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Novel potent pyrimido[4,5-*c*]quinoline inhibitors of protein kinase CK2: SAR and preliminary assessment of their analgesic and anti-viral properties

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ABSTRACT

We describe the discovery of novel potent substituted pyrimido[4,5-*c*]quinoline ATP-competitive inhibitors of protein kinase CK2. A binding model of the inhibitors with the protein was elaborated on the basis of SAR and revealed various modes of interaction with the hinge region. Representative analog **14k** (CK2 IC_{50} = 9 nM) showed anti-viral activity at nanomolar concentrations against HIV-1. Orally available compound **7e** (CK2 IC_{50} = 3 nM) reduced pain in the phase II of a murine formalin model. These preliminary data confirm that properly optimized CK2 inhibitors may be used for anti-viral and pain therapy.

One of the oldest kinases described,[1] protein kinase CK2 is a heterotetrameric Ser/Thr kinase comprised of two catalytic subunits (α and/or α′) and two regulatory (β) subunits. A large body of work reported the important role of CK2 in many disease processes (reviewed in Refs. [2,3]) and proposed to target the protein with selective inhibitors for therapy. Despite the large number of inhibitors described in the literature,[4–10] few molecules possessed potencies and drug properties suitable for exploring the modulation of the enzyme in animals.

While a large part of published research work has been devoted to cancer,[11–17] inflammatory disorders and viral infections also represent large pools of patients that may benefit from new CK2 inhibitor drugs. Several reports have linked CK2 to inflammatory pain through the modulation of nociceptive signaling pathways.[18] CK2 enhances NMDA channel function in hippocampal neurons[19] and the enzymatic activity of neuronal nitric oxide synthase (nNOS) through the phosphorylation of calmodulin.[20] Other systems affected by CK2 include heme oxygenase type 2 (HO-2)[21] and the serotonin 5-HT3 receptor channel.[22] Intrathecal administration of CK2 inhibitors TBB and DRB has been shown to reduce formalin-stimulated pain behaviors in mice.[18]

It has also been shown that CK2 plays a role in the stimulation of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase[23] and protease.[24] The HIV Nef protein, critical for disease progression, was shown to be phosphorylated by CK2.[25] Critchfield et al. reported that the weak CK2 inhibitor DRB inhibited HIV-1 expression in chronically infected cells.[26]

We recently reported[27,28] the discovery of the first CK2 inhibitor to enter clinical trials, CX-4945 (**1**), a selective ATP-competitive inhibitor orally efficacious in animal models of cancer. The high affinity of CX-4945 for CK2 (K_i = 0.38 nM) was rationalized by a binding model supported by SAR (Fig. 1). This model prompted us to expand our chemical series by seeking further interactions with the kinase hinge region through chemical modification of the 3- and 4-positions of the left ring. Herein we report the preliminary findings of these optimization efforts, leading to the discovery of novel potent pyrimido[4,5-*c*]quinoline CK2 inhibitors. An

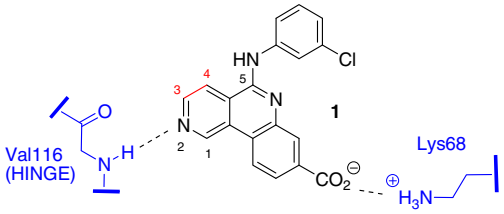

Figure 1. Schematic representation of CX-4945 (**1**) binding model in the co-factor site of CK2.[28] Binding resulted from interactions with hydrophobic residues (not shown), an ionic bridge with positively charged Lys68, and hydrogen bonding with the hinge Val116 amide.

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evaluation of their analgesic and anti-viral properties illustrates the potential of CK2 as a target for multiple indications.

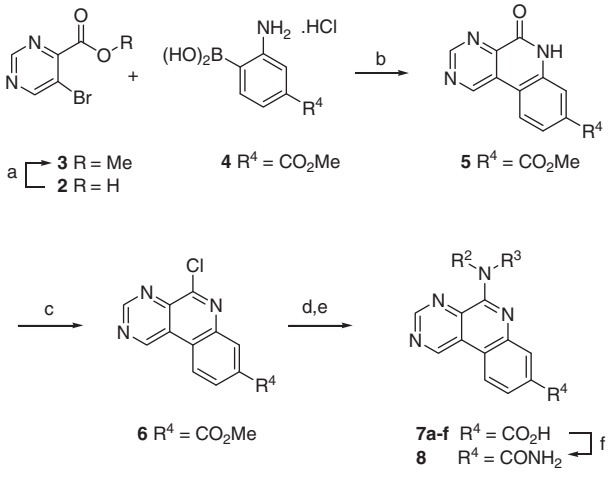
We first considered adding a second nitrogen in the left ring to modulate the basicity of the scaffold. For that purpose, methyl 5-bromopyrimidine-4-carboxylate **3** (Scheme 1) was prepared from 5-bromopyrimidine-4-carboxylic acid **2**[29] via its corresponding acyl chloride. Palladium catalyzed coupling between **3** and commercially available 2-amino-4-(methoxycarbonyl) phenylboronic acid hydrochloride **4** resulted in the one-pot formation of cyclized methyl 5-oxo-5,6-dihydropyrimido[4,5-*c*]quinoline-8-carboxylate **5**.[30]

This material was converted to chloropyrimidoquinoline **6** using phosphorus oxychloride. Substitution of the chlorine in **6** by various substituted anilines and subsequent ester hydrolysis provided final molecules **7a–f**. Acid **7e** was converted to methyl amide **8** using standard conditions.

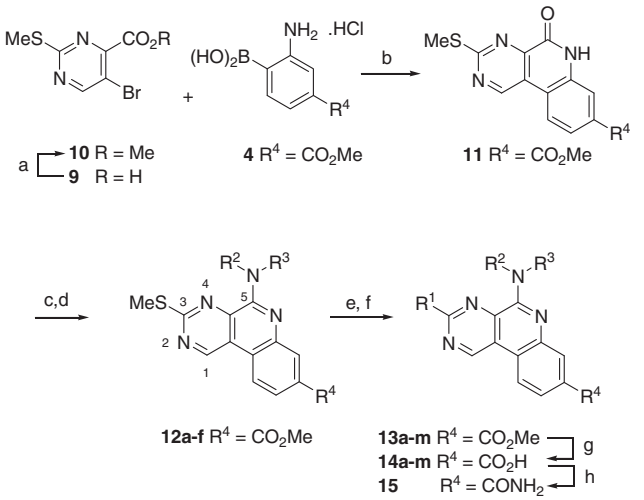
Applying the same chemistry to commercially available 5-bromo-2-(methylthio) pyrimidine-4-carboxylic acid **9** (Scheme 2) produced intermediates **12a–f** bearing a methyl sulfide group on C-3. MCPBA oxidation of the thioether to a sulfone activated this site for the easy introduction of *N*- or *O*-nucleophiles. Ester hydrolysis afforded carboxylate inhibitors **14a–m**, of which **14k** was converted to amide **15**.

To study the effect of a methyl group positioned between the pyrimidine nitrogen atoms, compound **21** was prepared by an alternate synthetic route (Scheme 3). 4-Methyl-3-nitrobenzoic acid **16** was converted to intermediate **17** in four steps using a base-catalyzed addition to diethyl oxalate, a vinylether synthesis using triethyl formate followed by displacement of the ethyl ether group by pyrrolidine. In the following step, the pyrimidine ring was formed by reacting intermediate **17** with acetamidine hydrochloride in basic conditions. Hydrogenation of **18** reduced the nitro to an amine group and also hydrolyzed the ester of the neighboring ring to its corresponding acid. This intermediate could be converted to the desired lactam **19** by intramolecular amide formation utilizing EDCI in DMF. Chemistries similar to Schemes 1 and 2 provided compound **21**.

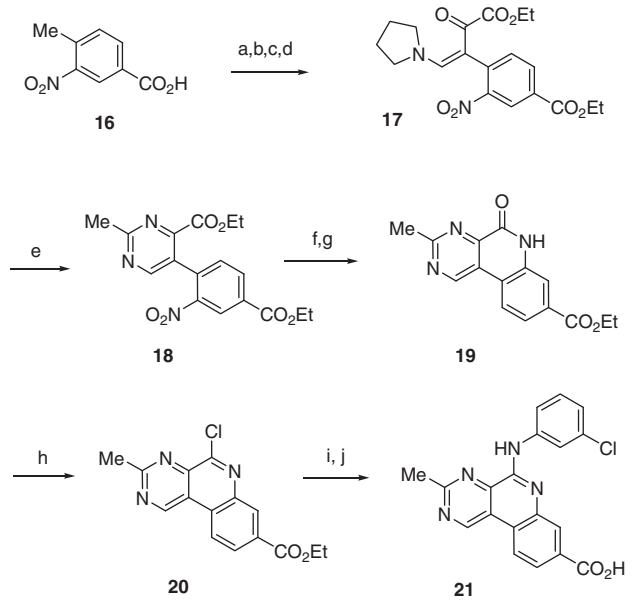
The resulting compounds were tested in an enzymatic assay using recombinant CK2 holoenzyme ($\alpha\alpha\beta\beta$). The data summarized in Table 1 show that pyrimidines **7a–f** substituted in C-5 by anilines bearing small groups in the *meta* position were potent inhibitors of CK2. IC_{50} values for these compounds varied from 3 to



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, then MeOH, 39%; (b) Cs_2CO_3, $PdCl_2$(dppf), dioxane, reflux, 80%; (c) $POCl_3$, toluene, DIEA, reflux, 71%; (d) NHR^2R^3 (for structure of R^2 and R^3 see Table 1), NMP, microwaves 120 °C; (e) LiOH, MeOH, THF, H_2O, rt, 15–78% from **6**; (f) HOBt, Et_3N, EDCI, NH_4Cl, NMP, 70 °C, 50%.



Scheme 2. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, then MeOH 0 °C to rt, 66%; (b) Cs_2CO_3, $PdCl_2$(dppf), dioxane, reflux, 65%; (c) $POCl_3$, reflux, 94%; (d) HNR^2R^3 (for structure of R^2 and R^3 see Table 1), NMP, μwave 120–160 °C, 43–87%; (e) MCPBA, CH_2Cl_2, rt; (f) when R^1 = -NH-alkyl, -$N(Me)_2$, -NHPh, $-NH_2$ free amine or amine HCl salt (+DIEA) in NMP, rt to 60 °C, when R^1 = EtO, EtONa, EtOH, μwave 100 °C; (g) NaOH or LiOH, H_2O, EtOH, 50–80 °C, 6–85% from **11**; (h) HOBt, Et_3N, EDCI, NH_4Cl, NMP, 70 °C, 31%.



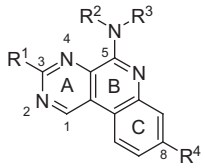
Scheme 3. Reagents and conditions: (a) EtOH, HCl, 93%; (b) $(CO_2Et)_2$, EtOH, EtONa, 0 °C to rt, 91%; (c) $(EtO)_3CH$, Ac_2O, reflux; (d) Pyrrolidine, EtOH, rt, 43% from **16**; (e) $MeC(NH_2)$ = NH·HCl, dioxane, K_2CO_3, 100 °C; (f) 50 psi H_2, Pd/C wet, MeOH; (g) EDCI, DMF, 80 °C, 17% from **17**; (h) $POCl_3$, 120 °C, 70%; (i) 3-chloroaniline, NMP, μwave 120 °C; (j) 6N NaOH, EtOH, 60 °C, 56% from **20**.

10 nM, a range of activity close to their pyridine analogs (1–5 nM[28]).

The methyl group on C-3 in **21** induced a 24-fold decrease in activity when compared with unsubstituted analog **7a**. A more dramatic loss (>60×) was observed with larger tertiary dimethylamino (**14c**) and ethoxy (**14l**) moieties on the same position.

Hydrogen bond donors such as primary (**14a**) or secondary (**14b**) amino groups on C-3 restored nanomolar inhibition, showing that a more favorable interaction with the hinge region had taken place. Increasing the size of the amino group substituents (**14d**, **14e**, **14m**) caused a decrease in potency. The relatively compact cyclopropyl-amino group however increased inhibitory activity

Table 1
CK2 inhibitory activity of 3,5,8 trisubstituted pyrimido[4,5-*c*]quinolones[31]



Compd	R^1	$-NR^2R^3$	R^4	CK2 IC_{50} (μM)
7a	H	–HN(3-Cl-phenyl)	$-CO_2H$	0.007
7b	H	–HN(3-Cl, 4-F-phenyl)	$-CO_2H$	0.010
7c	H	–HN(3,5-Difluorophenyl)	$-CO_2H$	0.009
7d	H	–HN(3-F-phenyl)	$-CO_2H$	0.007
7e	H	–HN(3-Acetylenyl-phenyl)	$-CO_2H$	0.003
8	H	–HN(3-Acetylenyl-phenyl)	$-CONH_2$	>0.5
7f	H	–HN(3-CF_3-phenyl)	$-CO_2H$	0.011
21	Me	–HN(3-Cl-phenyl)	$-CO_2H$	0.172
14a	H_2N–	–HN(3-Cl-phenyl)	$-CO_2H$	0.004
14b	MeNH–	–HN(3-Cl-phenyl)	$-CO_2H$	0.018
14c	Me_2N–	–HN(3-Cl-phenyl)	$-CO_2H$	>0.75
14d	EtHN–	–HN(3-Cl-phenyl)	$-CO_2H$	0.030
14e	*i*-PrHN–	–HN(3-Cl-phenyl)	$-CO_2H$	0.134
14f	*c*-PrHN–	–HN(3-Cl-phenyl)	$-CO_2H$	0.013
14g	*c*-PrHN–	–HN-Phenyl	$-CO_2H$	0.016
14h	*c*-PrHN–	–(Me)N-phenyl	$-CO_2H$	0.105
14i	*c*-PrHN–	–HN(3-F-phenyl)	$-CO_2H$	0.086
14j	*c*-PrHN–	–HN(3-Acetylenyl-phenyl)	$-CO_2H$	0.046
14k	*c*-PrHN–	–HN(3-CF_3-Phenyl)	$-CO_2H$	0.009
14l	EtO–	–HN(3-CF_3-phenyl)	$-CO_2H$	>0.75
13k	*c*-PrHN–	–HN(3-CF_3-phenyl)	$-CO_2Me$	>0.75
15	*c*-PrHN–	–HN(3-CF_3-phenyl)	$-CONH_2$	>0.75
14m	PhenylHN–	–HN-Phenyl	$-CO_2H$	0.185

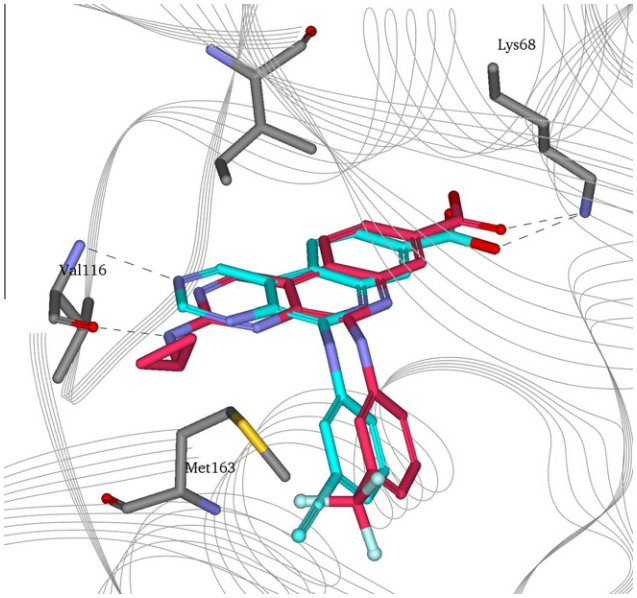


Figure 2. Binding model[32] of **7e** (blue) and **14k** (red) in the ATP- pocket of CK2, based on overlay with previously published structures.[34–38]

as shown with molecule **14f** (IC_{50} = 13 nM). This result led us to prepare a subset of analogs substituted on C-3 by a cyclopropylamino group and bearing various moieties on C-5 (**14f–k**).

A broad range of activities was observed for the resulting compounds. The most potent analog was 3-trifluoromethyl anilino substituted **14k** (IC_{50} = 9 nM). The least potent analog **14h** (IC_{50} = 105 nM) carried tertiary amino groups on C-5, not suitable for optimal interactions with the tight ATP-binding pocket of CK2, as shown with similar classes of inhibitors.[28]

Direct comparison between C-3 cyclopropylamino analogs **14f**, **14i**, **14j**, **14k** and their respective unsubstituted pyrimidines **7a**, **7d**, **7e**, **7f**, revealed a non-linear SAR between the two series, indicating that they likely exhibited a slightly different binding mode. This was best illustrated by the large difference of activity between **7e** (IC_{50} = 3 nM) and its cyclopropylamino analog **14j** (IC_{50} = 46 nM); while **7f** (IC_{50} = 11 nM) had an IC_{50} value equivalent to its cyclopropylamino analog **14k** (IC_{50} = 9 nM). Finally, the lack of CK2 inhibition by analogs bearing in C-8 a carboxamide (**8**, **15**) or an ester (**13k**) demonstrated the need for an acidic moiety at that position.

These results could be explained by a binding model[32] of inhibitors **7e** and **14k** complexed with CK2 (Fig. 2). Compound **7e** was found to be a potent ATP competitive inhibitor of CK2 with a K_i value of 0.42 ± 0.04 nM, confirming that the pyrimido[4,5-*c*]quinolines were ATP-site binding inhibitors. Structural similarity strongly argued in favor of **7e** interacting with CK2 in a manner highly similar to CX-4945 (**1**).[28] The interaction with Lys68 through an ionic bridge was supported by the inactivity of carboxamide **8**. The potent activity of **7e** and its analogs **7a–d**, **7f** showed that the nitrogen N-4 of the A ring had a marginal effect on the hydrogen bond between N-2 and the hinge region.

The decline in activity of analogs **21**, **14c**, **14l** resulted from unfavorable interactions of their C-3 substituents with the carbonyl of Val116 in the hinge region, lowering the ability of the adjacent N-2 to act as a hydrogen bonding acceptor. The restoration of potency observed with H-bond donors in molecules such as **14k**, could be explained by a slight shift of the core away from the hinge, allowing the NH substituent to be in optimal position for hydrogen bonding with the carbonyl of Val116. As a result of the overall move of the scaffold, aniline substituents on C-5 were positioned in a slightly different position, affecting their interactions with hydrophobic pockets and explaining the difference in SAR observed between **7e** and **14k** series.

Potent analogs **7e** and **14k** were tested for their ability to modulate CK2 in a cellular assay. In BxPC3 cells, compounds **7e** and **14k** decreased phosphorylation of Akt(S129), a CK2 specific phosphorylation site[27,28,33] with EC_{50} values of 0.27 and 0.17 μM, respectively.

The pharmacokinetic properties of **7e** and **14k** were evaluated in rodents (Table 2). Compound **7e** demonstrated a favorable profile with a low clearance, high volume of distribution and low to moderate inter species oral exposure (%*F* = 11–39). Compound **14k** displayed a higher clearance and a lower oral absorption.

Compound **7e** was tested in a mouse model of formalin induced pain[40,41] (Fig. 3). The compound was administered orally as a single dose 1 h prior to injection of formalin. During the first 10 min phase (phase I) post-injection, the compound did not affect rates

Table 2
Pharmacokinetic parameters for compounds **7e** and **14k**[39]

Compd	Species (*n* = 3)	iv (mg/kg)	po (mg/kg)	Cls (L/h/kg)	Vss (L/kg)	$T_{1/2}$ (H)	po C_{max} (ng/mL)	po AUC (ng · h/mL)	*F* (%)
7e	Mouse	5.4	10.8	0.32	4.7	10.2	800	2213	11
	Rat	1.6	8.1	0.04	0.32	6.4	6668	73,945	39
14k	Mouse	3.6	7.1	1.40	0.50	1.4	28	54	1

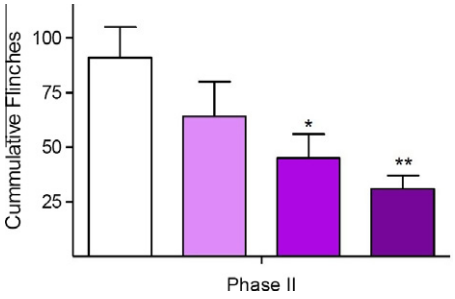


Figure 3. Effect of **7e** in phase II (11–40 min) of the formalin response in mice. (□) Vehicle (water 10 ml/kg), po; (▭) **7e** (25 mg/kg, po); (■) **7e** (75 mg/kg, po); (■)**7e** (150 mg/kg, po); Shown are the mean ± SEM (n = 12–17) flinches for the group indicated (*/**: p <0.05/0.01, one way ANOVA, Dunnett's *post hoc* test vs vehicle).

Table 3
Antiviral activity of compound **14k** against HIV-1 clinical isolates in fresh human PBMC[42]

Virus (subtype)	Endpoint	IC_{50} (μM)	TC_{50} (μM)	Therapeutic index TC_{50}/IC_{50}
00KNH1209 (A)	RT	0.40	>100	>248
	p24	0.17	>100	>605
94US3393IN (B)	RT	0.54	>100	>184
	p24	0.19	>100	>527
98IN022 (C)	RT	0.26	>100	>387
	p24	0.20	>100	>491
00KENKU3006 (D)	RT	0.24	>100	>419
	p24	0.13	>100	>757
93TH073 (E)	RT	0.08	>100	>1246
	p24	0.14	>100	>726
93BR019 (BF)	RT	0.28	>100	>355
	p24	0.39	>100	257
G3 (G)	RT	0.24	>100	>410
	p24	0.10	>100	>1018

of pain induced flinching. However, **7e** significantly reduced pain during the phase II (11–40 min) in a dose-dependent manner compared to vehicle treated mice, suggesting an anti-inflammatory based mechanism. A single dose of morphine (3 mg/kg, subcutaneously injected) served as a positive control, and reduced formalin-induced flinching in both phases of the test.

Preliminary anti-viral screening identified promising activity for **14k** against CCR5-tropic HIV-1 clinical isolates in human peripheral blood mononuclear cells (PBMC). This compound was further evaluated against a broader assortment of clinical isolates (Table 3). Overall, compound **14k** inhibited virus production at sub micromolar IC_{50} values and had no cytotoxicity at high test concentration of 100 μM in PBMC, providing a favorable therapeutic index.

In summary, we have discovered multiple series of novel potent pyrimido[4,5-*c*]quinolones, that act mechanistically as ATP-competitive inhibitors of protein kinase CK2. Among these new chemical entities, several representatives inhibited the protein with IC_{50} values below 10 nM. Molecular modeling and SAR suggested that C-3 alkyl-amino substituted inhibitors such as **14k** interacted slightly differently with the hinge region of the protein, an observation that might potentially affect their kinase selectivity profile.

We have shown that representative analogs **7e** and **14k** were active in cells, providing valuable tools for investigating the pharmacology of CK2. Compound **14k** showed in vitro anti-viral activity with IC_{50} values as low as 80 nM against HIV-1 viruses with an excellent therapeutic index. Orally available **7e** demonstrated dose dependent reduction of pain in vivo. These results confirm the therapeutic potential of CK2 inhibitors, and suggest that properly optimized inhibitors may deliver drugs for multiple disease indications. Additional characterization and optimization of our chemical series will be the subject of future reports.[43]

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39. The sodium salt of **7e** was administered as a DI water solution. The free acid of **14k** was administered as a solution in water containing 0.4–0.8% L-arginine. After administration in animals, blood samples were centrifuged and the plasma was analyzed by LC–MS/MS technique. The pharmacokinetic parameters were derived by noncompartmental analysis. Abbreviations: iv, intravenous dose; po, oral dose; 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.
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42. Work carried out at the Southern Research Institute, Maryland. Acute infection of HIV-1 isolates (CCR5-tropic HIV-1 subtype) using fresh human PBMC (PMA and IL-2 stimulated). Antiviral activity was determined as a reduction in reverse transcriptase activity (RT) or p24 ELISA quantification after 7-day incubation. AZT was used as the positive control for all the assays. IC_{50}, 50% inhibition of virus replication; TC_{50}, 50% host cell cytotoxicity.
43. Compounds **14k** and **7e** appear to be more selective for CK2 than CX-4945 in a kinase selectivity panel. A full biochemical and crystallographic characterization of the molecules is ongoing and will be the subject of another paper.