.* **2002**, *364* (1), 41–47.
(38) Zien, P.; Bretner, M.; Zastapilo, K.; Szyszka, R.; Shugar, D. Selectivity of 4,5,6,7-tetrabromobenzimidazole as an ATP-competitive potent inhibitor of protein kinase CK2 from various sources. *Biochem. Biophys. Res. Commun.* **2003**, *306* (1), 129–133.
(39) DePierre, J. W., Mammalian Toxicity of Organic Compounds of Bromine and Iodine. In *Organic Bromine and Iodine Compounds*, Neilson, A. H., Ed.; Springer-Verlag: Berlin-Heidelberg-New York, 2003; Vol. 3R, pp 221−232.
(40) Vangrevelinghe, E.; Zimmermann, K.; Schoepfer, J.; Portmann, R.; Fabbro, D.; Furet, P. Discovery of a potent and selective protein kinase CK2 inhibitor by high-throughput docking. *J. Med. Chem.* **2003**, *46* (13), 2656–2662.
(41) Nie, Z.; Perretta, C.; Erickson, P.; Margosiak, S.; Almassy, R.; Lu, J.; Averill, A.; Yager, K. M.; Chu, S. Structure-based design, synthesis, and study of pyrazolo[1,5-*a*][1,3,5]triazine derivatives as potent inhibitors of protein kinase CK2. *Bioorg. Med. Chem. Lett.* **2007**, *17* (15), 4191–4195.
(42) Nie, Z.; Perretta, C.; Erickson, P.; Margosiak, S.; Lu, J.; Averill, A.; Almassy, R.; Chu, S. Structure-based design and synthesis of novel macrocyclic pyrazolo[1,5-*a*] [1,3,5]triazine compounds as potent inhibitors of protein kinase CK2 and their anticancer activities. *Bioorg. Med. Chem. Lett.* **2008**, *18* (2), 619–623.
(43) Zhu, D.; Hensel, J.; Hilgraf, R.; Abbasian, M.; Pornillos, O.; Deyanat-Yazdi, G.; Hua, X. H.; Cox, S. Inhibition of protein kinase CK2 expression and activity blocks tumor cell growth. *Mol. Cell. Biochem.* **2009**, *333* (1−2), 159–167.
(44) Prudent, R.; Cochet, C. New protein kinase CK2 inhibitors: jumping out of the catalytic box. *Chem. Biol.* **2009**, *16* (2), 112–20.
(45) Prudent, R.; Sautel, C.; Cochet, C. Structure-based discovery of small molecules targeting different surfaces of protein-kinase CK2. *Biochim. Biophys. Acta* **2009**, *3*, 493–498.
(46) Hodgetts, K. J.; Kershaw, M. T. Synthesis of 2-aryl-oxazolo[4,5-*c*]-quinoline-4(5*H*)-ones and 2-aryl-thiazolo[4,5-*c*]quinoline-4(5*H*)-ones. *Org. Lett.* **2003**, *5* (16), 2911–2914.
(47) Alessi, M.; Larkin, A. L.; Ogilvie, K. A.; Green, L. A.; Lai, S.; Lopez, S.; Snieckus, V. Directed ortho metalation-boronation and Suzuki−Miyaura cross coupling of pyridine derivatives: a one-pot protocol to substituted azabiaryls. *J. Org. Chem.* **2007**, *72* (5), 1588–1594.
(48) Rodgers, J.; Robinson, D.; Arvanitis, A.; Masduskuie, T.; Shepard, S.; Storace, L.; Wang, H.; Rafalski, M.; Jalluri, R.; Combs, A.; Crawley, M., Tetracylclic Inhibitors of Janus Kinases. PCT patent application WO2005/105814, 10 November 2005.
(49) Jagtap, P.; Szabo, C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nature Rev. Drug Discovery* **2005**, *4* (5), 421–440.
(50) Cepeda, V.; Fuertesa, M.; Castillac, J.; Alonsoa, C.; Quevedob, C.; Sotoa, M.; Pérez, J. Poly(ADP-Ribose) Polymerase-1 (PARP-1) Inhibitors in Cancer Chemotherapy. *Recent Pat. Anti-Cancer Drug Discovery* **2006**, *1*, 39–55.
(51) Niefind, K.; Guerra, B.; Ermakowa, I.; Issinger, O. G. Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme. *EMBO J.* **2001**, *20* (19), 5320–5331.
(52) Mazzorana, M.; Pinna, L. A.; Battistutta, R. A structural insight into CK2 inhibition. *Mol. Cell. Biochem.* **2008**, *316* (1−2), 57–62.
(53) Battistutta, R.; Mazzorana, M.; Cendron, L.; Bortolato, A.; Sarno, S.; Kazimierczuk, Z.; Zanotti, G.; Moro, S.; Pinna, L. A. The ATP-Binding Site of Protein Kinase CK2 Holds a Positive Electrostatic Area and Conserved Water Molecules. *ChemBioChem* **2007**, *8* (15), 1804–1809.
(54) Niefind, K.; Raaf, J.; Issinger, O. G. Protein kinase CK2: From structures to insights. *Cell. Mol. Life Sci.* **2009**, *66* (11−12), 1800–1816.
(55) Battistutta, R. Structural bases of protein kinase CK2 inhibition. *Cell. Mol. Life Sci.* **2009**, *66* (11−12), 1868–1889.
(56) Siddiqui-Jain, A., Drygin, D.; Streiner, N.; Chua, P.; Pierre, F.; O'Brien, S.; Bliesath, J.; Omori, M.; Huser, N.; Ho, C.; Proffitt, C.; Schwaebe, M.; Ryckman, D. ; Rice, W. G. ; Anderes, K. CX-4945, an Orally Bioavailable Selective Inhibitor of Protein Kinase CK2, Inhibits Prosurvival and Angiogenic Signaling and Exhibits Antitumor Efficacy. *Cancer Res.* **2010**, *70* (24), 10288−10298.
(57) Di Maira, G.; Salvi, M.; Arrigoni, G.; Marin, O.; Sarno, S.; Brustolon, F.; Pinna, L. A.; Ruzzene, M. Protein kinase CK2 phosphorylates and upregulates Akt/PKB. *Cell Death Differ.* **2005**, *12* (6), 668–677.
(58) Tested at MicroConstants, San Diego, CA .
(59) Tested at CEREP, Seattle, WA .
(60) Lim, J. K., Padgett, C. S.; Marschke, R. F.; Northfelt, D. W.; Andreopoulou, E.; Von Hoff, D. D.; Anderes, K., Chen, T. K.; O'Brien, S., Utility of Clinical Biomarkers for Detecting Protein Kinase CK2 Inhibition: A Report from the Phase I Trial of CX-4945. AACR 101st Annual Meeting, Washington, DC, April 17−21, 2010.
(61) Barreau, M.; Cotrel, C.; Jeanmart, C. 1,2-Dithiole derivatives. U.S. Patent US4,110,450, Aug 29, 1978.
(62) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22* (23), 3099–3108.