

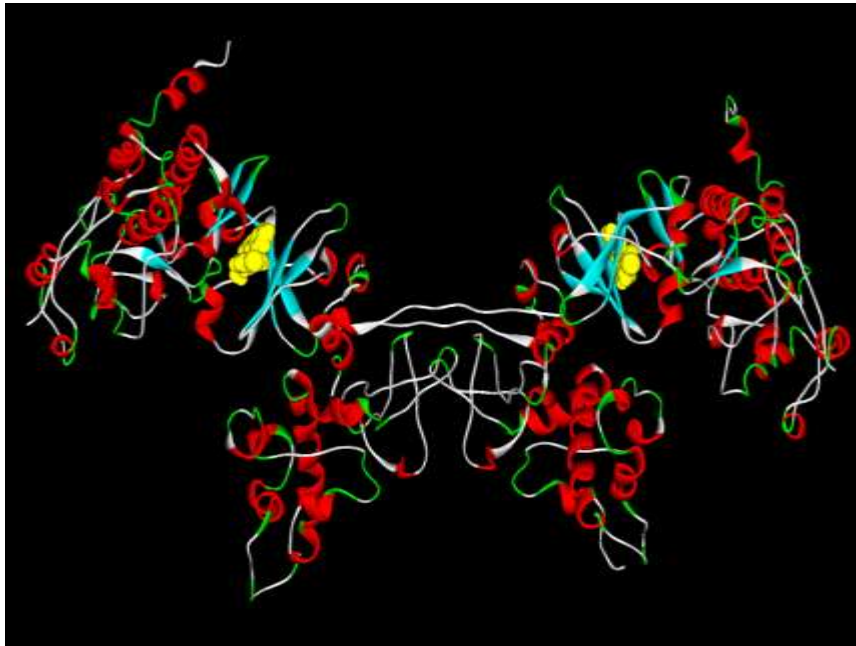


**Discovery of CX-4945, the First Clinical Stage  
Inhibitor of Protein Kinase CK2 for the Treatment  
of Cancer**

**Fabrice Pierre**

Fifth Annual *Emerging Targets for the Kinase Inhibitor Pipeline*  
Boston, 2 November 2011

# CK2 Serine-Threonine Protein Kinase Complex



- CK2α and CK2α' are catalytic subunits
- CK2β is a regulatory element
- All are separate gene products
- CK2 complex exists as a tetramer
- α and α' share sequence homology of 85%

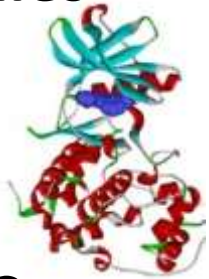
# CK2 Has Distinctive Cancer Biology

## ▶ **Cancers and Protein Kinase CK2**

- Constitutively active - does not require activation by modifications or mutations
- CK2 essential for survival and maintenance of cancer phenotype
- Mere overexpression will drive the cancer phenotype
- Overexpressed in many cancers – but not mutated

## ▶ **CK2 Not Effectively Targeted by Other Drugs or Companies**

- Not mutated, so many considered it a “Housekeeping Gene”
- Others failed due to architecture of unique ATP binding site
- Requires new chemical space for kinase inhibitors



## ▶ **Promotes Multiple Pathways and Processes that Drive Cancer**

- Strong mechanistic rationale for combination with other drugs
- Provides biomarkers to show hitting target and desired pathways
- *Enables extensive combinability with multiple classes of drugs*

# CK2 Drives Multiple Oncogenic Pathways



**CK2**

**Overexpressed  
In Tumors**



**Essential for  
Tumor Survival &  
Maintenance**

**Drives Multiple  
Oncogenic Pathways  
Enables “Rational  
Combinability”**

- **Mechanistic rationale for extensive combinability with multiple drugs**
- **Applicable to combination therapy in numerous cancer indications**

Kinase	Compound											
	Staurosporine (10,000 nM)	G-6903 (10,000 nM)	IC87 (10,000 nM)	Imatinib (10,000 nM)	PP2 (10,000 nM)	PD-318220 (10,000 nM)	Dasatinib (10,000 nM)	Nilotinib (10,000 nM)	BB-2516 (10,000 nM)	Staurosporine (10,000 nM)	Typhostin AG5476 (10,000 nM)	TG0202 (10,000 nM)
ABL1	95	20	9	55	95	30	6	10	99	99	3	3
ABL1 G226K	93	21	3	55	97	37	7	13	99	99	9	5
ABL1 G250E	89	24	10	49	96	31	3	11	97	99	9	1
ABL1 T315I	10	46	10	26	50	74	4	9	91	97	9	6
ABL1 Y253F	50	35	3	63	95	43	11	25	96	99	9	7
ABL2 (Arg)	93	29	8	74	95	34	13	29	97	97	9	9
ACVR1B (ALK4)	1	15	8	-6	37	17	4	55	98	9	9	3
ADRB1 (GRK2)	2	3	33	1	2	42	2	1	79	2	9	9
ADRB2 (GRK2)	-3	-2	25	-1	-1	16	-1	-2	73	-1	9	5
AKT1 (PKB alpha)	5	9	4	4	4	95	2	9	99	-1	9	10
AKT2 (PKB beta)	2	11	50	1	4	79	0	10	97	3	2	4
AKT3 (PKB gamma)	-4	52	73	1	11	99	0	10	99	2	4	4
ALK	13	59	3	27	36	94	57	19	94	48	19	19
AURKB (Aurora B)	7	16	59	33	6	28	0	4	100	69	15	15
BLK	72	45	8	58	99	76	-2	10	101	69	1	1
BRK	-8	24	9	37	9	44	-9	9	99	-9	7	7
BRAP	71	33	39	44	44	-20	39	95	101	53	22	22
BRAP Y596E	97	16	30	61	67	45	27	95	98	47	-1	1
BTX	-4	15	7	50	92	40	-2	8	101	39	3	3
CAMK1D (CaMKI delta)	6	75	14	11	7	27	1	-11	105	29	0	0
CaMK2A (CaMKII alpha)	2	45	15	9	6	95	2	0	100	13	2	2
CaMK2B (CaMKII beta)	-1	23	13	9	1	45	9	9	97	9	9	9
CaMK2D (CaMKII delta)	-5	52	25	33	-2	99	9	10	98	23	20	20
CAMK4 (CaMKIV)	3	24	6	3	2	-23	4	9	77	6	7	7
COX10 (JAK2)	13	30	24	8	19	-3	82	16	99	18	30	30
COX10 (JAK2)	-5	16	20	-7	8	13	99	0	95	-3	29	29
CRKL	-4	18	20	-9	4	24	99	4	109	-2	28	28
CHK1 (CHK1)	4	13	79	11	1	104	5	19	91	-9	12	12
CHK2 (CHK2)	4	59	66	37	19	95	13	6	105	27	11	11
CLK1	19	25	10	3	7	99	35	10	95	1	45	45
CLK2	27	58	28	8	22	19	50	5	99	19	33	33
CFHR1 (PRK8)	103	76	25	44	92	87	2	25	101	54	4	4
CK1	43	20	9	45	95	107	19	63	99	41	9	9
CK1A (CK1 alpha 1)	5	3	4	3	4	93	10	17	99	17	3	3
CK1D (CK1 delta)	7	10	7	6	92	-6	47	90	12	17	7	7
CK1E (CK1 epsilon)	2	24	15	45	96	39	14	20	95	9	15	15
CK1G1 (CK1 gamma 1)	2	-3	-5	1	3	23	4	9	9	9	3	3
CK1G2 (CK1 gamma 2)	7	-1	1	-2	0	-12	57	9	42	8	7	7
CK1G3 (CK1 gamma 3)	10	9	8	4	2	39	15	12	4	7	14	14
CK1M1 (CK1 alpha 1)	5	1	3	2	2	45	9	-1	12	4	1	1
CK1M2 (CK1 alpha 2)	41	29	7	7	19	16	-6	11	43	29	1	1
CK1M3 (CK1 alpha 3)	-1	6	14	3	2	7	0	2	101	9	2	2
DYRK3	6	16	55	29	-3	97	-6	20	97	36	15	15
DYRK4	-1	-8	-2	-3	-3	-7	-2	0	24	-2	-3	-3
EGFR (ErbB1)	23	33	3	91	91	39	8	74	94	39	0	0
EGFR L858R (ErbB1 L858R)	15	29	11	125	95	91	-13	7	75	99	-1	-1
EGFR L858R (ErbB1 L858R)	29	36	5	93	97	39	11	75	97	97	-1	-1
EPHA1	19	57	29	87	100	91	12	51	99	51	15	15
EPHA2	59	46	8	87	103	70	19	51	101	71	24	24
EPHA3	20	-1	-4	30	79	1	0	3	97	26	-3	-3
EPHA4	33	7	7	76	94	49	9	31	97	74	7	7
EPHA5	71	25	7	71	94	41	14	33	98	68	15	15
EPHA6	84	23	13	84	95	17	15	40	97	77	17	17
EPHB1	25	33	12	64	92	73	15	22	97	67	7	7
EPHB2	31	20	6	76	97	59	7	17	95	74	3	3
EPHB3	24	3	8	18	91	9	6	18	48	6	8	8
EPHR1	12	11	-1	83	95	55	4	15	99	75	-1	-1
EPHR2 (HER2)	6	3	-5	95	99	-42	0	20	99	95	2	2
EPHR3 (HER3)	59	22	-1	99	99	49	9	52	99	99	-8	-8
FER	17	51	9	10	8	64	16	0	99	13	1	1
FES (FPS)	19	30	47	5	41	29	39	-1	100	9	-2	-2
FGFR1	-2	69	18	11	63	73	6	2	100	66	7	7
FGFR2	6	98	30	30	90	69	10	9	110	65	7	7
FGFR3	5	65	15	0	91	21	13	8	99	15	1	1
FGFR4	-11	10	-4	-15	47	-62	-1	-10	97	-6	-13	-13
FGFR	31	44	17	86	100	65	7	37	99	94	7	7
FLT1 (VEGFR1)	3	59	4	6	8	68	10	12	104	21	9	9
FLT3	25	34	67	63	65	99	18	7	100	65	11	11
FLT3 D558Y	39	16	75	69	75	99	20	12	100	76	20	20
FLT4 (VEGFR3)	22	59	33	38	76	-29	9	22	93	78	7	7
FRK (PTK5)	24	15	5	70	99	9	4	45	98	77	2	2
FYN	54	12	6	20	99	19	3	4	100	37	1	1
GRK4	-29	23	40	-30	-21	93	-16	-4	93	-4	6	6
GRK5	0	17	5	-1	-1	69	3	-1	99	0	3	3
GRK6	-1	41	35	1	1	6	9	-4	95	6	5	5
GRK7	4	37	40	1	11	51	14	1	100	9	5	5
GRK9 (GRK3 alpha)	17	102	8	11	16	102	24	69	98	5	31	31
GRK3B (GRK3 beta)	3	100	8	7	100	7	55	101	9	10	1	1
HCK	62	25	12	74	100	32	10	24	102	84	9	9
HGFHR	0	8	-7	1	1	16	13	-4	97	-7	-7	-7
INSR (IR) beta	3	8	10	-1	10	21	17	13	98	3	10	10
INSR	11	17	2	10	10	21	17	13	98	3	10	10
INSR (IR) alpha	9	3	-6	6	4	9	16	2	98	-9	9	9
IRAK4	14	24	-2	59	15	91	27	13	95	49	12	12
ITK	2	-2	-4	6	6	-6	8	3	93	-3	1	1
JAK2	1	15	2	7	4	90	0	2	97	7	-3	-3
JAK3	2	45	2	3	3	-1	13	-1	91	13	-1	-1
KDR (VEGFR2)	35	100	59	73	92	100	14	50	101	59	21	21
KIT	45	24	5	2	45	27	7	3	95	19	5	5
KIT T670R	15	14	9	6	13	-41	-1	-2	95	40	5	5
LCK	98	63	10	84	99	76	10	37	102	75	9	9
LYN A	100	46	17	93	100	49	11	34	102	91	10	10
LYN B	95	34	13	81	95	43	7	24	101	67	5	5
MAP2K1 (MEK1)	5	10	19	0	13	28	24	14	100	7	5	5
MAP2K2 (MEK2)	15	22	22	8	34	36	29	14	100	15	15	15
MAP2K4 (MKK4)	-1	5	9	-7	-7	29	-4	9	101	-9	6	6
MAP3K8 (COT)	21	1	47	10	34	-39	42	29	95	45	6	6

# CK2: Unique Active Site

CK2 represents a novel area of space where other drugs including Staurosporine do not inhibit



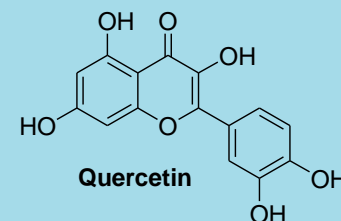
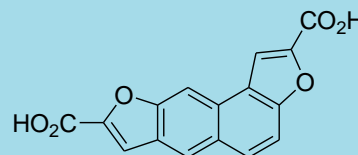
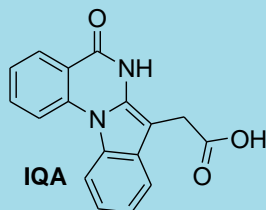
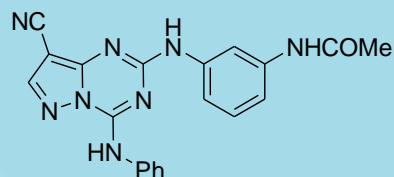
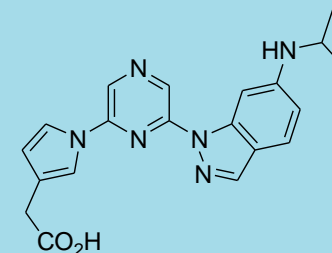
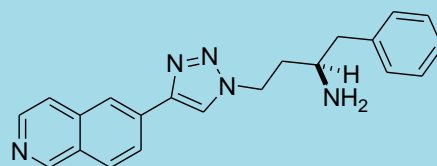
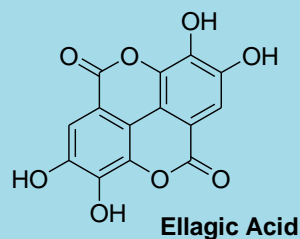
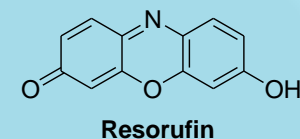
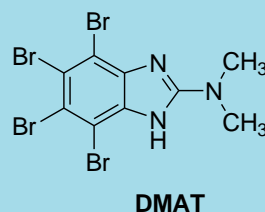
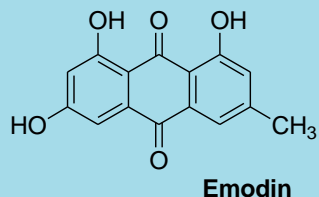
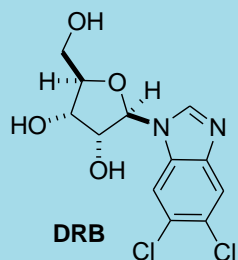
10 uM Standard Drug inhibition of 125 Kinases

Red = >80% inhibition  
White = 40-80% inhibition  
Blue = < 40% inhibition

75-100% inhibition  
40%-75% inhibition  
100% inhibition

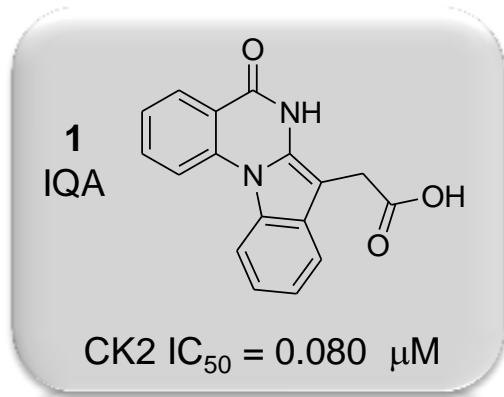
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# Many ATP-Competitive Inhibitors of CK2 Reported in Literature

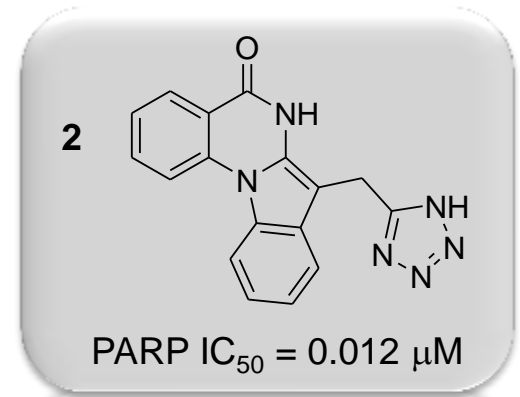
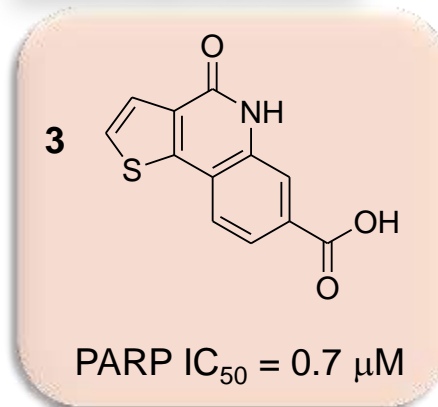


References and reviews: Zhu, D. *et al.*, *Mol. Cell Biochem.* **2009**, 333(1-2), 159-167. Lopez-Ramos, M. *et al.* *Faseb J.* **2010**. Cozza, G. *et al.*, *Med. Res. Rev.* **2010**, 30(3), 419-462.

# Lead Discovery



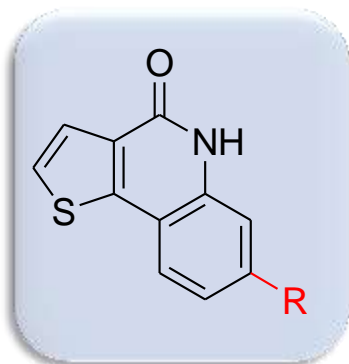
Vangrevelinghe, E et al., *J.Med.Chem.*, 2003



Jagtap, P. et al., *Nat.Rev.Drug.Discov.*, 2005

- ▶ IQA **1** similar to poly(ADP-ribose)polymerase (PARP) inhibitor **2**
- ▶ PARP inhibitor **3** picked up from Cylene library for CK2 screening
- ▶ Compound **3** inhibits CK2

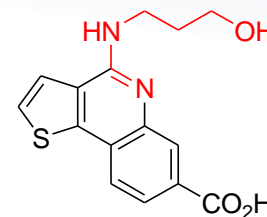
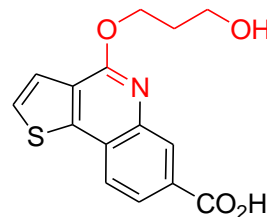
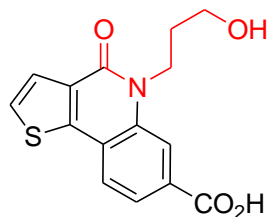
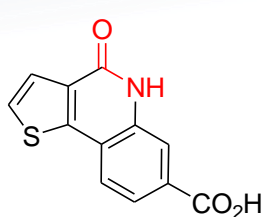
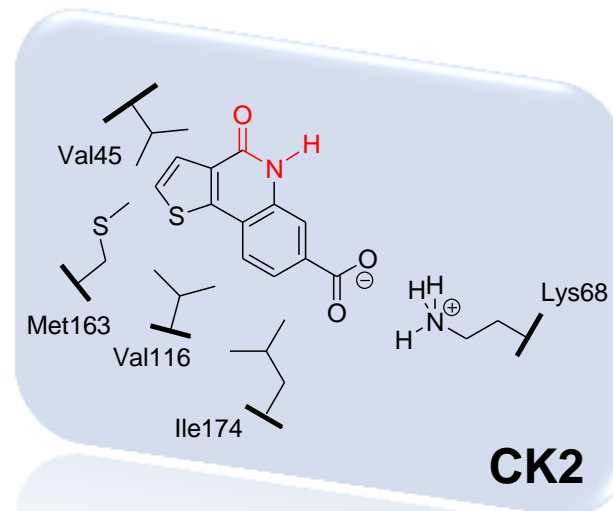
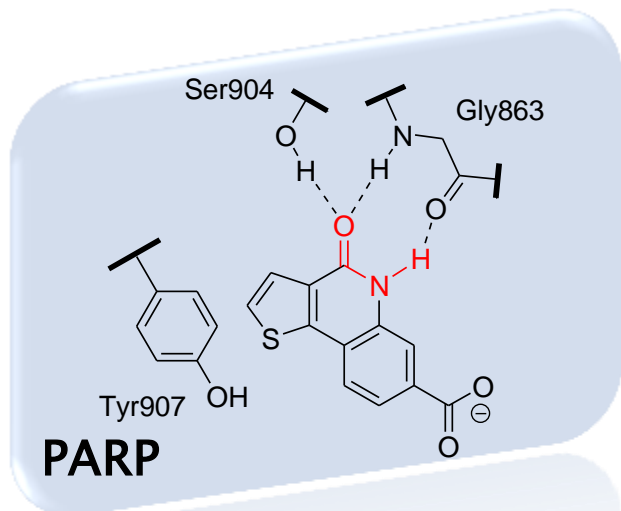
# Carboxylic Acid Essential for CK2 Inhibition



R	PARP IC <sub>50</sub> (μM)	CK2 IC <sub>50</sub> (μM)
-H	>20	>10
-CO <sub>2</sub> H	0.70	2.1
-CH <sub>2</sub> OH	0.40	>10
-CONH <sub>2</sub>	0.06	>10
-CONH(CH <sub>2</sub> ) <sub>2</sub> OMe	0.07	>10

- ▶ Other moieties increase inhibition of PARP

# Lactam Modification Increases CK2 Selectivity



PARP %inh @1  
μM

58%

21%

7%

12%

CK2 IC<sub>50</sub> (μM)

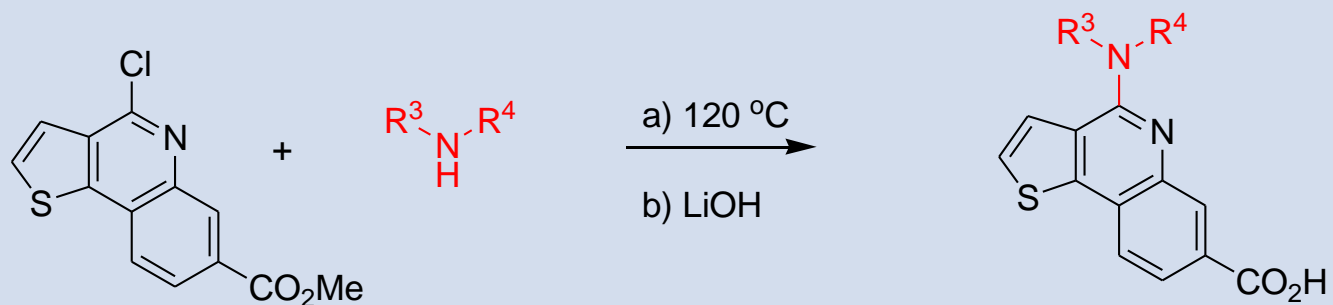
2.1

1.50

0.99

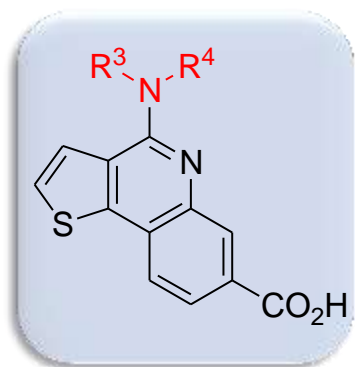
0.75

# Straightforward Chemistry for Optimization

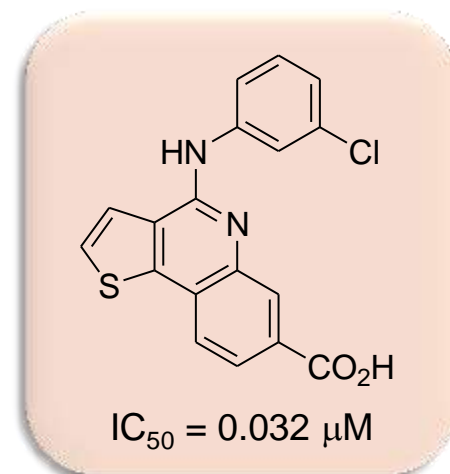


- ▶ 2-steps to prepare various analogs

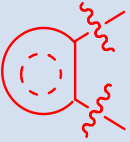
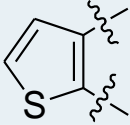
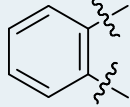
# Aniline Moiety Increases Potency

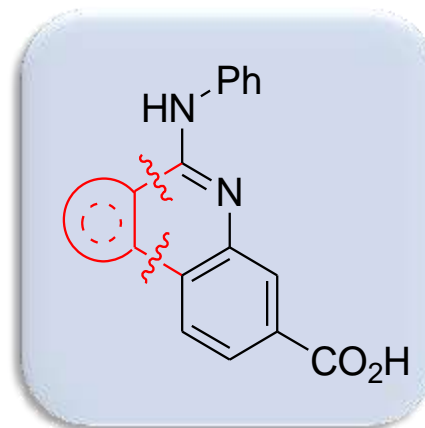


$\text{-NR}^3\text{R}^4$	CK2 $\text{IC}_{50}$ ( $\mu\text{M}$ )
$\text{-NH-(CH}_2)_2\text{OH}$	1.26
$\text{-NH-(CH}_2)_2\text{NMe}_2$	0.102
pyrrolidino	1.78
$\text{-NH-Phenyl}$	0.092
$\text{-NMe-Phenyl}$	1.07
$\text{-NH-(2-Me-Phenyl)}$	0.970
$\text{-NH-(CH}_2)_2\text{Ph}$	0.516
$\text{-NH-(4-F-Phenyl)}$	0.219
$\text{-NH-(3-F-Phenyl)}$	0.068
$\text{-NH-(4-Cl-Phenyl)}$	0.178
$\text{-NH-(3-Cl-Phenyl)}$	0.032
$\text{-NH-(3-MeO-Phenyl)}$	0.077
$\text{-NH-(3-acetylenyl-Phenyl)}$	0.028
$\text{-NH-(3-(PhO)-Phenyl)}$	0.395
$\text{-NH-(3-(CONHMe)-Phenyl)}$	0.129



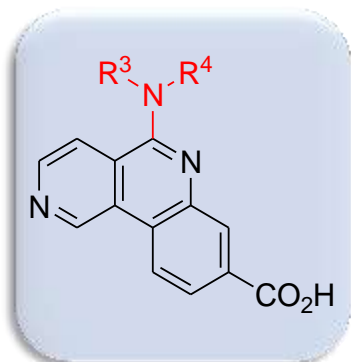
# Left Ring Optimization

	CK2 IC <sub>50</sub> (μM)
	0.092
	>1.0

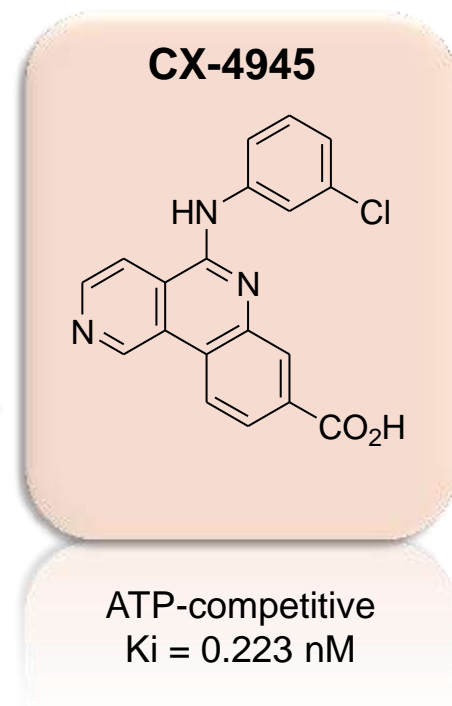


- ▶ Phenyl induces loss of activity
- ▶ 4-pyridinyl increase activity (15x)

# CX-4945 Very Potent CK2 Inhibitor

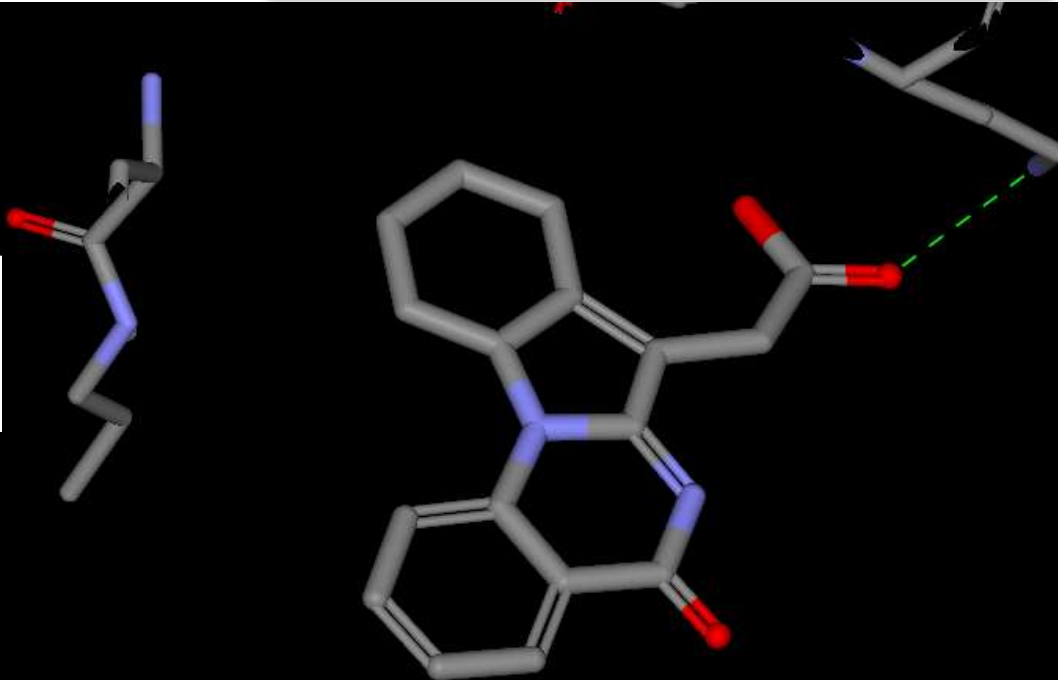


-NR <sup>3</sup> R <sup>4</sup>	CK2 IC <sub>50</sub> (μM)
-NH-Phenyl	0.006
-NH-(CH <sub>2</sub> ) <sub>2</sub> NMe <sub>2</sub>	0.025
-NH-Cyclopentyl	0.027
-NH-OMe	0.008
-NH-Cyclopropyl	0.016
-NH-(CH <sub>2</sub> ) <sub>2</sub> O-i-Pr	0.011
-NH(CH <sub>2</sub> )Phenyl	0.009
-NH(CH <sub>2</sub> ) <sub>2</sub> Phenyl	0.003
-NH(CH <sub>2</sub> ) <sub>3</sub> Phenyl	0.016
-NH-(3-MeO-Phenyl)	0.004
-NH-(3-Cl, 4-F-Phenyl)	0.004
-NH-(3-F-Phenyl)	0.005
-NH-(2-Cl-Phenyl)	0.008
-NH-(3-Cl-Phenyl)	0.001
-NH-(4-Cl-Phenyl)	0.007
-NH-(3-Acetylenyl-phenyl)	0.003
-NH-(3-CN-Phenyl)	0.004
-NH-(4-(PhO)-Phenyl)	0.069
-NH-(3-(PhO)-Phenyl)	0.019
-NH-(3-(SO <sub>2</sub> NH <sub>2</sub> )-Phenyl)	0.043



# IQA Binding in CK2

Hinge:  
Val 116

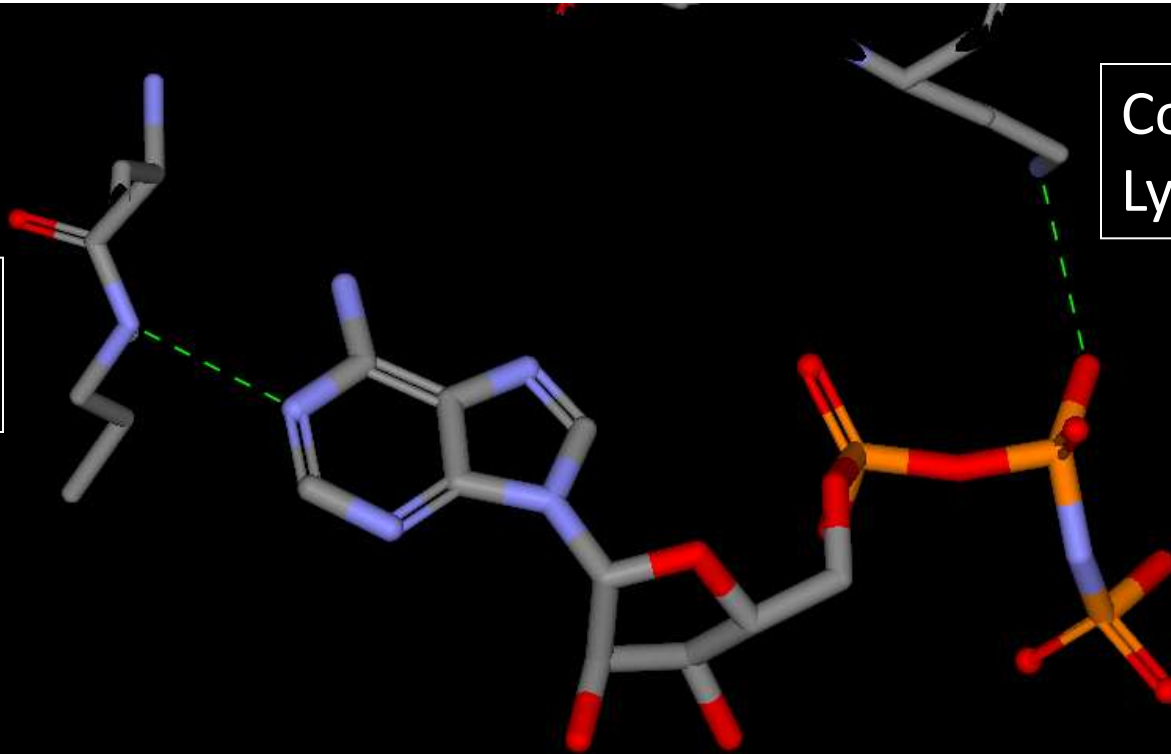


Conserved:  
Lys 68

Battistutta, R. *et al.* *Chembiochem* , **2007**, 8, (15), 1804-1809. Sarno, S. *et al.*, *Biochem. J.*, **2003**, 374, 639-646.

# ATP Binding in CK2

Hinge:  
Val 116

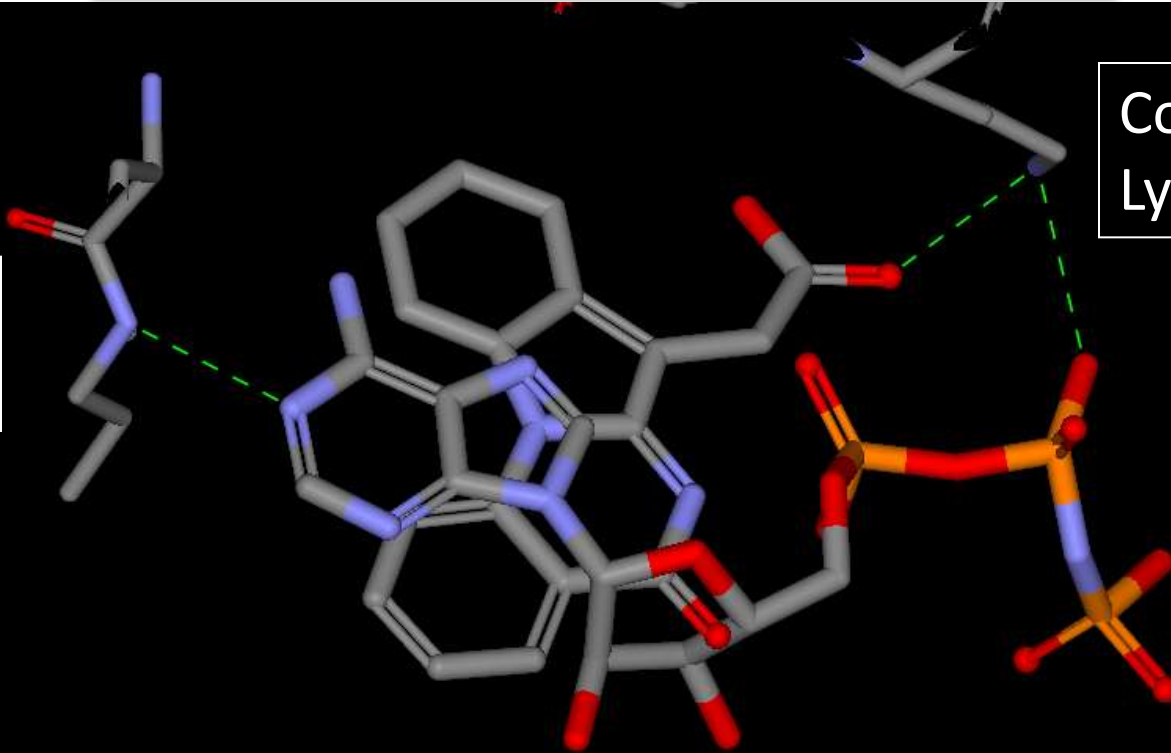


Conserved:  
Lys 68

# ATP & IQA Overlaid in CK2

Hinge:  
Val 116

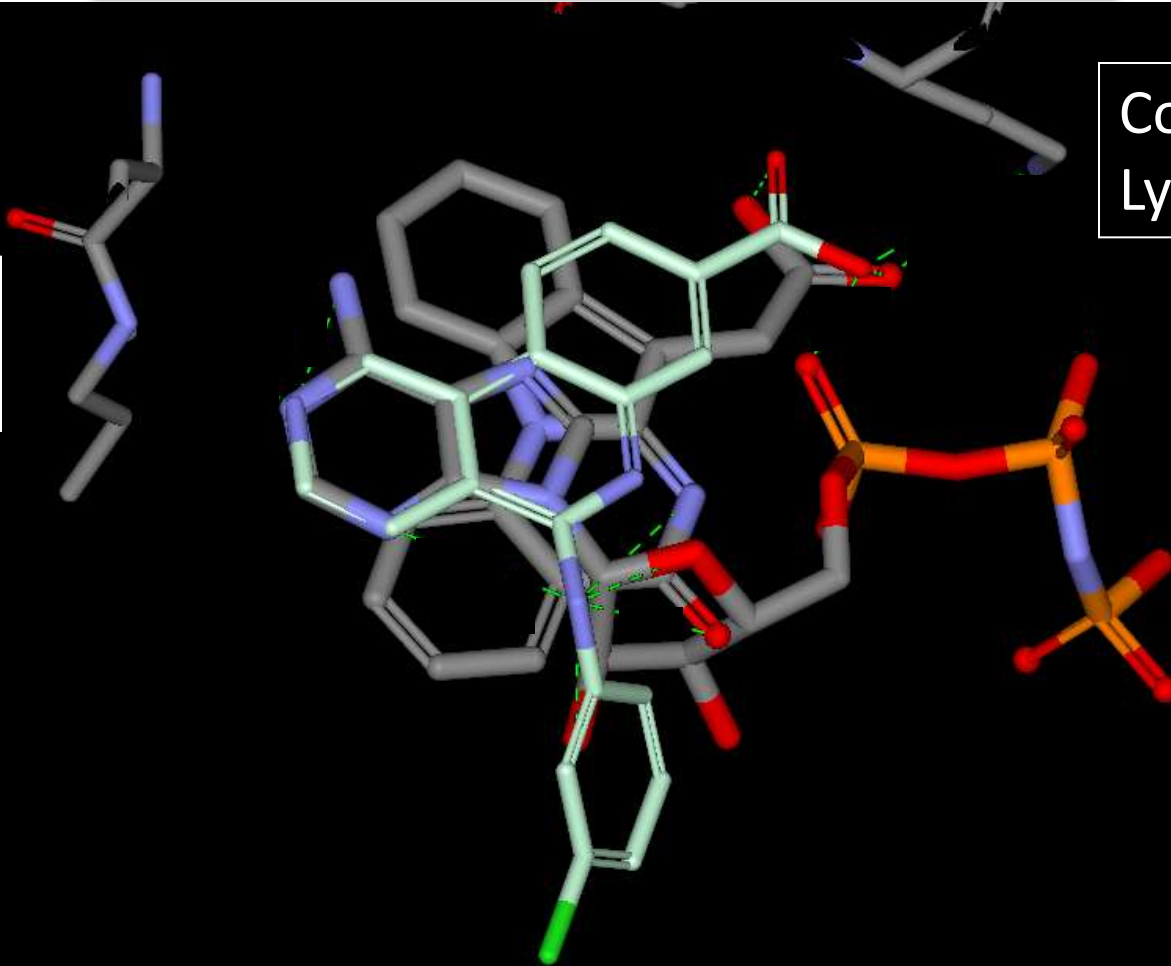
Conserved:  
Lys 68



# ATP & IQA Overlaid in CK2

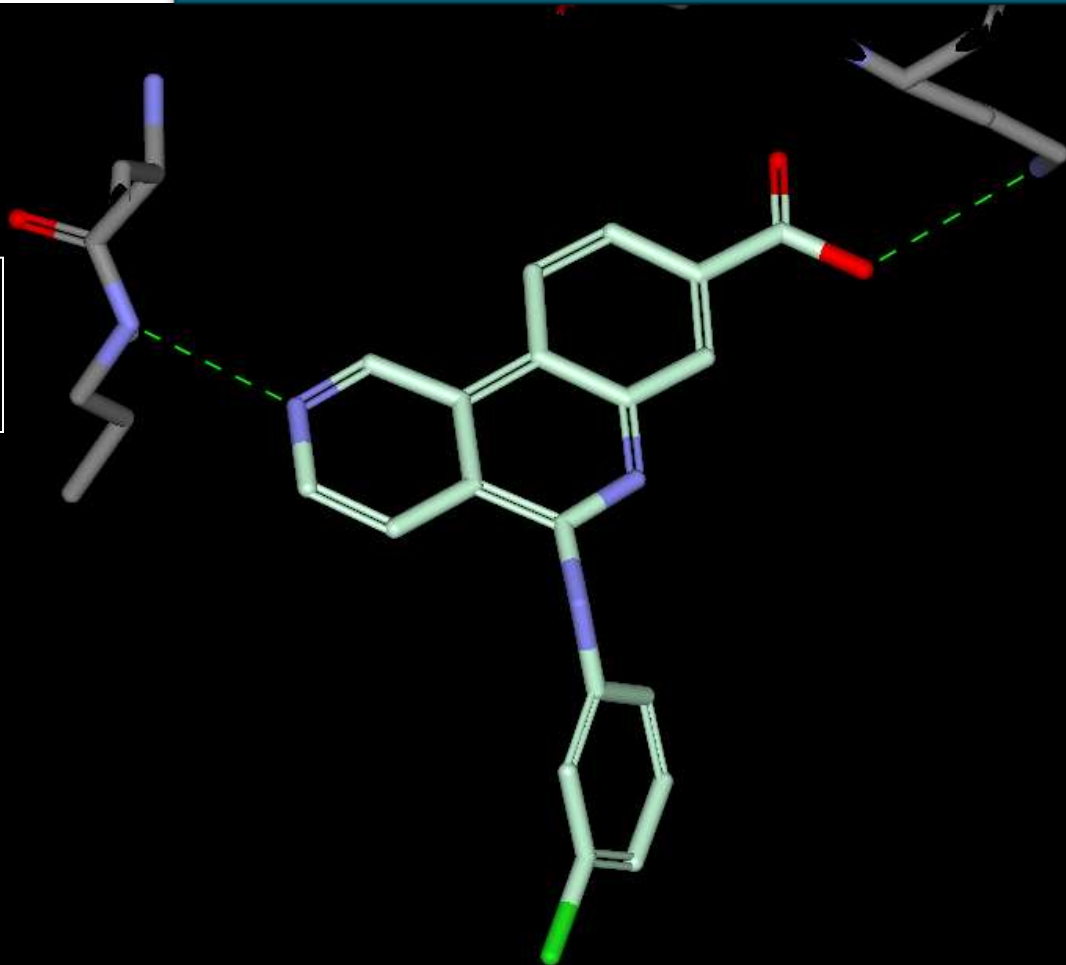
Hinge:  
Val 116

Conserved:  
Lys 68



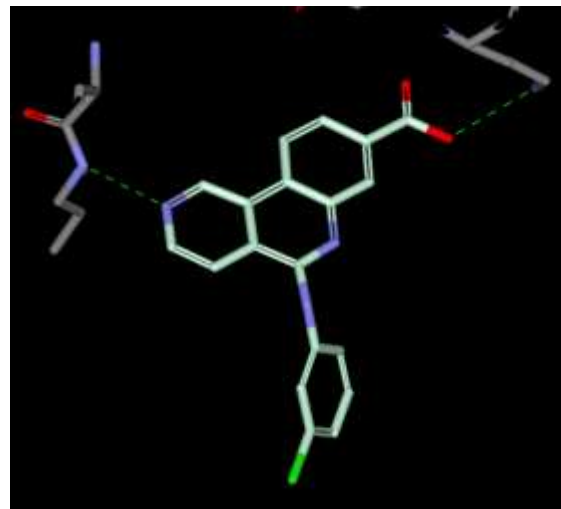
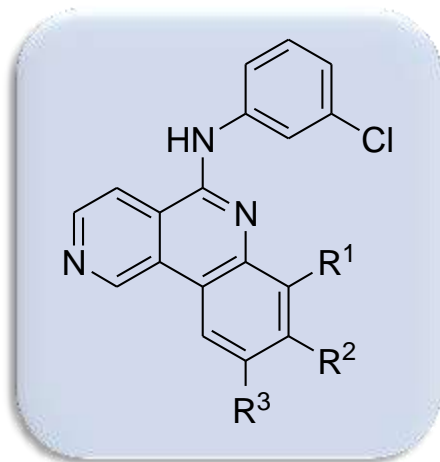
# CX-4945 Binding in CK2

Hinge:  
Val 116



Conserved:  
Lys 68

# Acidity and Position of Carboxylate Essential



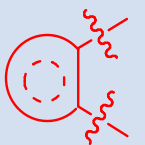
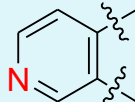
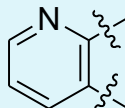
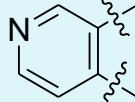
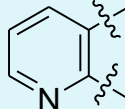
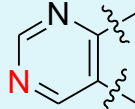
CX-4945

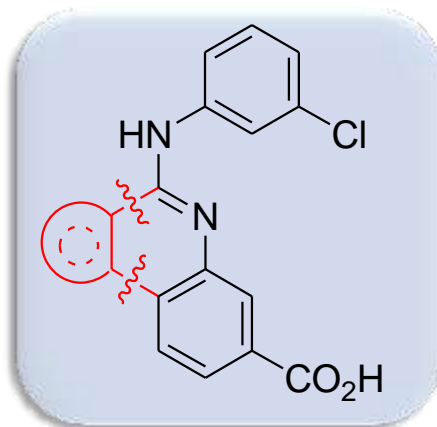
R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	CK2 IC <sub>50</sub> ( $\mu$ M)
H	CO <sub>2</sub> H	H	0.001
H	CO <sub>2</sub> Me	H	>0.5
H	CH <sub>2</sub> OH	H	>0.5
H	CONH <sub>2</sub>	H	0.417

- ▶ >400x loss of activity when acid replaced with non-acidic isosteres
- ▶ >350x loss of activity when acid moved to adjacent sites

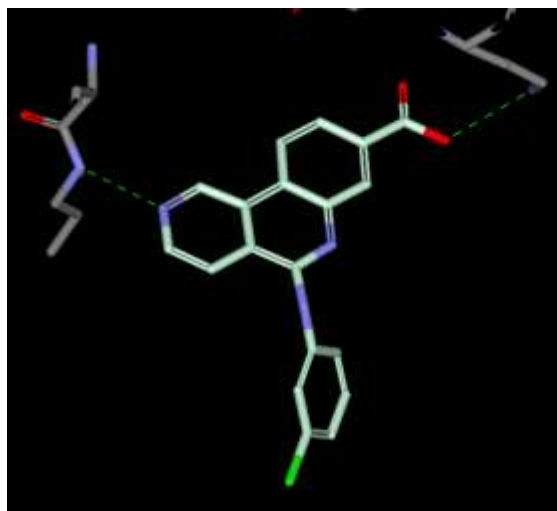
# Position of Pyridine Nitrogen Essential

CX-4945

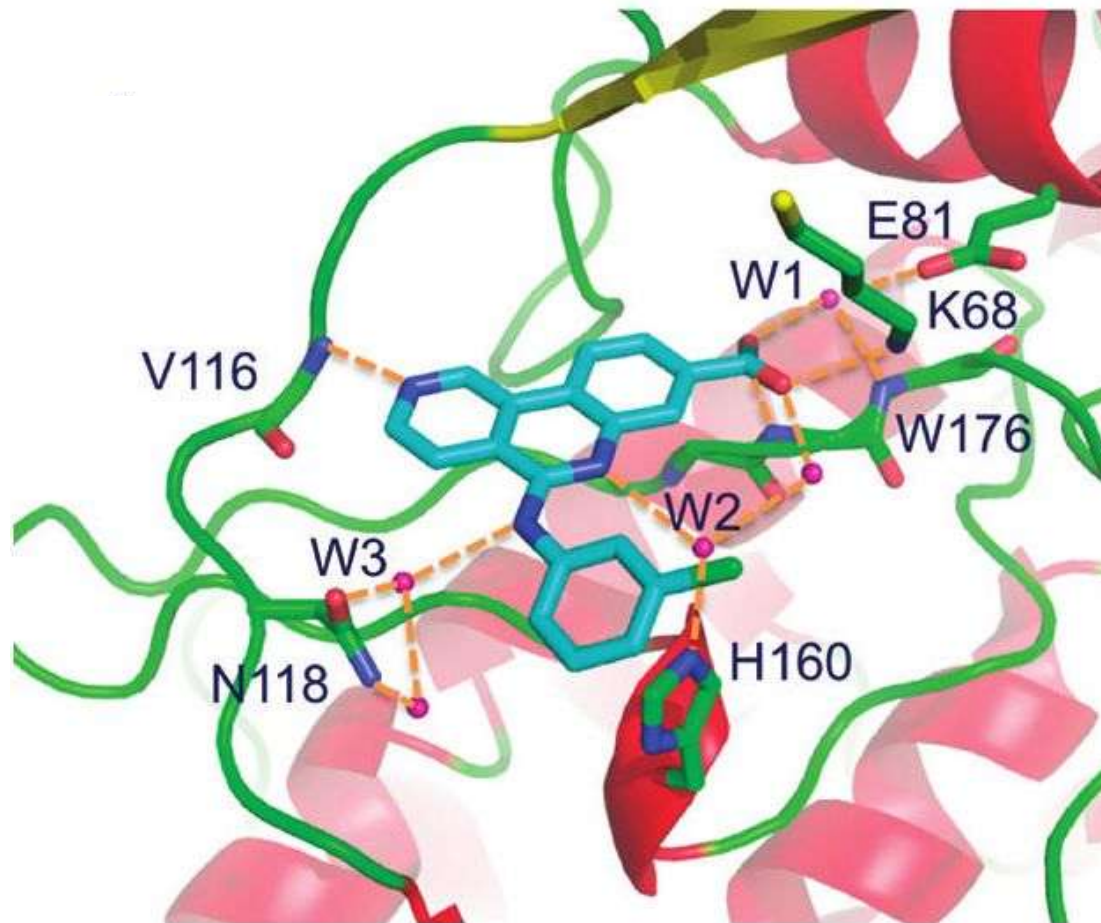
	CK2 IC <sub>50</sub> (μM)
	0.001
	>0.5
	>0.5
	>0.5
	0.007



- ▶ >500x loss of activity when Nitrogen removed from its optimal position



# Crystal structure of CK2 $\alpha$ -CX-4945

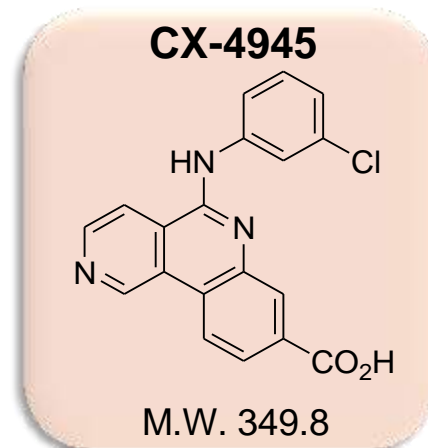


Battistutta R. *et al.*  
Biochemistry, 2011

- ▶ Main interaction of scaffold with hinge Val-116 and Lys-68 confirmed
- ▶ Network of water surrounding the inhibitor revealed by crystal structure

# CX-4945 Highly Selective CK2 Inhibitor with Favorable Drug Profile

CX-4945	Kinase	IC50 (nM)
	CK2 $\alpha$	1
	CK2 $\alpha'$	1
	DAPK3	17
	FLT3	35
	TBK1	35
	CLK3	41
	HIPK3	45
	PIM1	46
	Cdk1/Cyclin B	56
	DYRK2	91
	AKT1	>500
	AKT2	>500
	AKT3	>500
	mTOR	>500
	PDK1	>500
	p70S6K	>500
>90% inh @ 500 nM	PI3K (p110 $\beta$ /p85 $\alpha$ )	>500
50-90% inh @ 500 nM	PI3K (p120 $\gamma$ )	>500
>50% inh @ 500 nM	PI3K (p110 $\delta$ /p85 $\alpha$ )	>500



- ▶ Inhibit cell proliferation of various cancer cell lines
- ▶ Efficacious in multiple xenograft models of cancer
- ▶ Orally bioavailable across species (%F 20-48)
- ▶ No significant *in vitro* inhibition of 5 CYP isoforms and hERG channel
- ▶ Non-mutagenic (Ames)

# CX-4945 Biological Characterization

## ▶ Modulates PI3K-Akt signaling

- CK2 specific Akt (S129)
- Downstream p21 (T145)

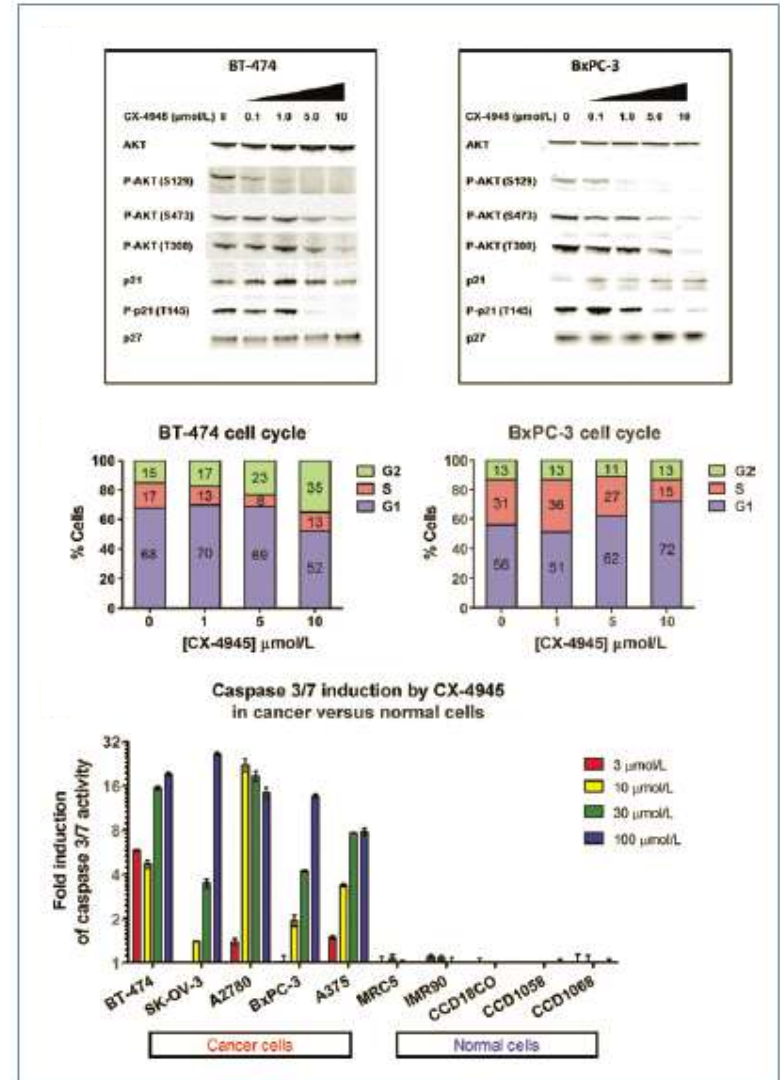
## ▶ Causes cell cycle arrest

- Cell Dependent

## ▶ Induces apoptosis

- Cancer Cells
- Not Normal Cells

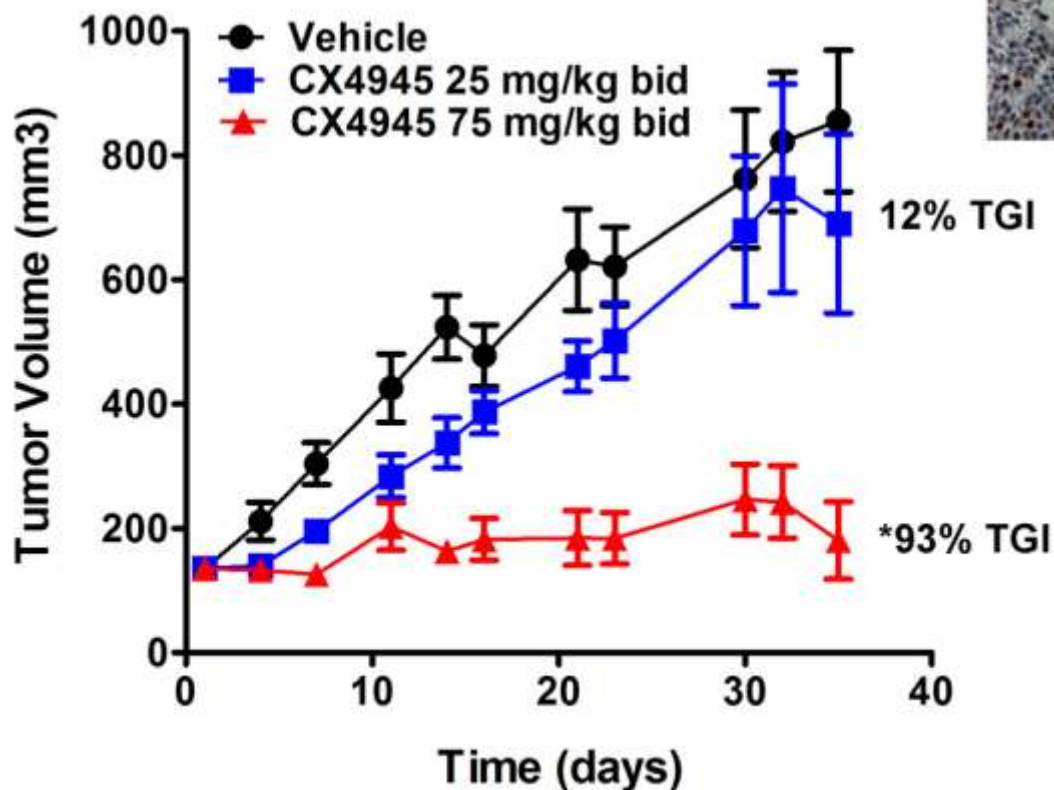
## ▶ Selectively Kills Cancer Cells



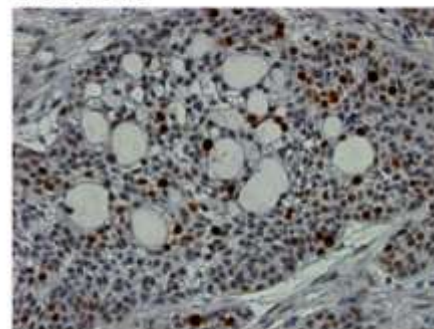
# CX-4945 Inhibits Tumor Growth and PD Markers *In Vivo*

## BxPC3 Xenograft

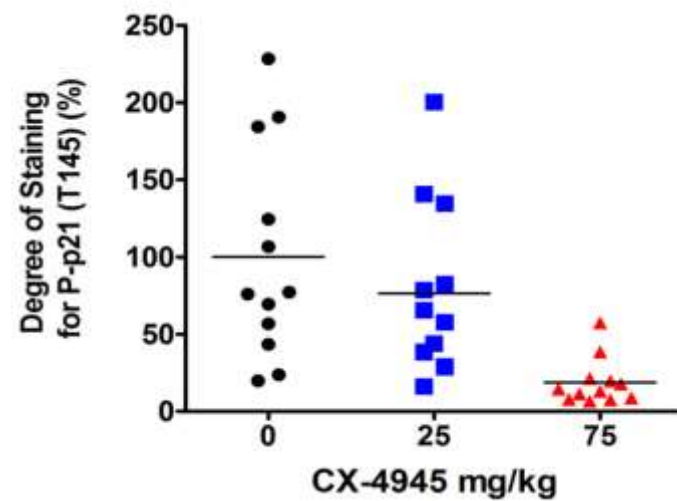
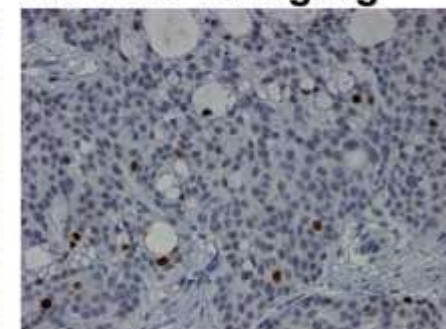
P53, CDKN2a, MAP2K4, SMAD4



Vehicle



CX-4945 75 mg/Kg



# CX-4945 Discovery Summary

- ▶ **First-in-Class** potent, selective and orally bioavailable ATP-competitive **Inhibitor of CK2** with favorable drug properties
- ▶ Discovered by the combined effect of structure- and chemistry-based optimization
- ▶ Established a binding model supported by SAR that rationalizes the strong interaction of CX-4945 within the ATP binding pocket
- ▶ Currently evaluated clinically as a new therapy for the treatment of cancer
- ▶ Breakthrough Discovery opening the door to new treatments for diseases in which CK2 is dysregulated

# Acknowledgments

## Chemistry

David M. Ryckman  
Pauline Bourbon  
Peter C. Chua  
Mustapha Haddach  
Jerome Michaux  
Johnny Nagasawa  
Fabrice Pierre  
Michael K. Schwaebe  
Eric Stefan  
Anne Vialettes  
Jeffrey P. Whitten

## Computational Sciences

Sean E. O' Brien



## President and CEO

William G. Rice

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John K. C. Lim  
Claire S. Padgett  
Daniel D. Von Hoff

## Cancer Biology

Kenna Anderes  
Adam Siddiqui-Jain  
Josh Bliesath  
Denis Drygin  
Caroline Ho  
Mayuko Omori  
Nanni Huser  
Chris Proffitt  
Nicole Streiner

## PK/ADME

Ryan Stansfield  
Ta Kung Chen  
Levan Darjania

# Publications

- ▶ “CX-4945, an Orally Bioavailable Selective Inhibitor of Protein Kinase CK2, Inhibits Survival and Angiogenic Signaling and Exhibits Antitumor Efficacy”, Siddiqui-Jain, A. *et al.*, *Cancer Research*, **2010**, 70(24), 10288-98.
- ▶ “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” , Pierre, F. *et al.*, *J. Med. Chem.*, **2011**, 54(2), 635-654.
- ▶ “Unprecedented selectivity and structural determinants of a new class of protein kinase CK2 inhibitors in clinical stage for the treatment of cancer” Battistutta, R. *et al.*, *Biochemistry*, **2011**, 50, 8478-8488.