Isoxazole Inhibitors of Bromodomains

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RSC Advances in Synthesis and Medicinal Chemistry
1 May 2012

INTRODUCING THE SGC

A model for open access public-private partnership

The SGC is a public-private partnership with a mandate to place protein structures of relevance to human health into the public domain, free from restrictions on use. Focus on proteins from human and human parasites.

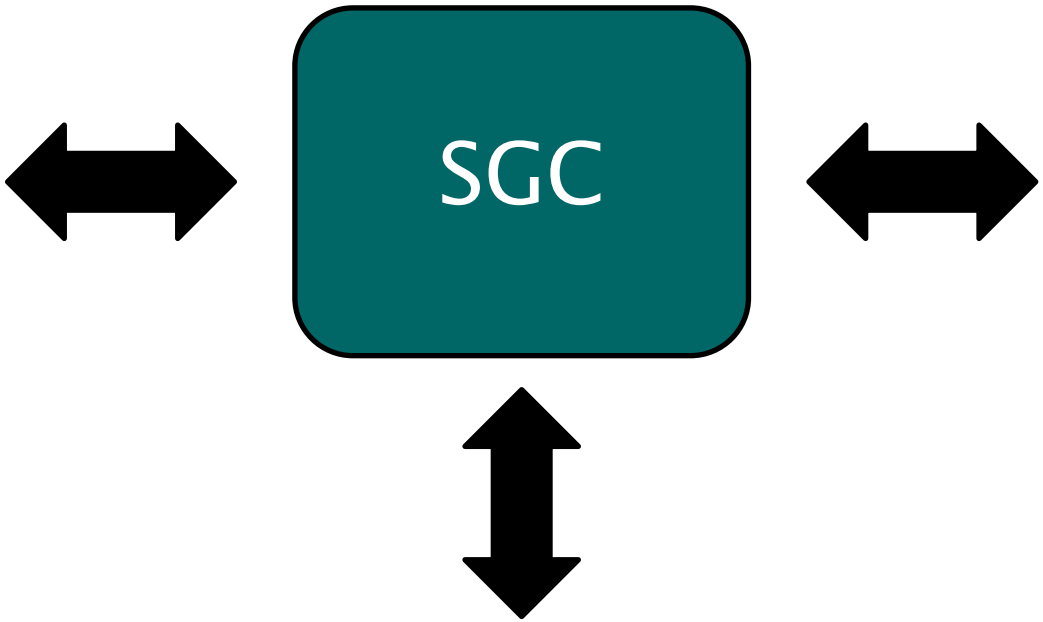
- To promote drug discovery by substantially increasing the number of medically relevant protein structures, as well as related reagents and protocols, available in the public domain
  - Human proteins (main effort)
  - Proteins from pathogens (e.g. Plasmodium)
  - Chemical probes
  - Biological probes

- 'Open Source' science
  - All structures/results are made freely available promptly
  - Funding partners receive no prior access or rights to data or progress information
  - **No IP**

PARTNERS

Pharma

GSK
Novartis
Pfizer
Eli Lilly
Abbott
Takeda



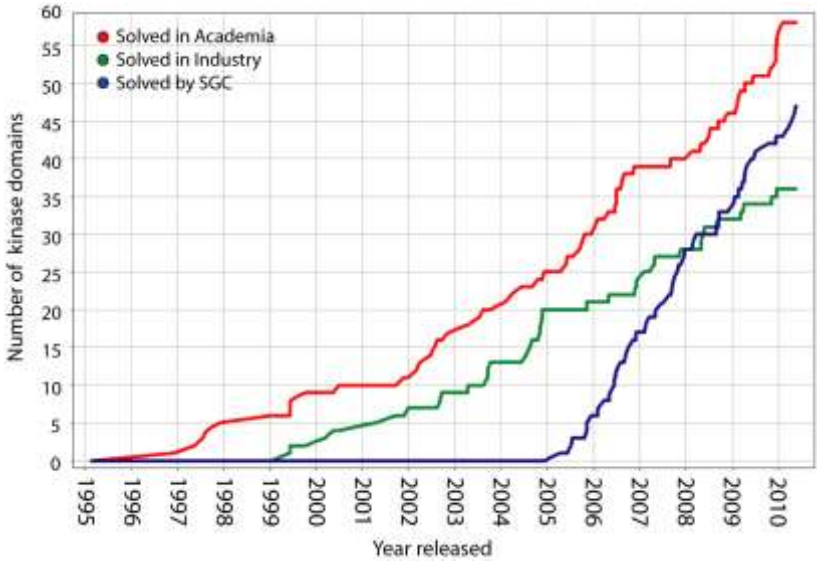
Academia

Oxford
University

Wellcome Trust

Leader in Structural Genomics

Kinase Structures



Epigenetic Structures

Family		Number of targets	Purified in SGC	Structures deposited [SGC/Total]
Lysine demethylase (KDM)		30	16	6/8
Bromodomain (BRD)		42	27	14/17
ROYAL	Tudor domain	36	15	6/19
	Chromo domain	34	20	10/16
	MBT domain	9	8	4/7
PHD		83	14	1/23
Histone acetyltransferase (HAT)		17	8	5/8
Histone methyltransferase (HMT)		60	31	12/18
TOTAL		311	139	58/116

EPIGENETICS

Wikipedia:

In biology, and specifically genetics, **epigenetics** is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence.

Underlying Mechanism of:

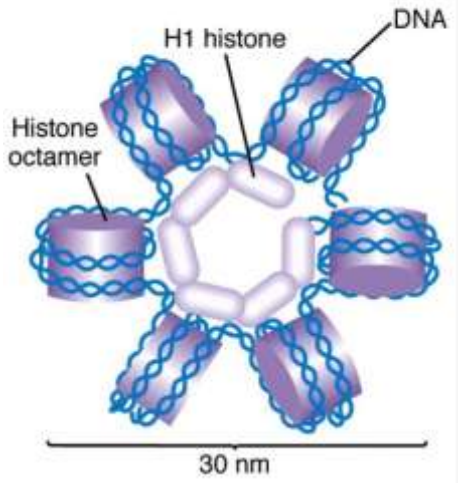
Cell differentiation
Disease progression

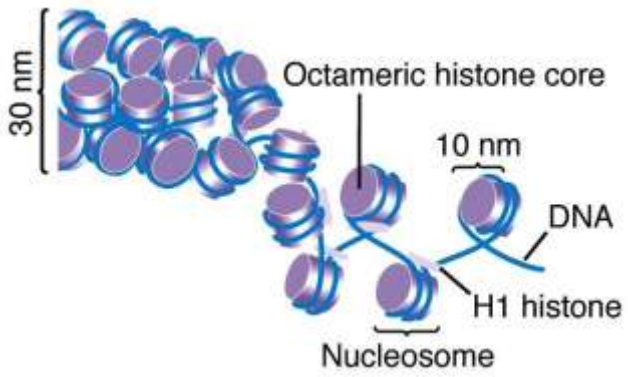
Molecular Mechanism:

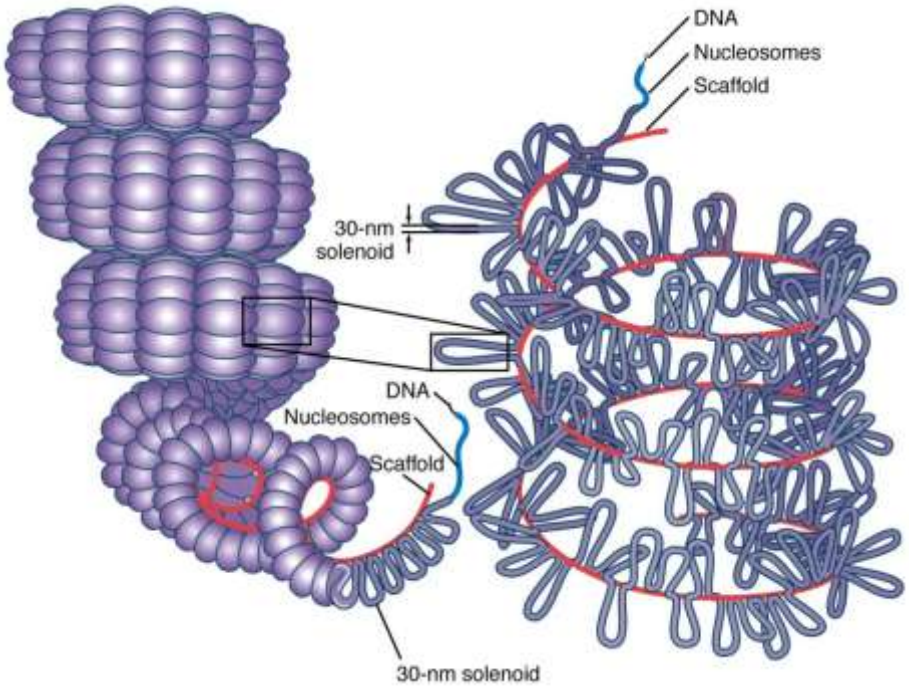
Reversible DNA modification
Reversible histone modification

Chromatin

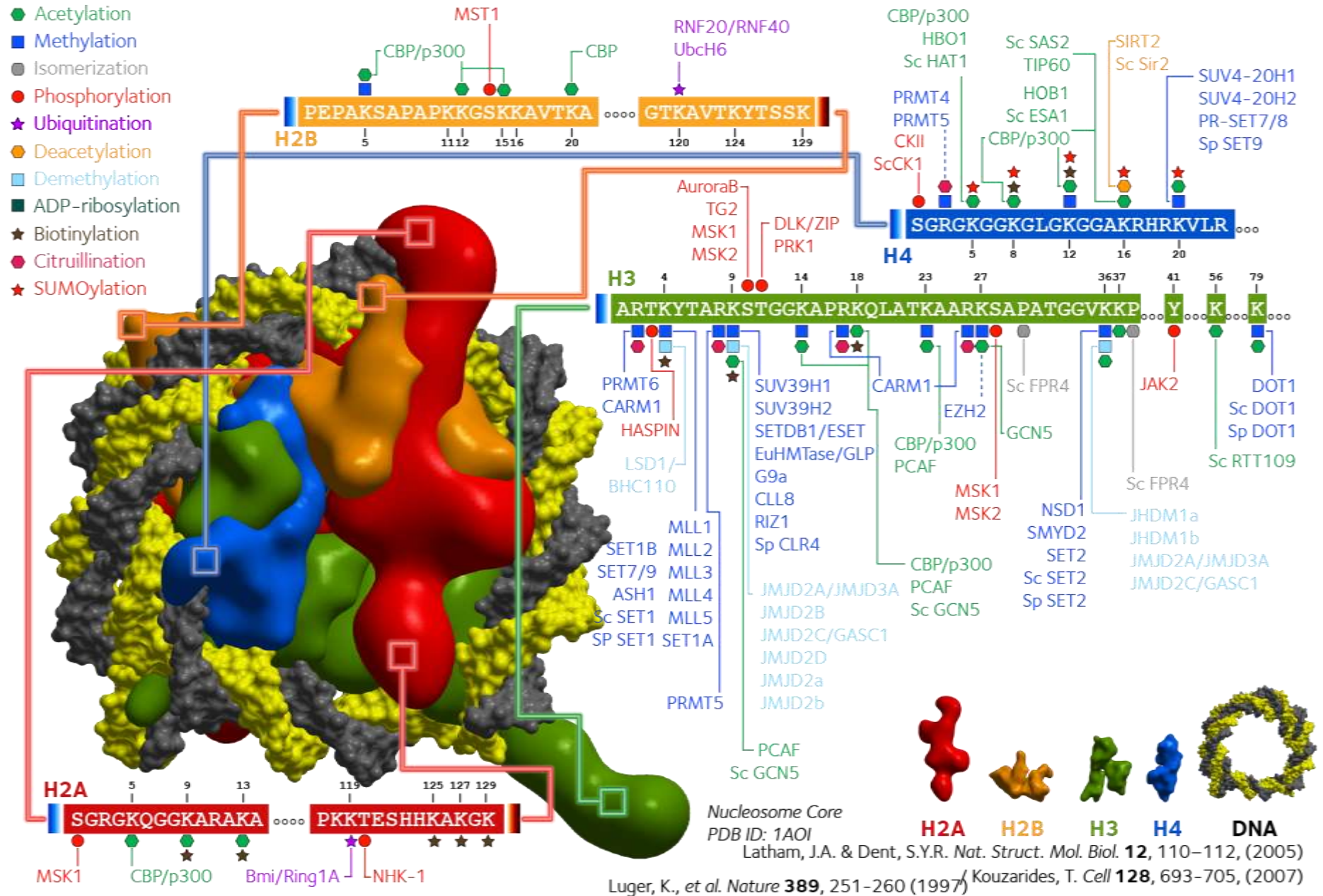
- The largest human cells are 0.1 µm (0.0000001 m) wide.
- There are 2 m of DNA in every cell.







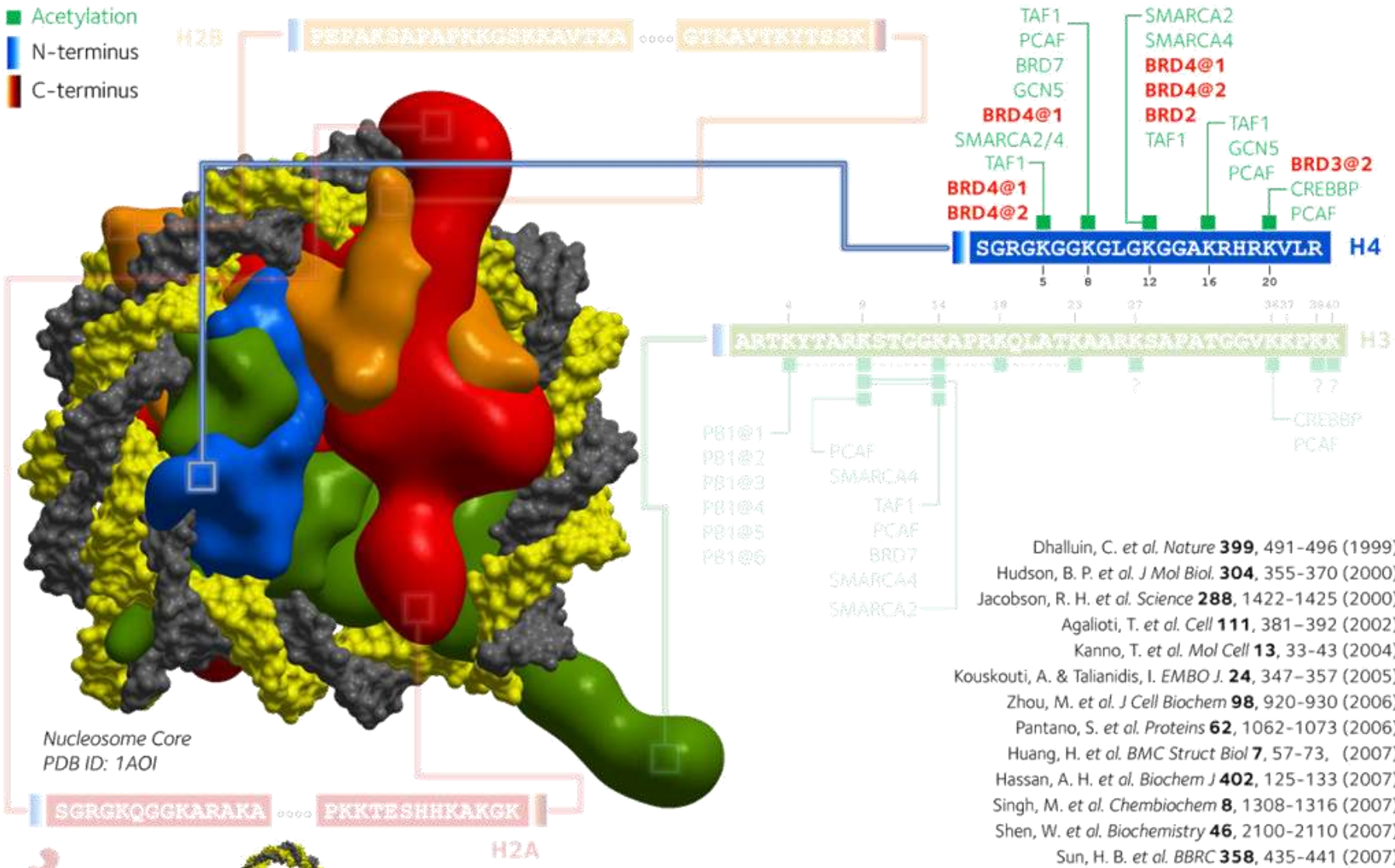
Histone Code: Writers and Erasers



Latham, J.A. & Dent, S.Y.R. *Nat. Struct. Mol. Biol.* **12**, 110–112, (2005)

Luger, K., *et al. Nature* **389**, 251–260 (1997) / Kouzarides, T. *Cell* **128**, 693–705, (2007)

Histone Code: Reader



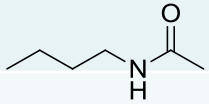
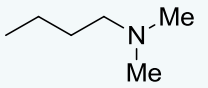
Luger, K., *et al. Nature* **389**, 251-260 (1997)

Dhalluin, C. *et al. Nature* **399**, 491-496 (1999)
Hudson, B. P. *et al. J Mol Biol.* **304**, 355-370 (2000)
Jacobson, R. H. *et al. Science* **288**, 1422-1425 (2000)
Agalioti, T. *et al. Cell* **111**, 381–392 (2002)
Kanno, T. *et al. Mol Cell* **13**, 33-43 (2004)
Kouskouti, A. & Talianidis, I. *EMBO J.* **24**, 347–357 (2005)
Zhou, M. *et al. J Cell Biochem* **98**, 920-930 (2006)
Pantano, S. *et al. Proteins* **62**, 1062-1073 (2006)
Huang, H. *et al. BMC Struct Biol* **7**, 57-73, (2007)
Hassan, A. H. *et al. Biochem J* **402**, 125-133 (2007)
Singh, M. *et al. Chembiochem* **8**, 1308-1316 (2007)
Shen, W. *et al. Biochemistry* **46**, 2100-2110 (2007)
Sun, H. B. *et al. BBRC* **358**, 435-441 (2007)
Kupitz, C. *et al. Biophysical Chemistry* **136**, 7-12 (2008)
Liu, Y. *et al. Biochemistry* **47**, 6403-6417 (2008)
Zeng, L. *et al. Structure* **16**, 643-652 (2008)
Leroy, G. *et al. Mol Cell* **30**, 51-60 (2008)

Epigenetic Code

Histones can be modified and recognized



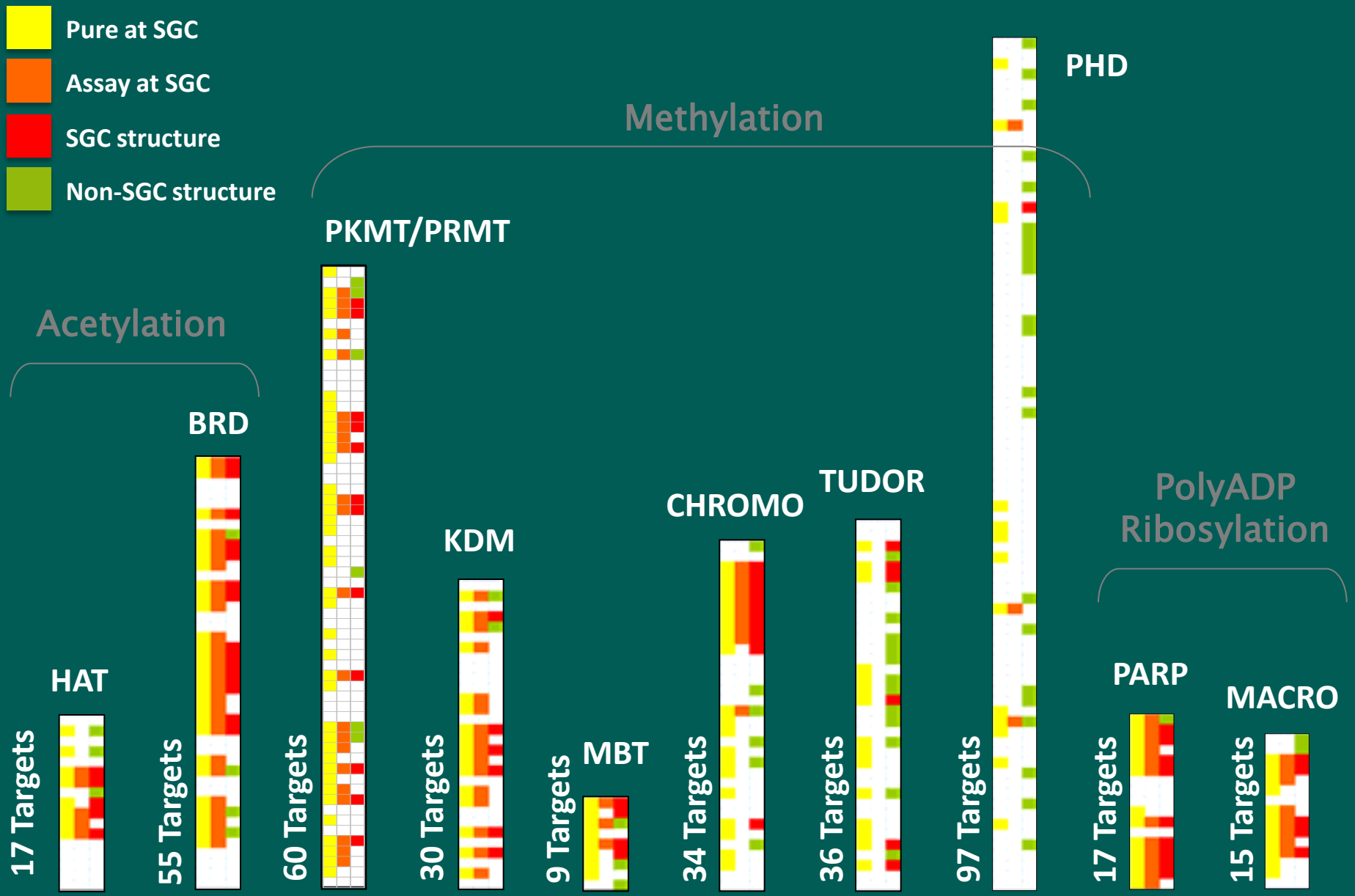
	Histone Modification	Write	Read	Erase
	Acetyl	HAT	Bromo	HDAC
	Methyl	HMT	Chromo, PHD, Tudor, MBT	HDM

Different modifications affect gene transcription

Modification	H3K4	H3K9	H3K27
KMe	Activation	Activation	Activation
KMe_3	Activation	Repression	Repression
KAc	Activation	Activation	Activation

HAT: histone acetyl transferase, **Bromo**: bromodomain; **HDAC**: histone deacetylase; **HMT**: histone methyl transferase **Chromodomain, PHD-domain, Tudor-domain, MBT-domain; HDM**: histone demethylase

SGC Progress for Epigenetics Targets



EPIGENETIC TARGETS IN DISEASE

- Early position in signalling cascades:
  - Epigenetic modifications regulate multiple genes implicated in chronic diseases
- Potential for many drugs, in many therapeutic areas

Bromodomain 4 activation predicts breast cancer survival

PNAS | April 29, 2008 | vol. 105 | no. 17

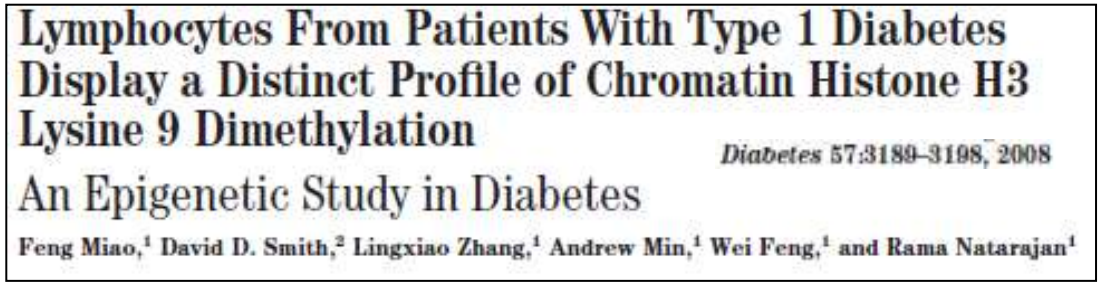
Nigel P. S. Crawford*, Jude Alsarraj*, Luanne Lukes*, Renard C. Walker*, Jennifer S. Officewala*, Howard H. Yang†, Maxwell P. Lee‡, Keiko Ozato†, and Kent W. Hunter*§

The EMBO Journal (2009) 28, 3341–3352 | © 2009 European Molecular Biology Organization | Some Rights Reserved 0261-4189/09
www.embojournal.org

Francesca De Santa, Vipin Narang Zhei Hwee Yap, Betsabeh Khoramian Tusi, Thomas Burgold, Liv Austenaa, Gabriele Bucci, Marieta Caganova, Samuele Notarbartolo, Stefano Casola, Giuseppe Testa, Wing-Kin Sung, Chia-Lin Wei,* and Gioacchino Natoli,*

Jmjd3 contributes to the control of gene expression in LPS-activated macrophages

THE EMBO JOURNAL
EMBO open



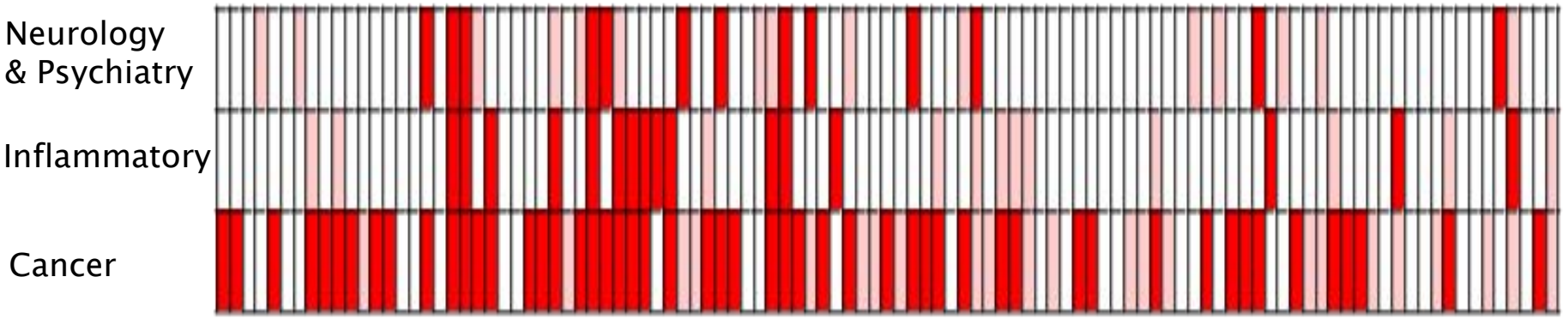
Lymphocytes From Patients With Type 1 Diabetes Display a Distinct Profile of Chromatin Histone H3 Lysine 9 Dimethylation

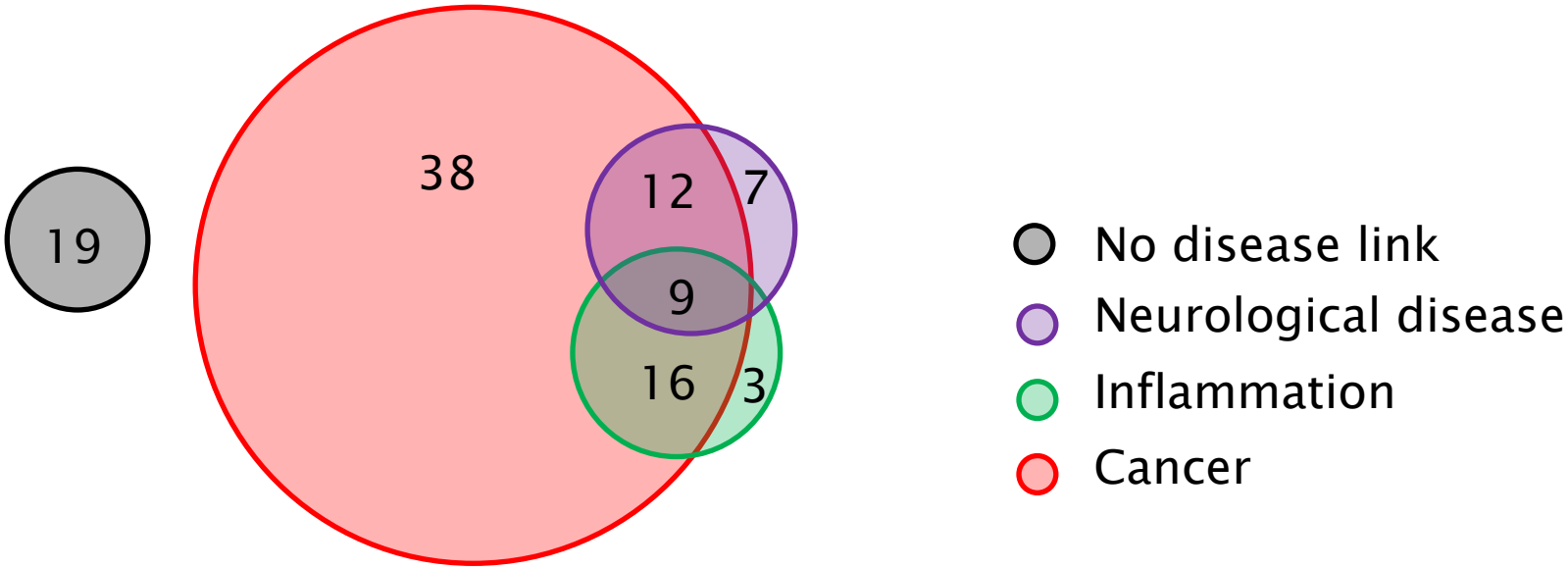
Diabetes 57:3189–3198, 2008

An Epigenetic Study in Diabetes

Feng Miao,[1] David D. Smith,[2] Lingxiao Zhang,[1] Andrew Min,[1] Wei Feng,[1] and Rama Natarajan[1]

Epigenetic Targets In Disease





SGC Chemical Probe Discovery

Probe Criteria

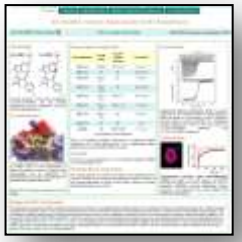
- **In vitro activity:** IC_{50} or Kd 100nM
- **In vitro selectivity:** 30-fold vs. other branches of phylogenetic tree
- **Cellular activity:** IC_{50} 1μM

Assay development

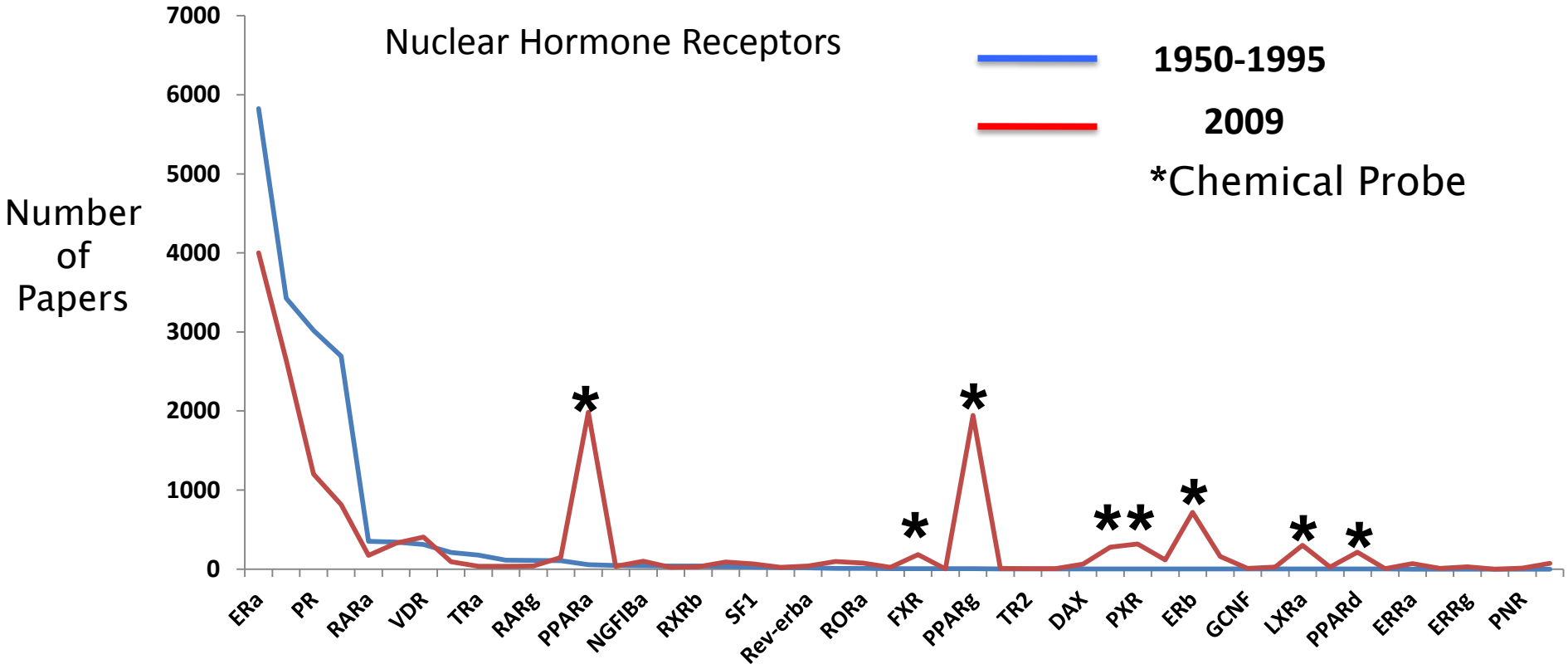
Focused sets
VLS
Fragments
HTS

Analogue purchase
Synthesis
SAR generation
SAR analysis
Complex structures
Selectivity

Secondary assays
Cellular assays
Selectivity



CHEMICAL PROBES HAVE BIG IMPACT



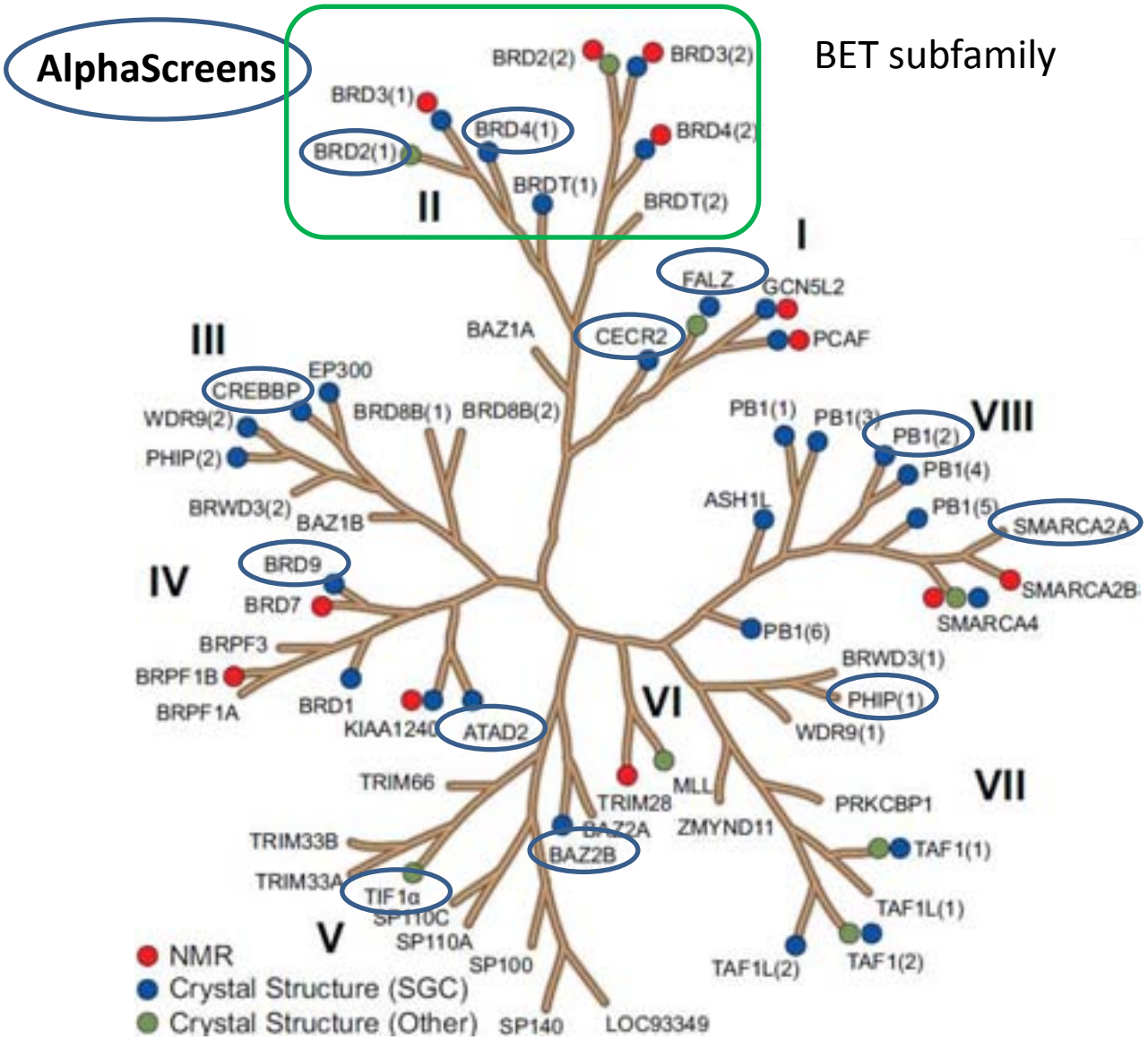
"Open access chemical and clinical probes to support drug discovery"
Nat. Chem. Bio. 2009, 5 (7), 436-440

Epigenetic Chemical Probes

Epigenetics Biology

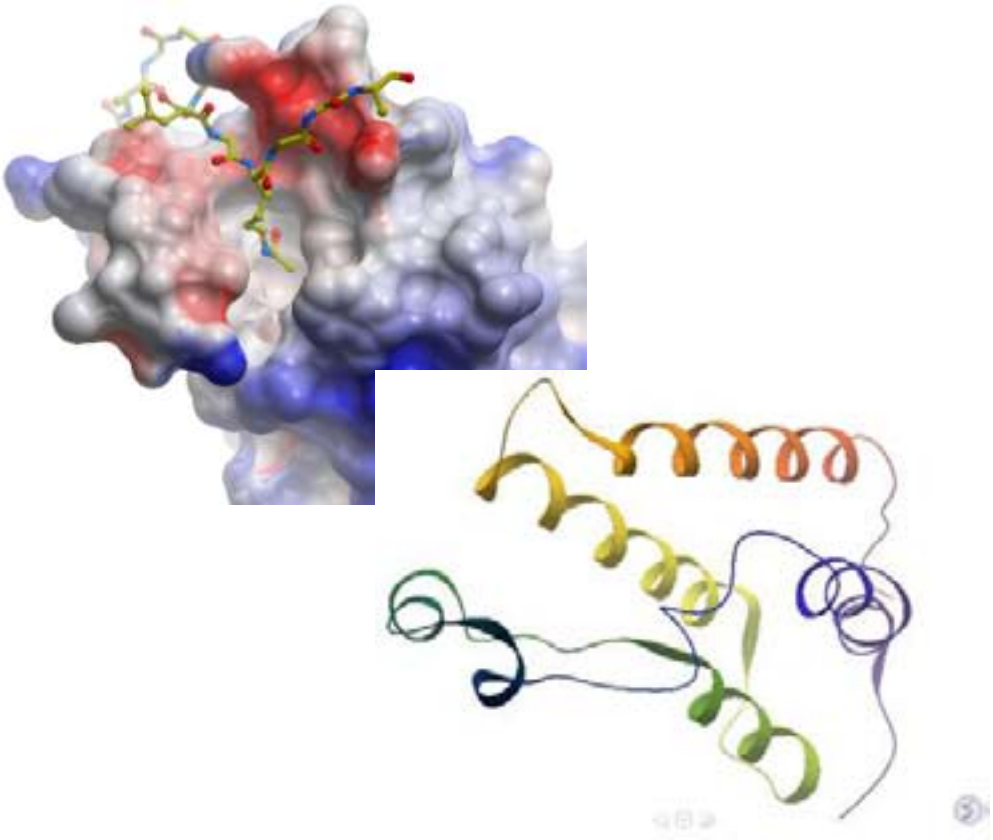
Chemical Probes

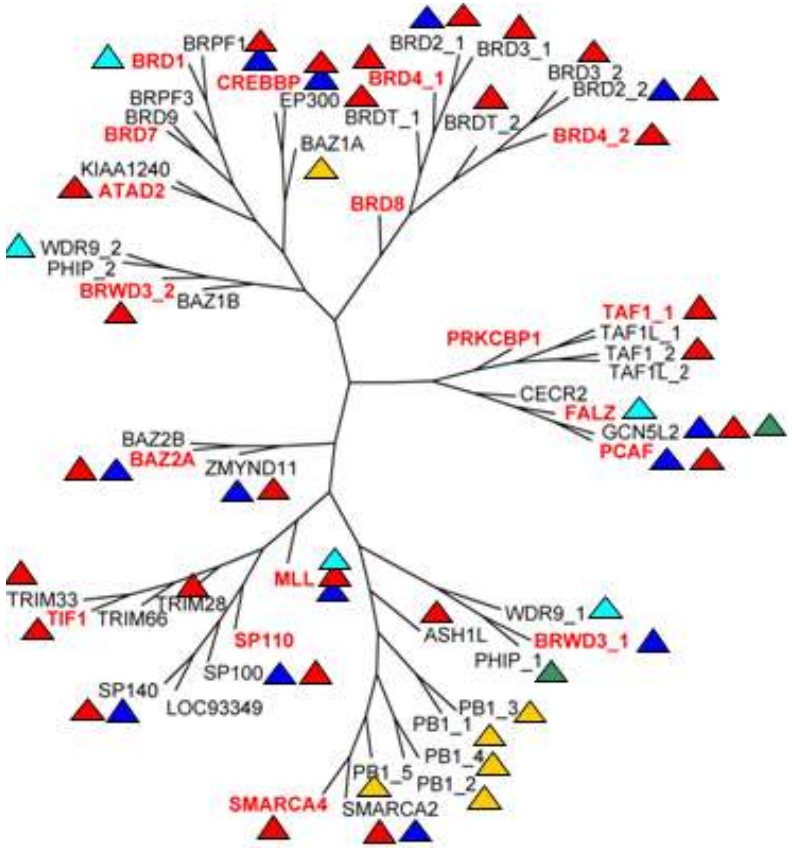
Bromodomain Structural Alignment Tree



ACETYL LYSINE READERS: BROMODOMAINS

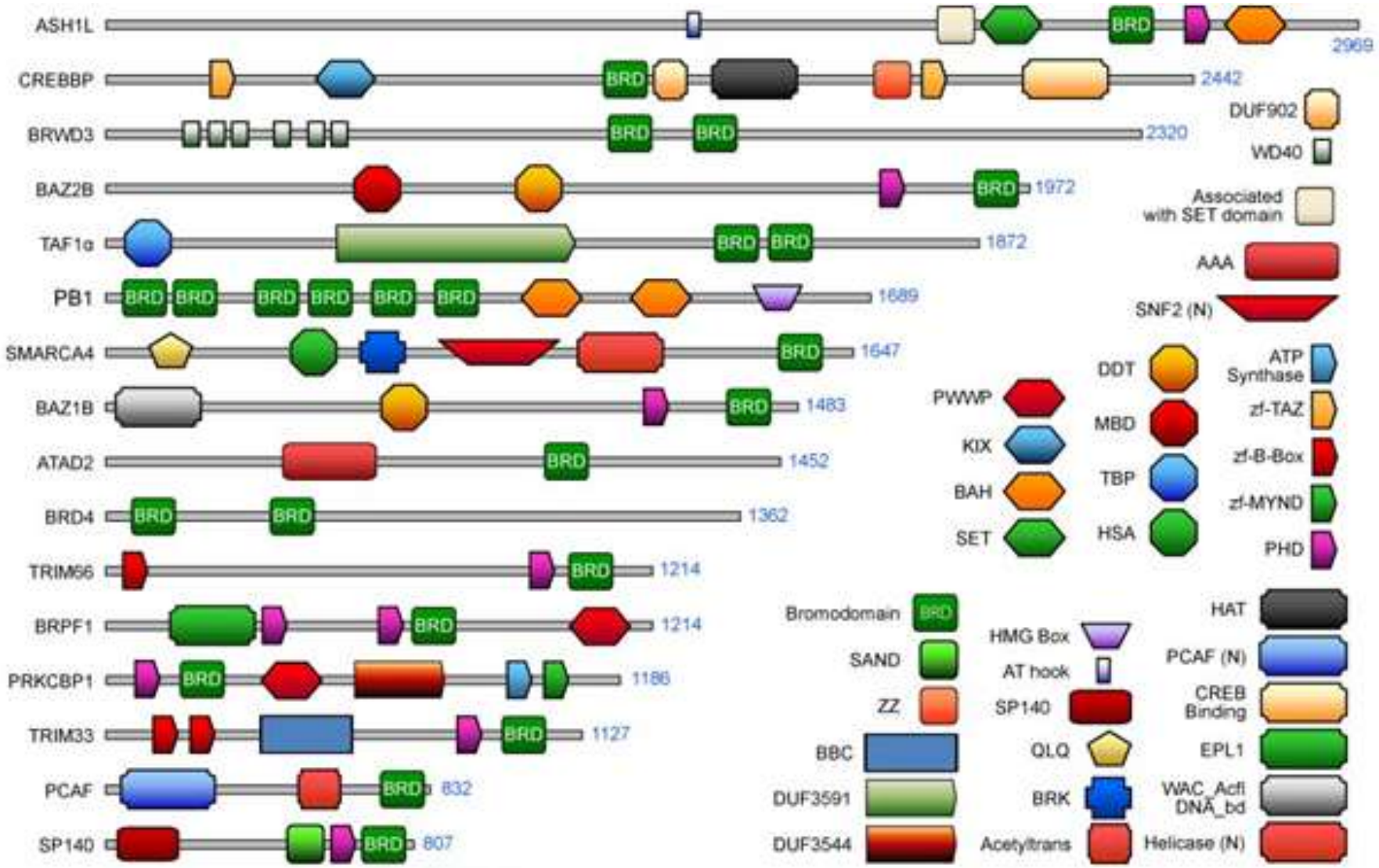
- 62 domains in Human
- ~120 residue Kac interaction module ("reader")
- Clinical POC targeting Kac regulation (HDACs)



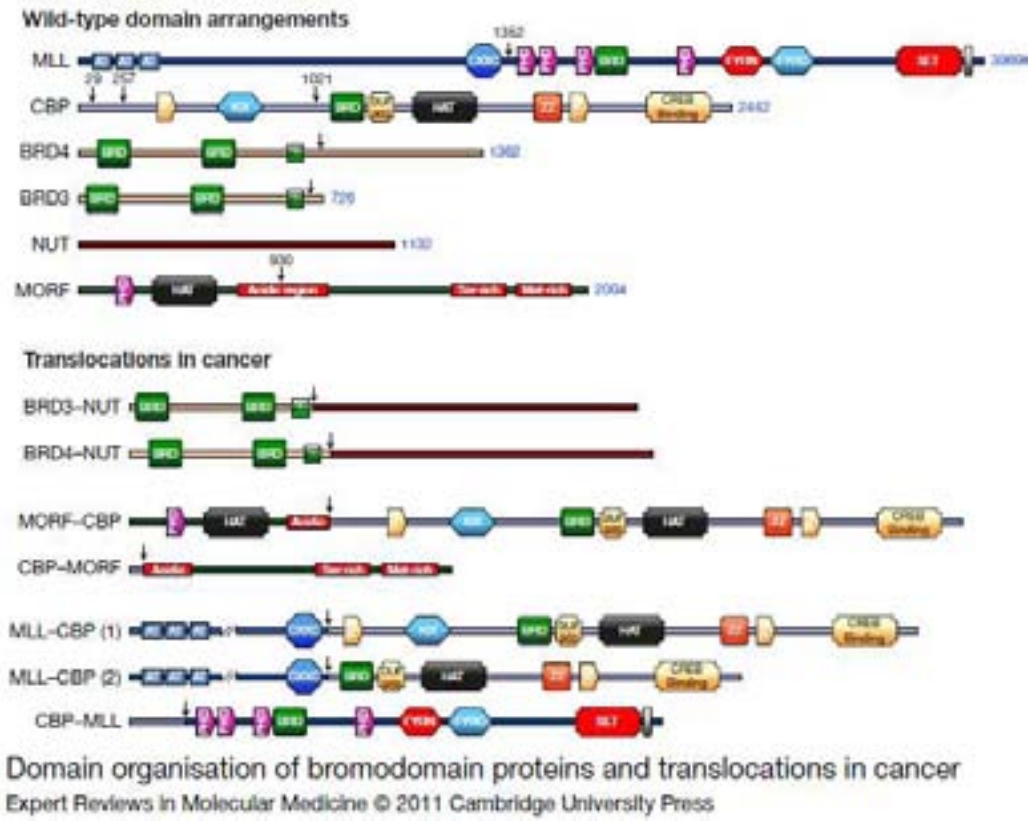


- Inflammation
- Cancer
- Metabolic disease
- Neurological diseases
- Cardiovascular diseases

Bromodomain Containing Proteins



Bromodomains and Cancer

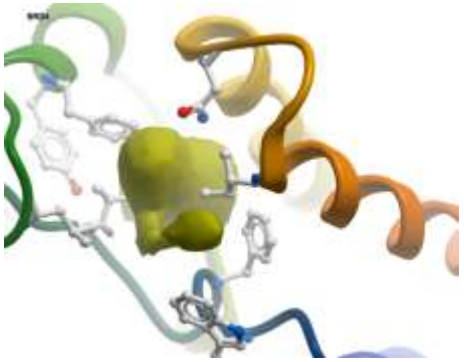


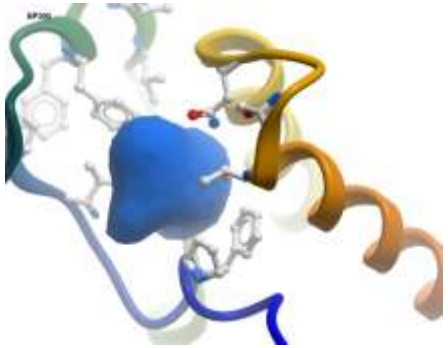
Domain organisation of bromodomain proteins and translocations in cancer

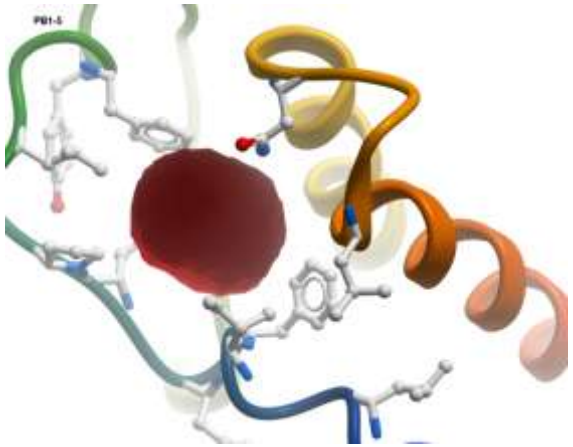
Domain organization of bromodomain proteins and translocations in cancer. Bromodomain modules are shown in green (labelled BRD). Other domain types are labelled directly in the figure and breakpoints are indicated by arrows. Wild type domain arrangements are shown in the upper panel.

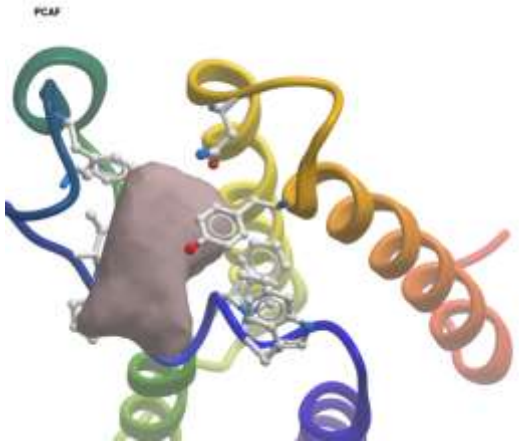
- Oncogenic fusions of CBP in acute leukemia.
- CBP contributes to tumorigenesis of fusion NUP98-HoxA9 and MOZ-TIF2 proteins.
- CBP mutations in relapsed acute lymphoblastic leukaemia (AML) and are very common in diffuse large B-cell lymphoma and follicular lymphoma.
- CBP and the related HAT EP300 are also highly expressed in advanced prostate cancer and expression levels have been linked with cancer patient survival.

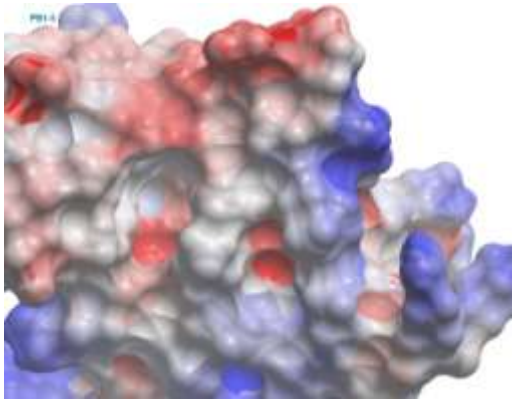
Acetyl Lysine Binding Sites

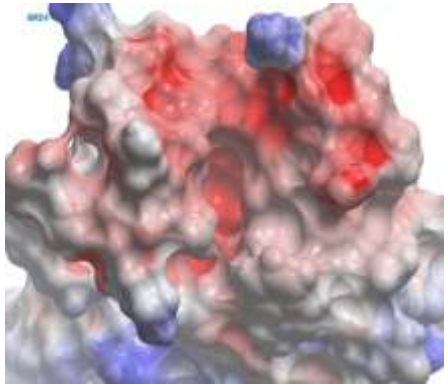




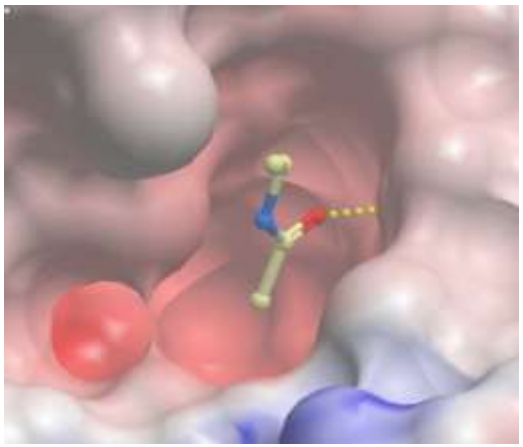


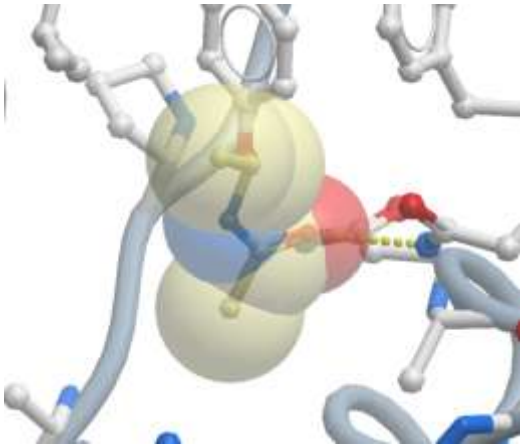


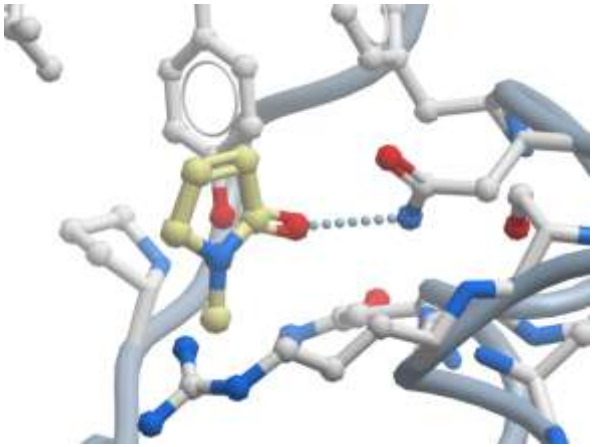


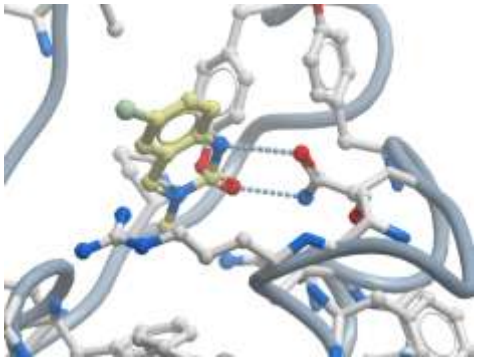


Acetyl Lysine Mimetics





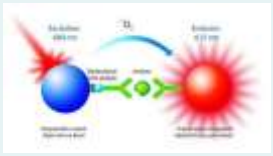
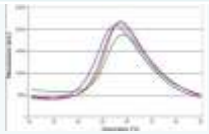
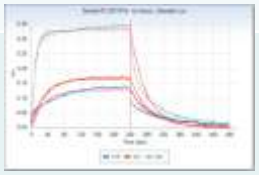
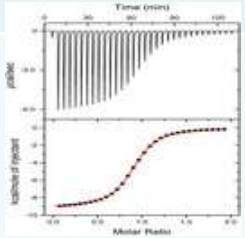




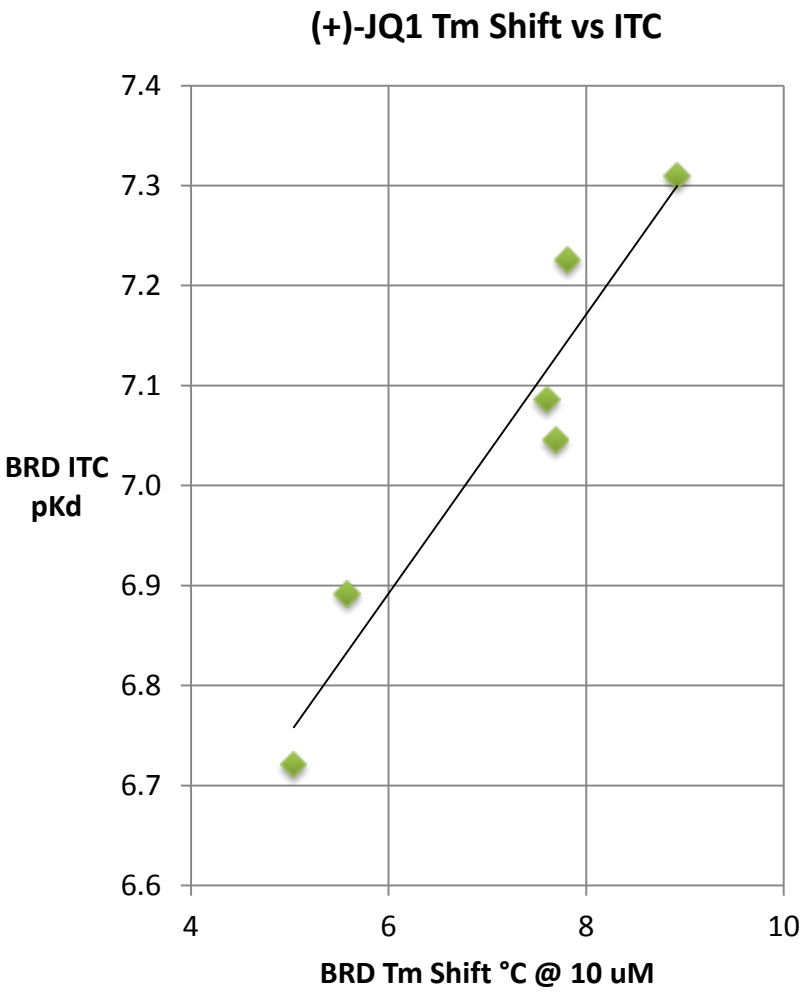
Ligand	BRD2	BRD4	BAZ2B	CREBBP	FALZ
Kac	3,740	7,210	2,990	522	4,350
DMSO	255,900	281,000	346,500	17,600	78,200
NMP	8,900	6,000	34,100	970	6,400
E07120	15.0	36.9	>>200	3.4	140

IC_{50} [μM]

Bromodomain Binding Assays

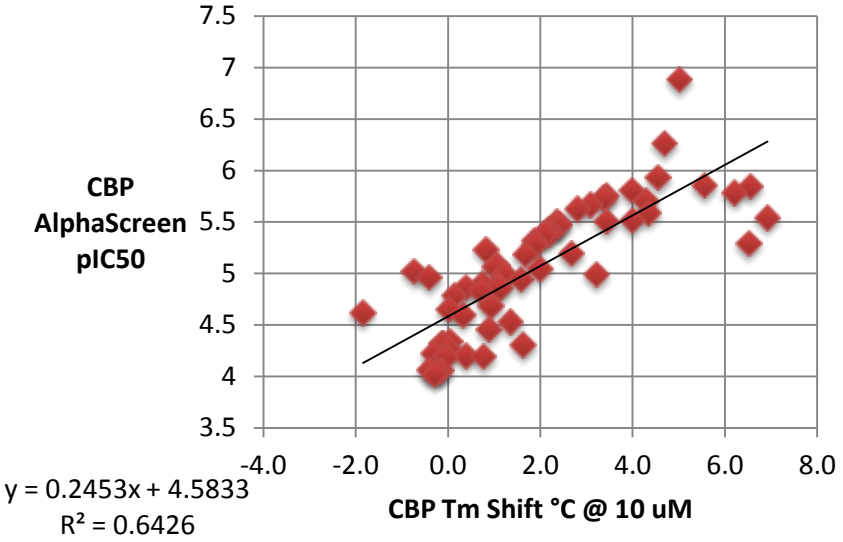
	Assay	Potent Output	Sensitivity	Through -put	Ligand needed	Drawbacks	Data Points
	Alpha-Screen	Low IC50	High	High	Yes	High false positive rate	4383 compounds 6 proteins
	Tm Shift	High Δ C	Medium	High	No	Indirect	4615 compounds 21 proteins
	Octet BLI	Low Kd	Medium	Medium	No	Biotinylated protein	219 compounds 4 proteins
	Micro ITC	Low Kd	Low	Low	No	Protein consumption	Very few

Bromodomain Binding Assays



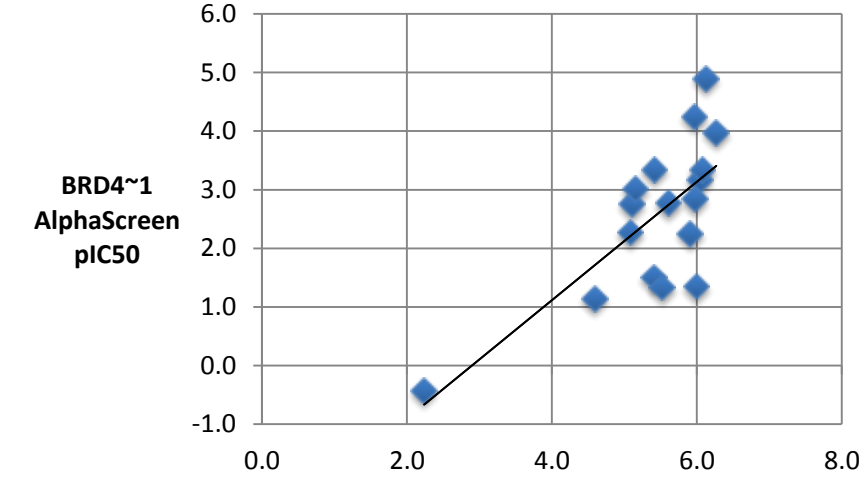
ITC pKd = 0.14*[Tm Shift C] +6.1

$R^2 = 0.92$



y = 0.2453x + 4.5833

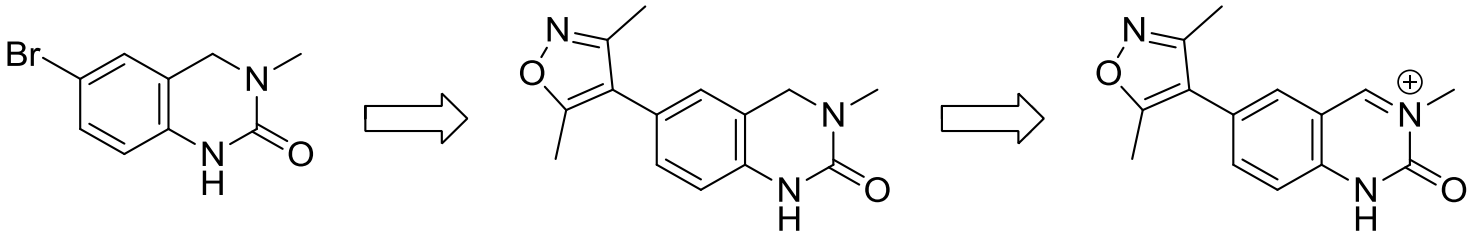
$R^2 = 0.6426$



y = 1.0102x - 2.9233

$R^2 = 0.5342$

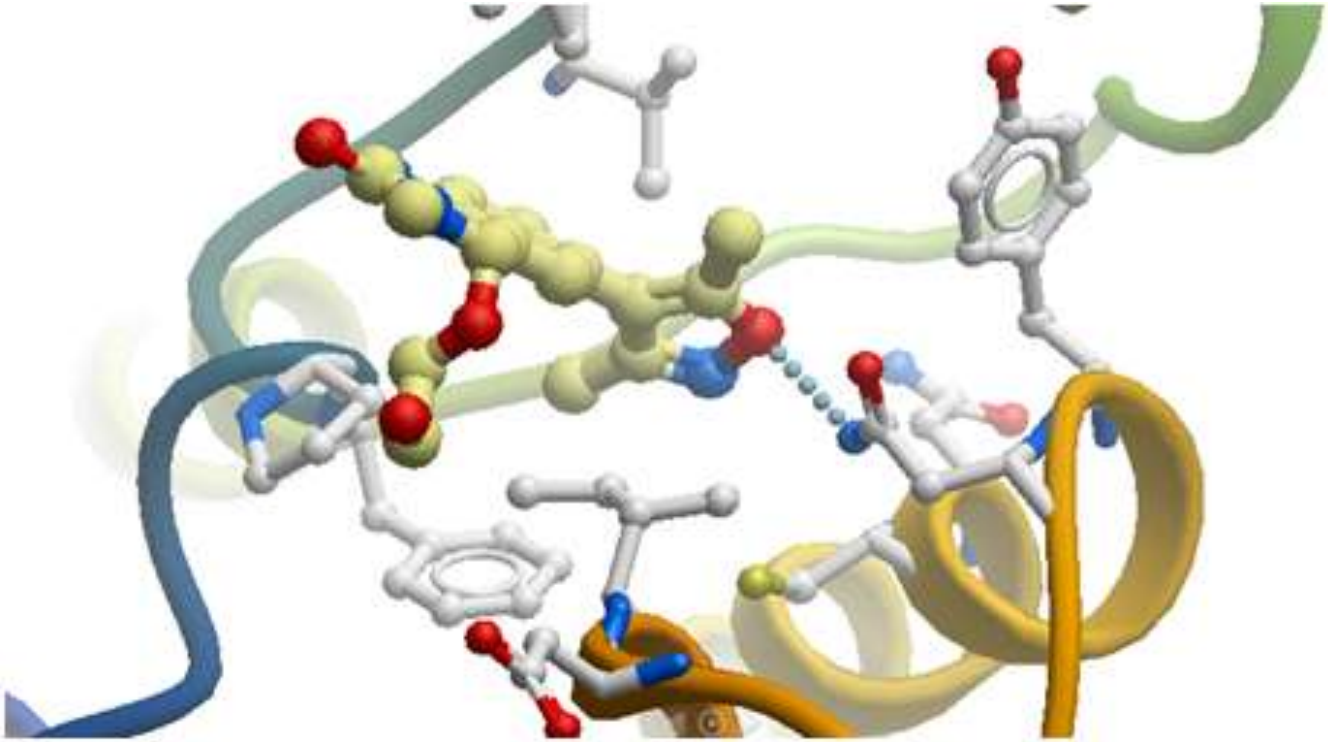
Discovery of Isoxazoles



BRD4* 36 uM

BRD4* 3.3 uM

BRD4* 0.7 uM



*AlphaScreen IC50

3,5-Dimethylisoxazoles as BET Inhibitors

Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

3,5-Dimethylisoxazoles Act As Acetyl-lysine-mimetic Bromodomain Ligands†

David S. Hewings,[‡,§] Minghua Wang,[‡] Martin Philpott,[‡] Oleg Fedorov,[‡] Sagar Uttarkar,[‡] Panagis Filippakopoulos,[‡] Sarah Picaud,[‡] Chaitanya Vuppusetty,[‡] Brian Marsden,[‡] Stefan Knapp,[‡] Stuart J. Conway,[*,§] and Tom D. Heightman[*,‡,∥]

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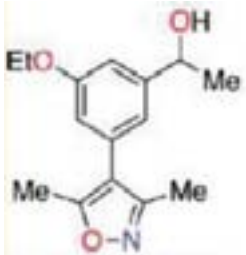
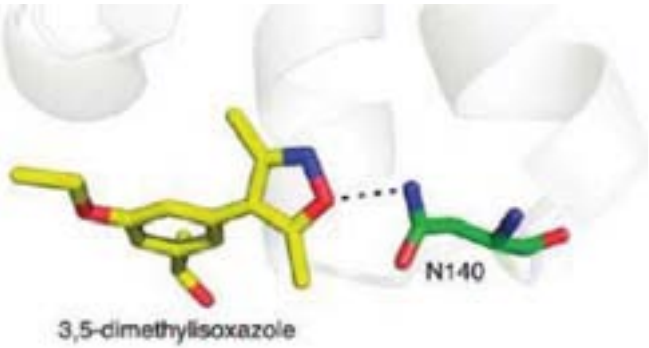


Compound 4d IC_{50}

BRD2(1) = 1.6 μM

BRD4(1) = 4.8 μM

CREBBP = 28.1 μM



LETTER

doi:10.1038/nature10509

Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia

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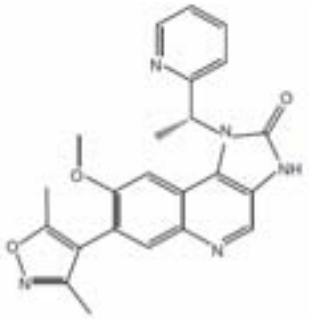
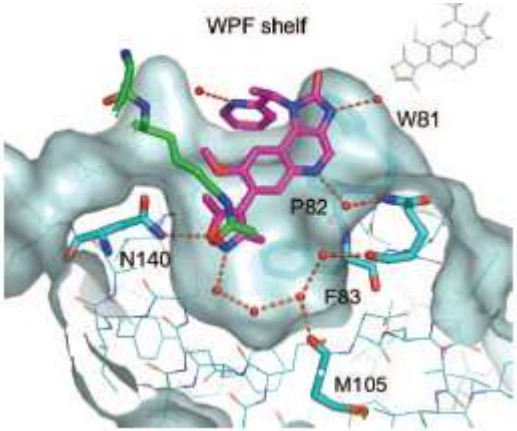
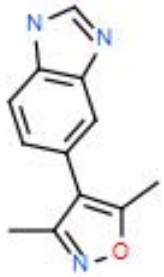
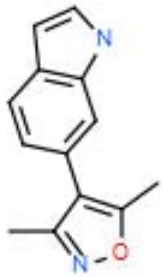
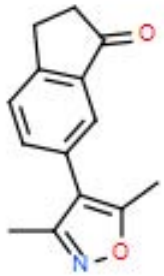


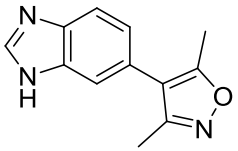


Commercial Isoxazoles For BRD4

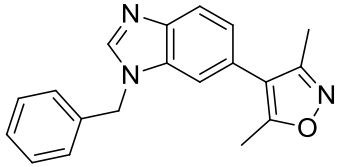
	mol	SGCGlobalIDs	BRD4 MinIC50	CREBBP MinIC50
1		BDOIA000079a	3.547	1.824
2		BDOIA000082a	8.029	3.95
3		BDOIA000081a	28.55	83.18

AlphaScreen IC50 µM

Isoxazoles For BRD4

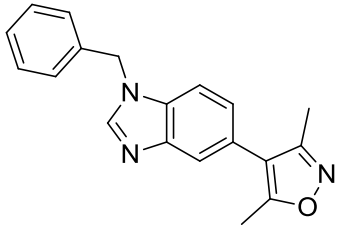


BDOIA79
BRD4* 3.5 uM

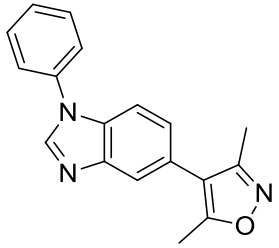


BDOIA116
BRD4* 1.4 uM
CBP* 24 uM

+



BDOIA114
BRD4* 0.91 uM
CBP* ND

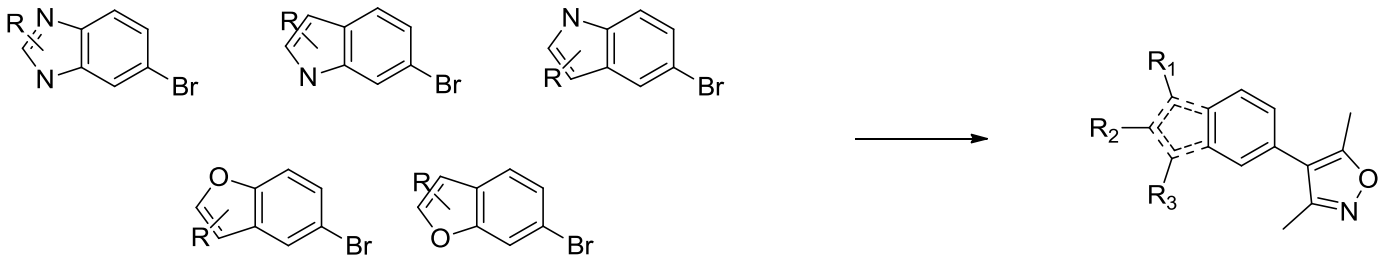


BDOIA115
BRD4* 2.6 uM
CBP* 3.8 uM

- 2 step synthesis for mixture
- 3-4 step synthesis for single regioisomer

*AlphaScreen IC50

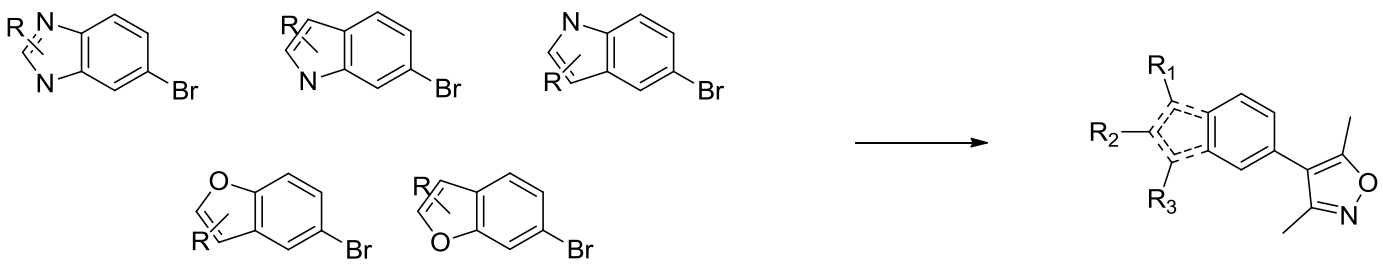
Isoxazoles For CBP

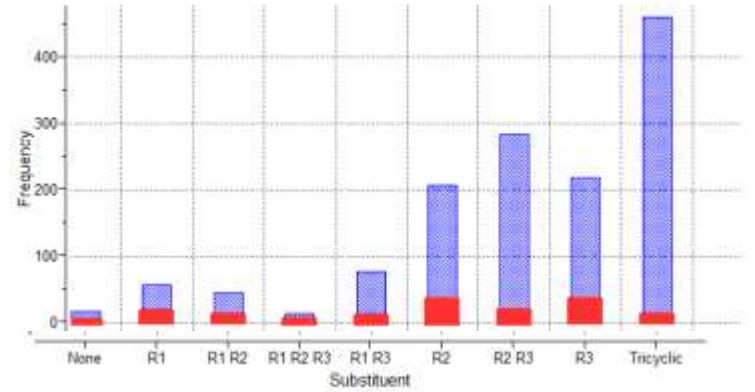


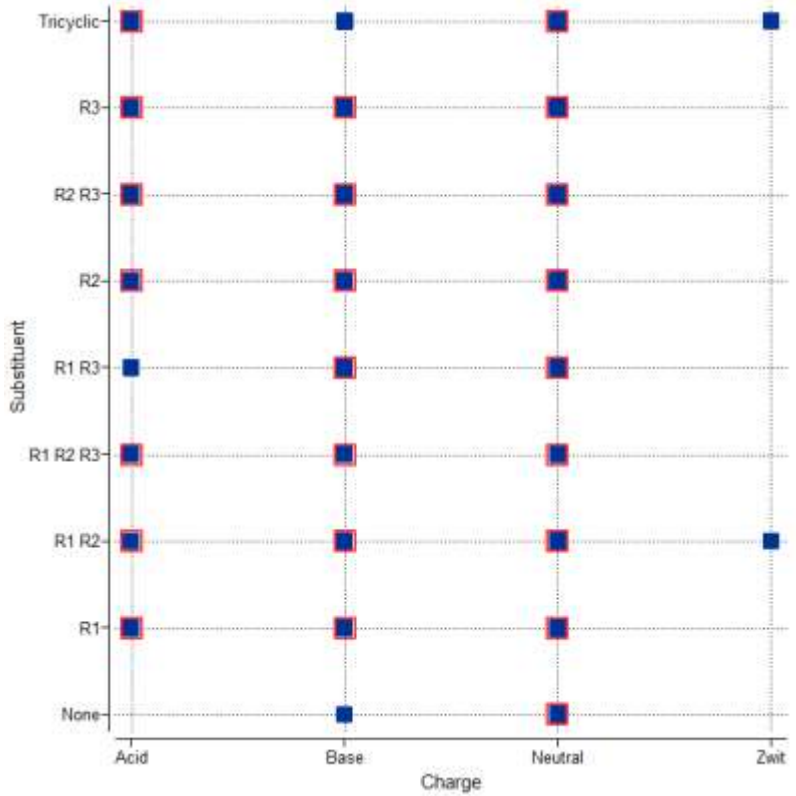
75 compounds selected from Pfizer collection

- > 50 mg available
- Published scaffolds
- Acid, base, neutral
- R1, R2, R3

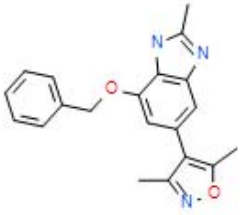
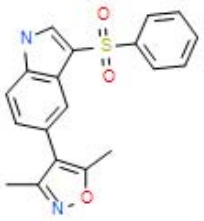
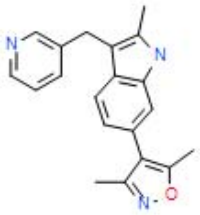
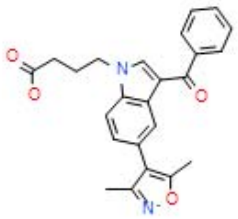
Isoxazoles For CBP



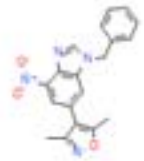
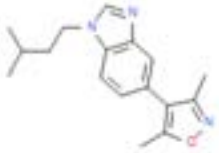
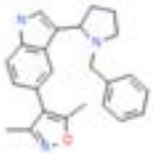


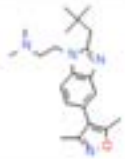
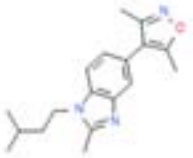


Non–Selective Isoxazoles

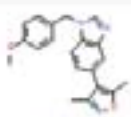
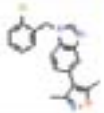
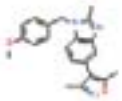
mol	SGCGlobalIDs	BRD4 AS IC50 uM	CBP AS IC50 uM
	BDOIA151	0.86	0.86
	BDOIA146	1.14	1.17
	BDOIA167	5.35	1.40
	BDOIA106	2.10	1.85

Selective Isoxazoles

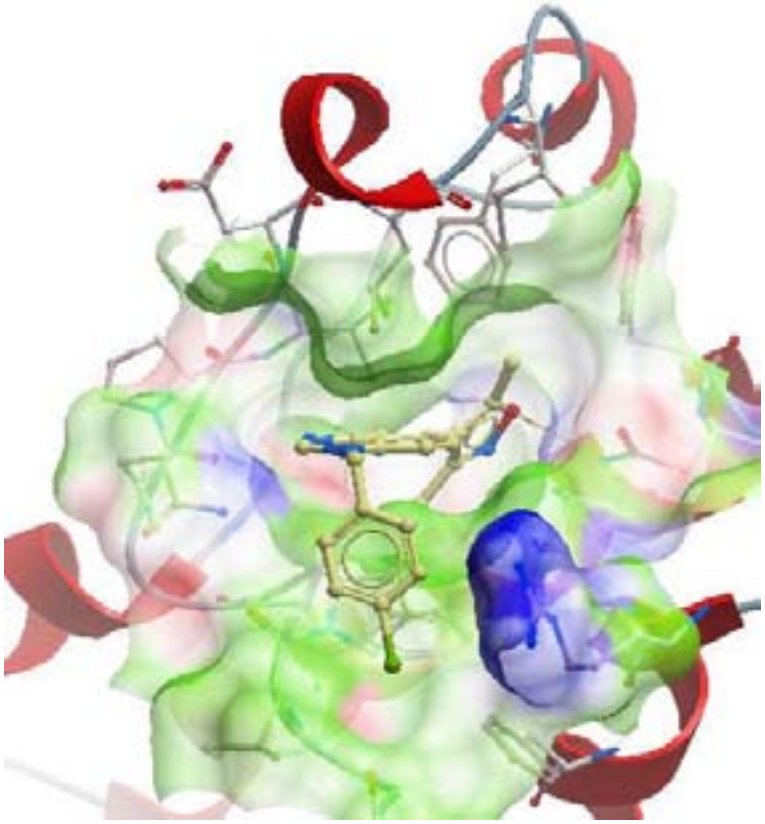
mol	SGCGlobalIDs	BRD4 AS IC50 uM	CBP AS IC50 uM
	BDOIA145	0.30	2.17
	BDOIA182	0.44	4.77
	BDOIA148	1.72	28.97

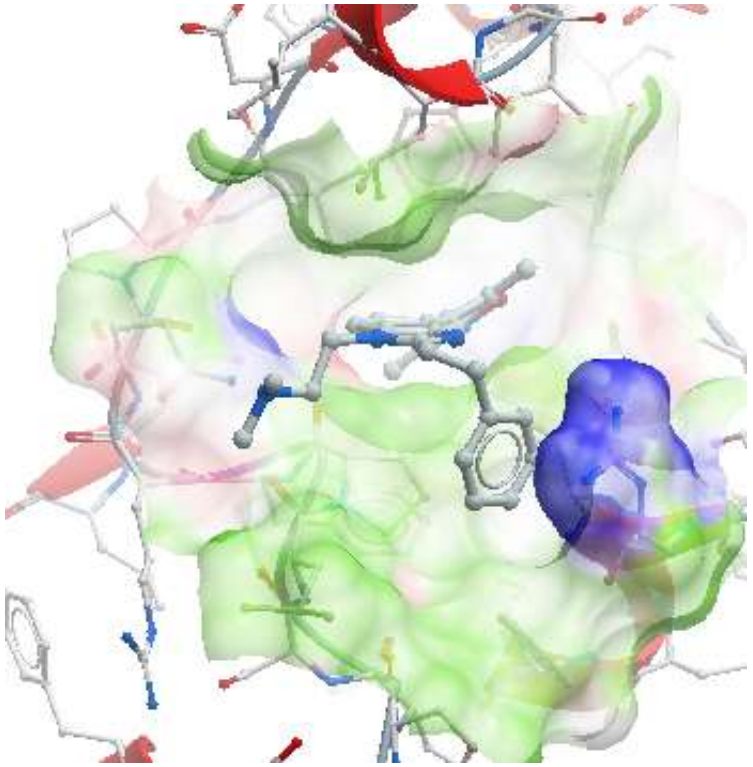
mol	SGCGlobalIDs	BRD4 AS IC50 uM	CBP AS IC50 uM
	BDOIA154	15.37	1.63
	BDOIA163	8.18	1.99

CBP Selective Isoxazoles

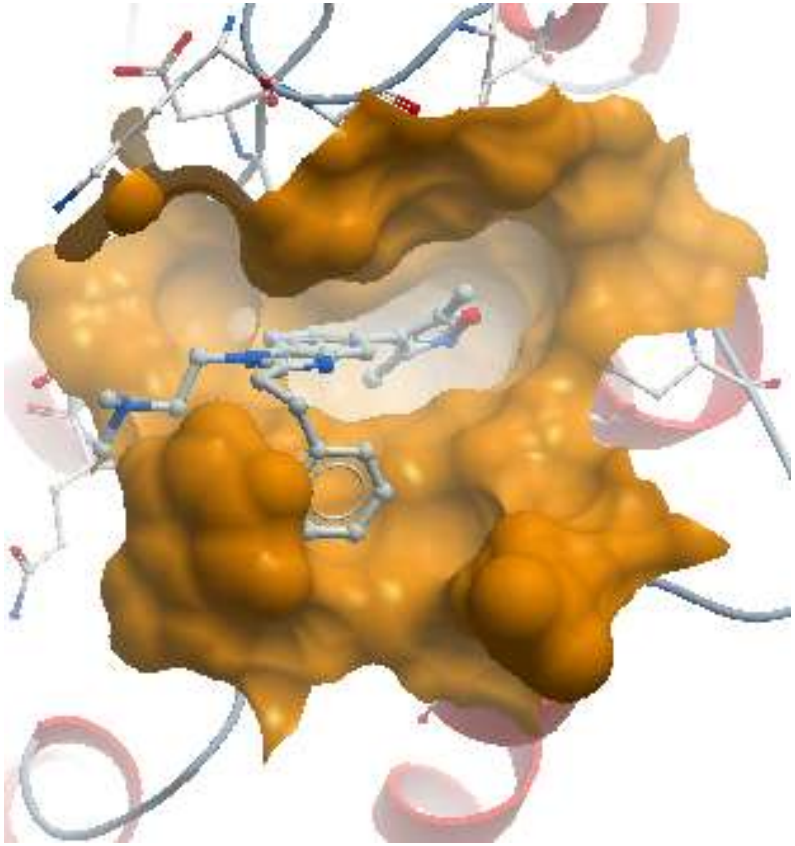
	5GCGlobalIDs	BRD4 AS IC50 uM	CBP AS IC50 uM	BRD4 Tm Shift @ 10 uM	BRD2 Tm Shift @ 10 uM	CREBBP Tm Shift @ 10 uM	BAZ2B Tm Shift @ 10 uM	BRD3 Tm Shift @ 10 uM	BRDT Tm Shift @ 10 uM	LOC93349 Tm Shift @ 10 uM	PB1 Tm Shift @ 10 uM	PCAF Tm Shift @ 10 uM
	BDOIA242	1.2	0.3	3.4	3.8	4.9	0.1	4.2	1.0	-0.2	0.1	0.0
	BDOIA244	1.4	0.27	3.5	4.1	4.7	-0.1	4.9	1.1	0.6	0.0	0.3
	BDOIA220		0.47	0.6		5.3					0.3	
	BDOIA268	7.0	0.9	3.0		4.5						

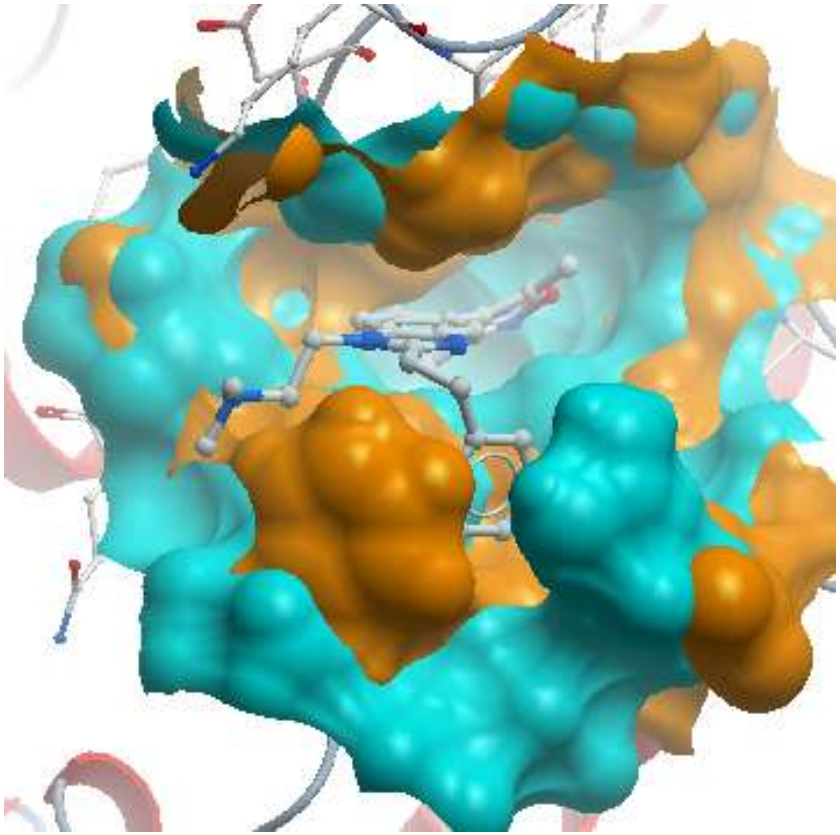
BDOIA220 Modelled in CBP and BRD4



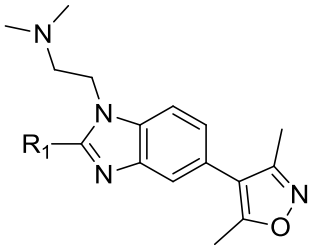


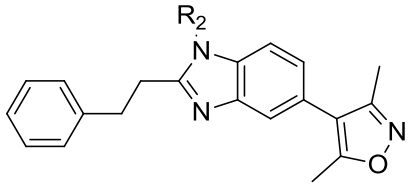
BDOIA220 Modelled in CBP and BRD4

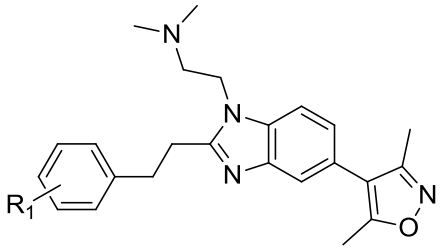


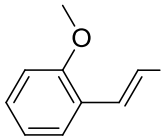


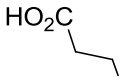
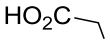
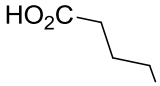
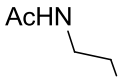
CBP Selective Isoxazole Optimization





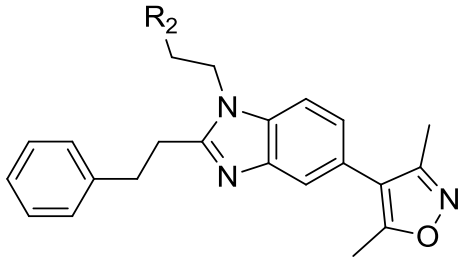


R1	ID	CREBBP IC50	CREBBP Tm Shift	BRD4_Tm Shift
Bn	BDOIA227		3.5	0.3
	BDOIA300	1.76		

R2	ID	CREBBP IC50 uM	CREBBP Tm Shift	BRD4 Tm Shift
	BDOIA348		4.2	2.9
	BDOIA347		2.4	1.1
	BDOIA328	1.96	4.0	1.9
	BDOIA325	1.34	5.2	3.3

ID	R1	CREBBP IC50 uM	CREBBP Tm Shift	BRD4 Tm Shift
BDOIA220	H	0.47	5.3	0.6
BDOIA299	3-MeO	0.23	4.0	1.9
BDOIA301	4-Me	0.30		
BDOIA302	2-Me	0.38		
BDOIA313	2-Cl	0.64	5.4	2.6
BDOIA314	3-Cl	0.82	5.5	1.8
BDOIA315	4-Cl	0.36	6.9	2.0
BDOIA316	2-CN	0.88	3.8	1.4
BDOIA317	2-OMe	1.32	2.9	1.5
BDOIA323	3-CN	1.40	4.4	2.2
BDOIA324	4-CN	1.35	4.7	2.0

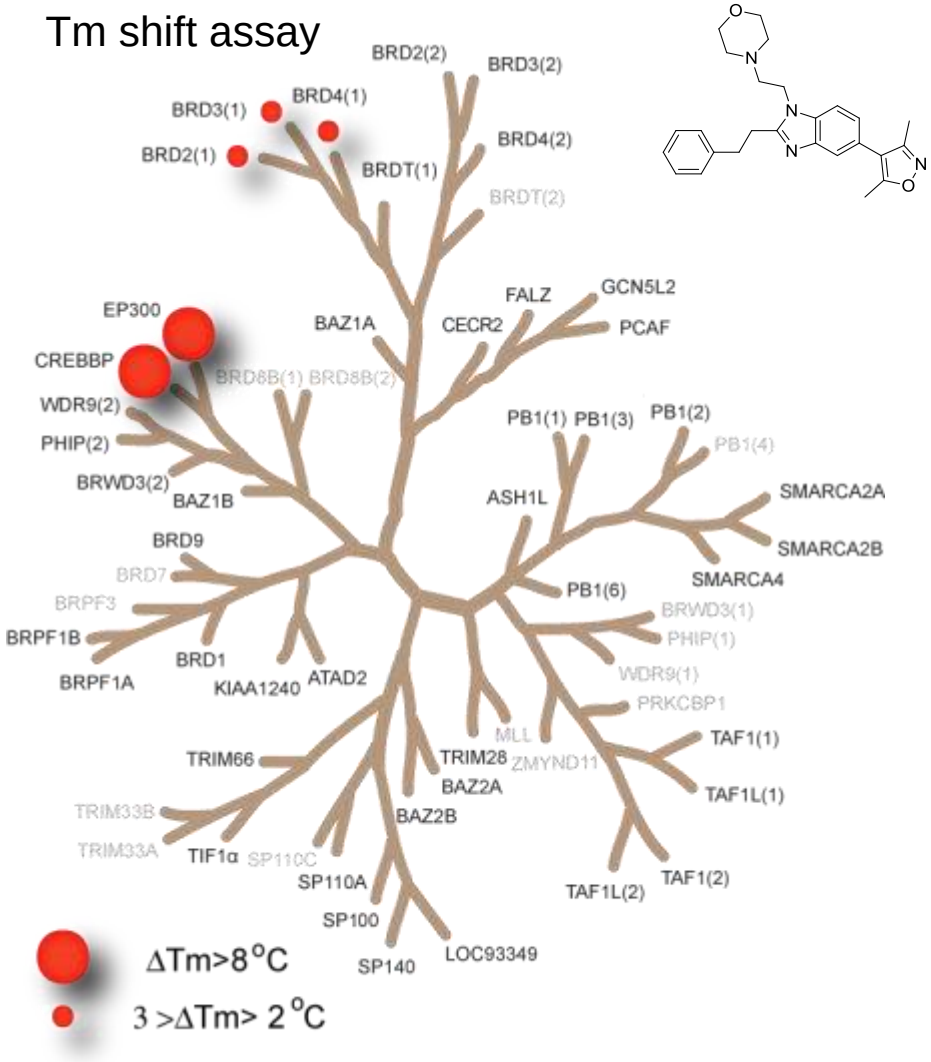
CBP Selective Isoxazole Optimization



ID	R2	CREBBP IC50 uM	CREBBP Tm Shift	BRD4 Tm Shift
BDOIA000322a	Piperazine	3.89	4.0	1.5
BDOIA000321a	Boc-Piperazine	1.21	5.5	2.2
BDOIA000320a	*N*-Me-Piperazine	1.64	4.1	2.2
BDOIA000319a	Azetidine	457.90	2.7	1.3
BDOIA000298a	Morpholine	0.11	6.7	3.5
BDOIA000297a	Pyrrolidine	0.57		
BDOIA000296a	Piperidine	0.28	6.1	1.8

BDOIA298 is a CBP/EP300 Inhibitor

Tm shift assay



AlphaScreen

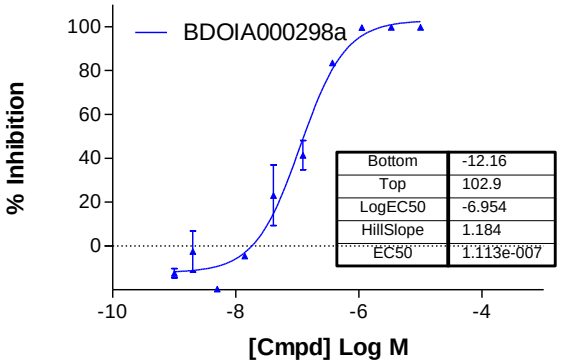
IC50	uM	Fold Selective
CREBBP	0.17	
BRD4	1.8	11
CECR2	10.3	92

% Inhibition	
CREBBP $\%I_{10}$	99.8
CECR2 $\%I_{12.5}$	47.0
BRD9 $\%I_{25}$	21.9
BAZ2A $\%I_{25}$	21.8
FALZ $\%I_{25}$	16.0
PHIP $\%I_{25}$	13.4
ATAD2 $\%I_{25}$	12.1
BRPF3	6.3

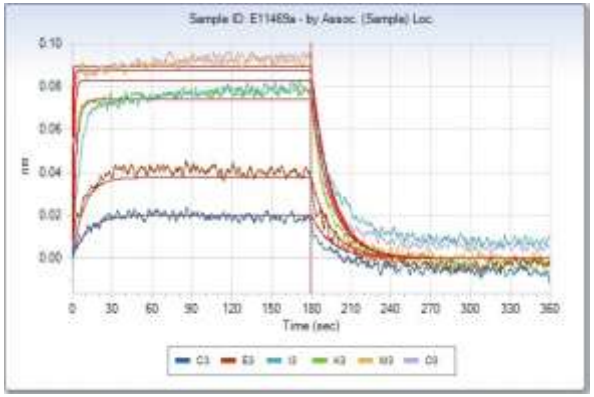
Selectivity panel: 43 bromodomains

BDOIA298 is a Potent CBP/EP300 Inhibitor

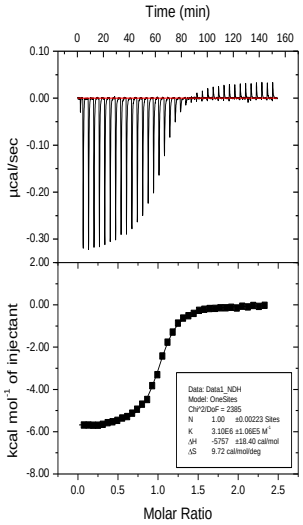
AlphaScreen IC_{50} 170 nM (n =5)



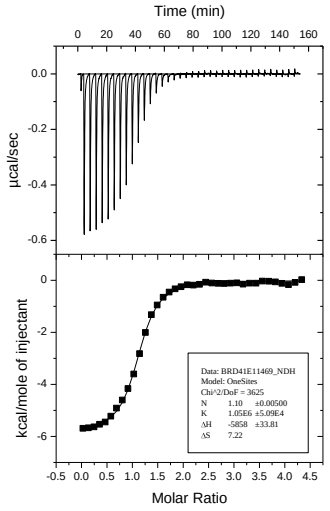
BLI Kd 105 nM (like SPR)



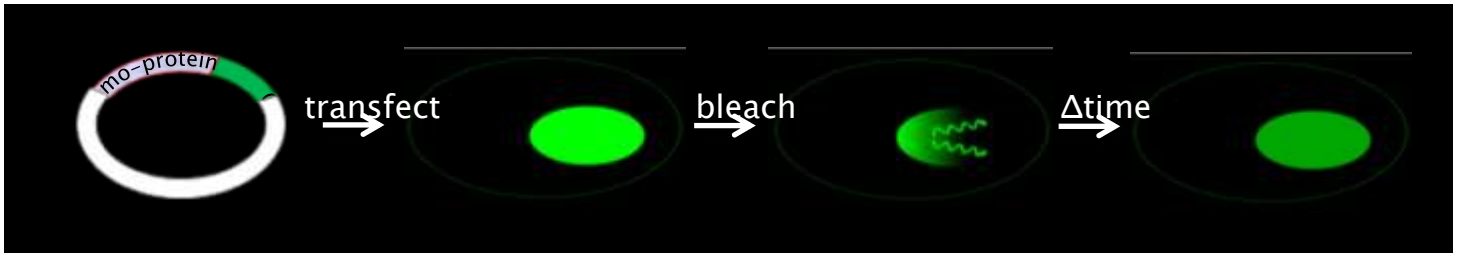
CBP ITC Kd 323 nM

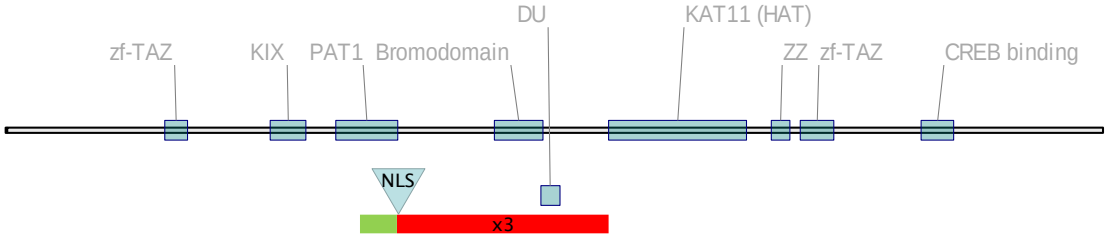


BRD4 ITC Kd 1050 nM



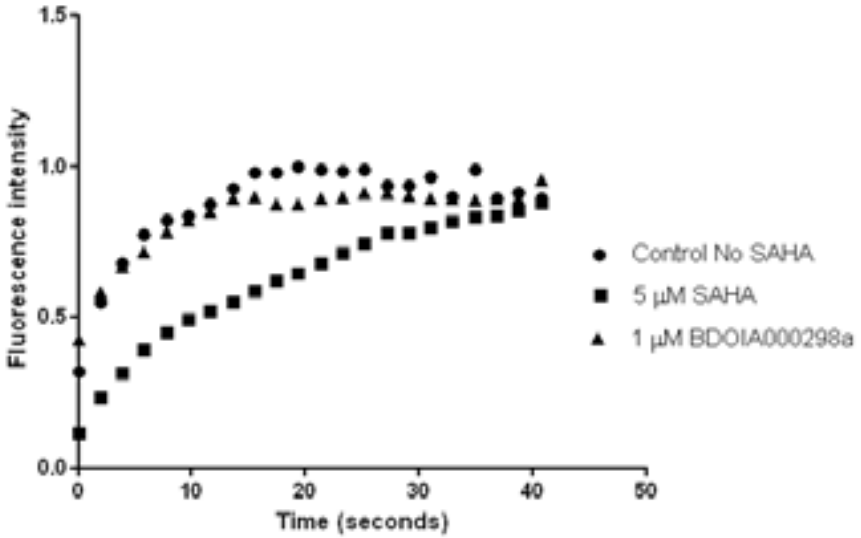
BDOIA298 Displaces CBP from Chromatin

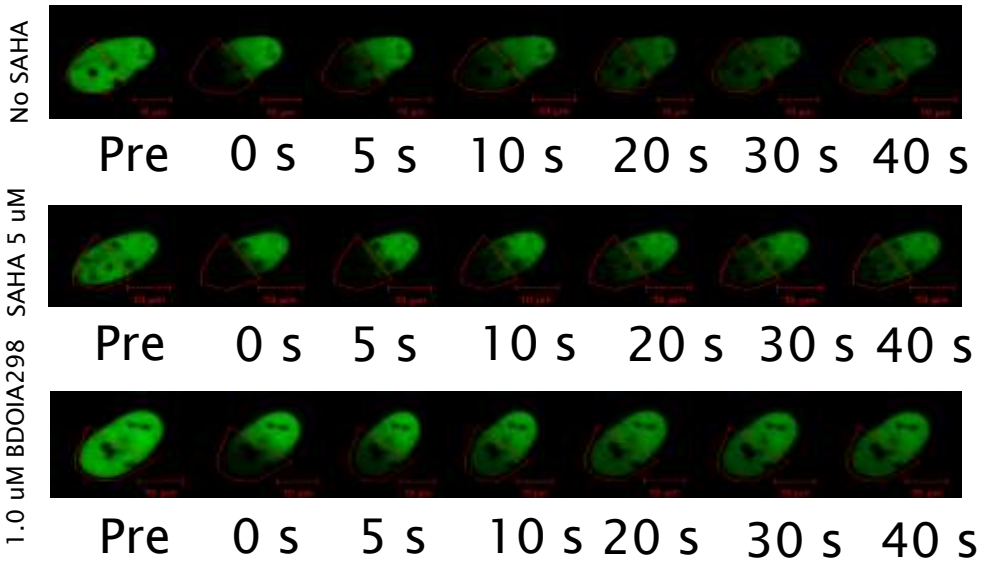




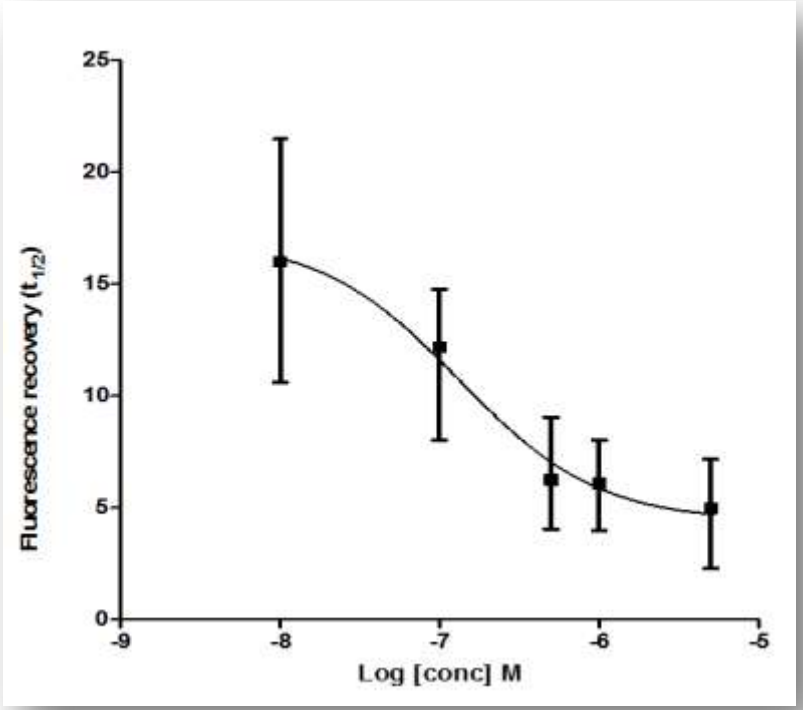
Full length protein nuclear, but no ΔFRAP with mutants or inhibitors

NLS+3x CREBBP bromodomain (with flanking sequence) gave nuclear expression





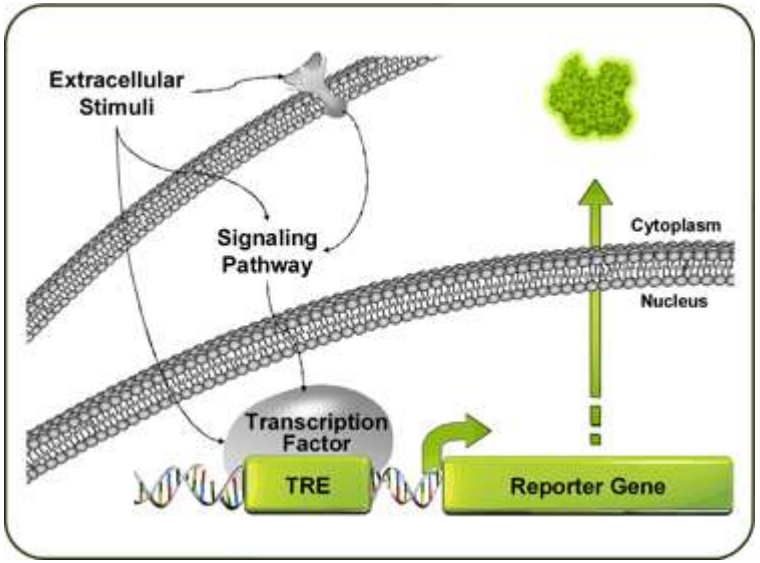
BDOIA298 Displaces CBP from Chromatin



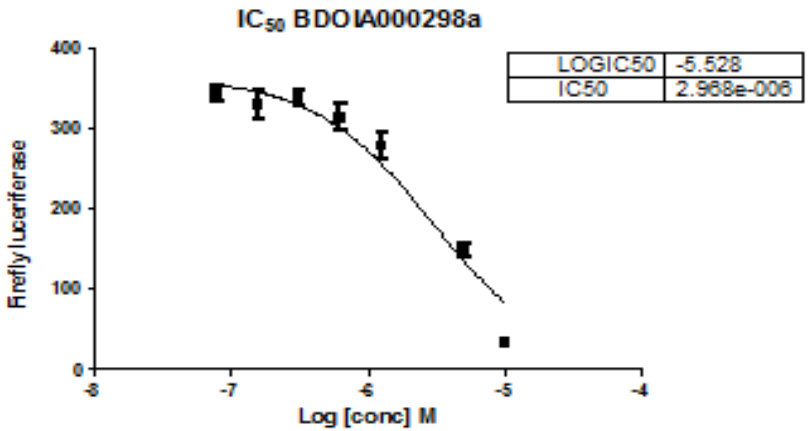
Cytotoxicity in HELA CC_{50} ~40 µM (non-toxic)

CBP Frap IC_{50} 100 nM

BDOIA298 Attenuates DNA Damage Induced p53 Reporter Expression



p53–reporter assay

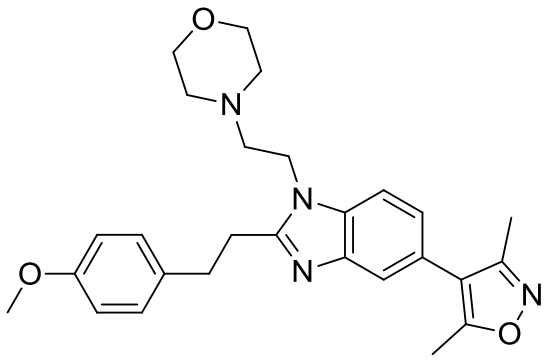


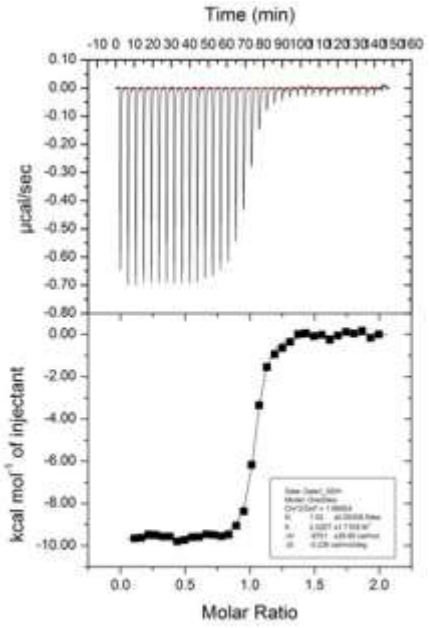
IC_{50}: 3 µM

CBP in stress response:

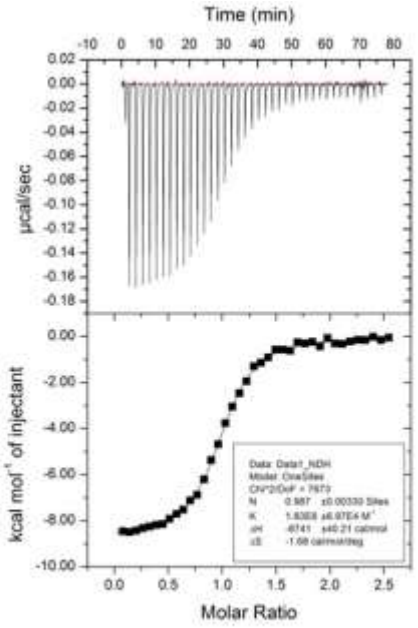
- CBP binding to p53 at the C-terminal acetylated lysine 382 upon DNA damage
- Results in p53 acetylation-dependent coactivator and transcriptional activation of the cyclin-dependent kinase inhibitor p21 in G1 cell cycle arrest.

BDOIA383 is More Selective for CBP



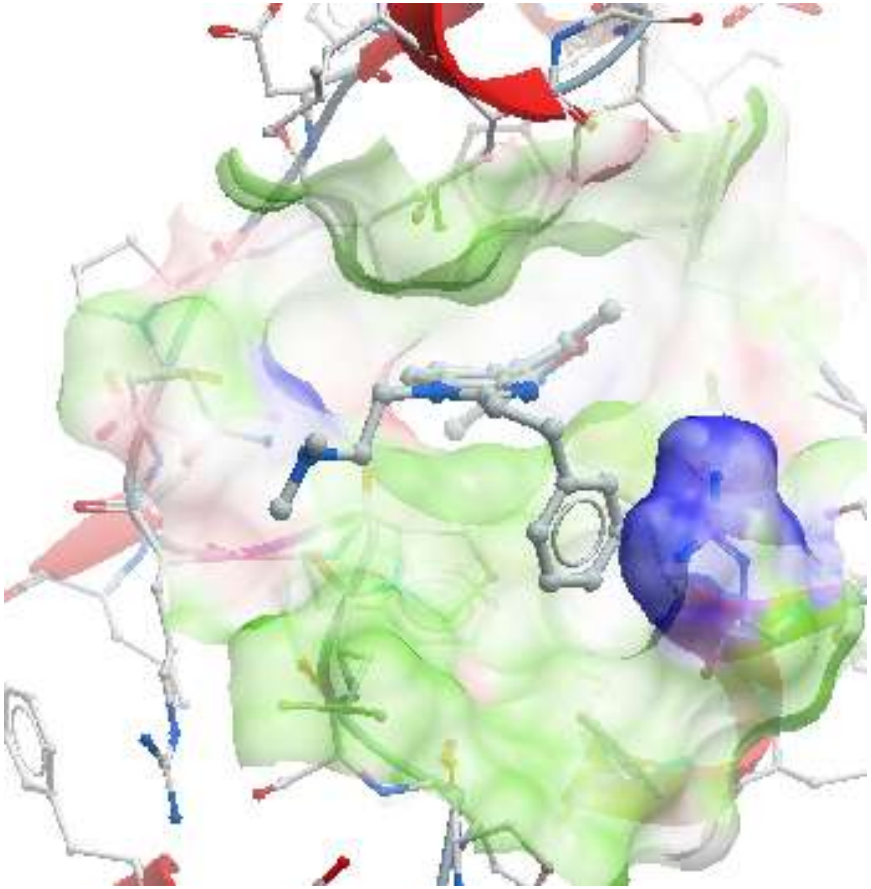


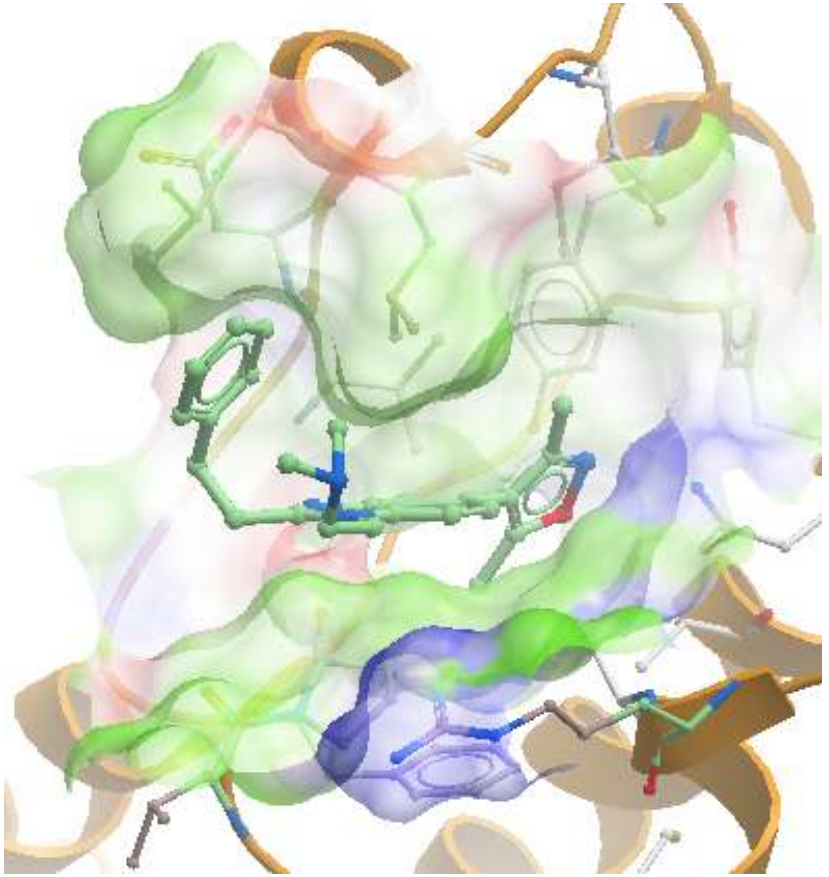
CREBBP - Kd = 50 nM



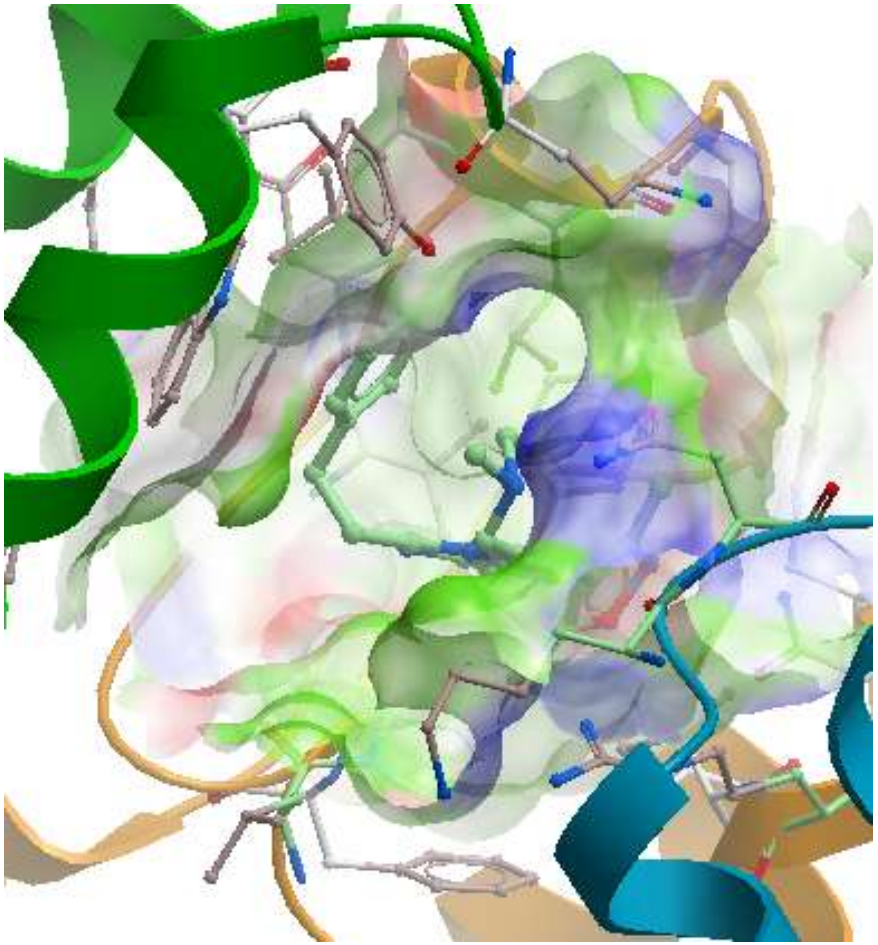
BRD4(1) - Kd = 546 nM

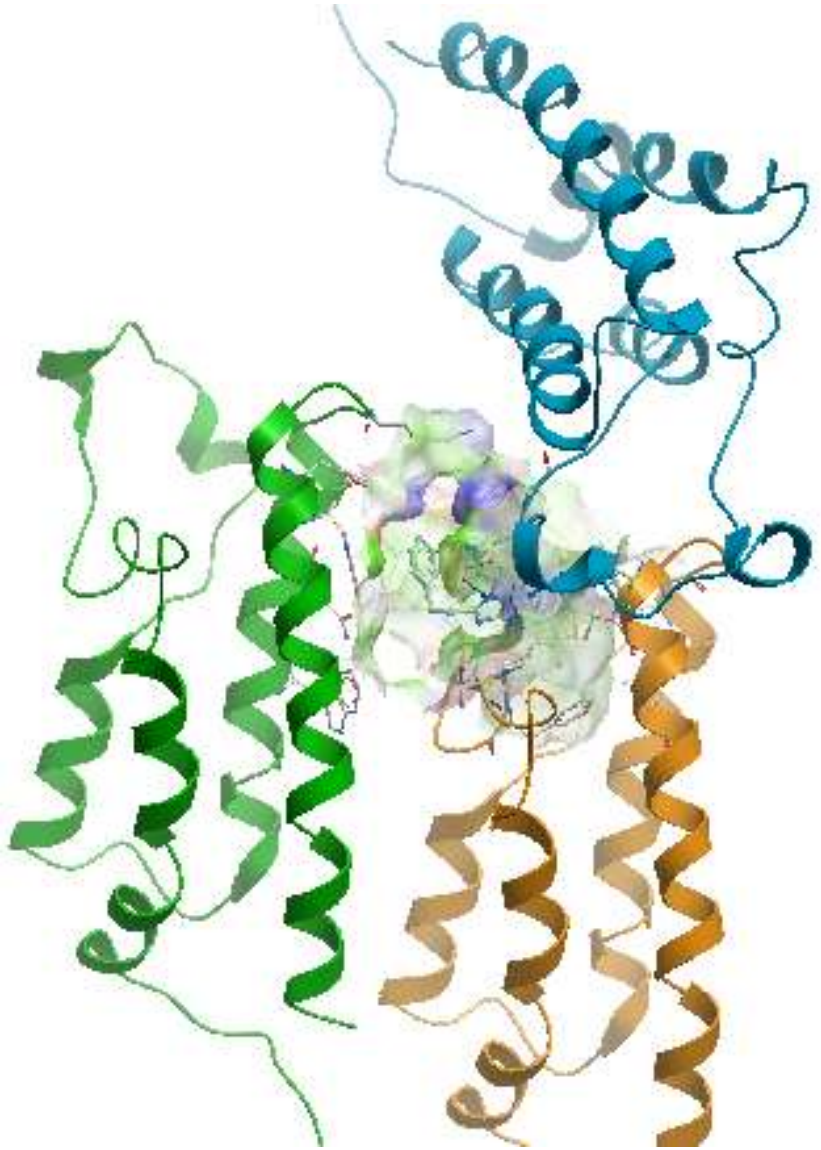
BDOIA220 in CBP





BDOIA220 in CBP





Summary

- Bromodomains and histones form a switchable protein-protein interaction important in transcriptional regulation.
- Ligand binding induces a more druggable pocket.
- Cyclic ureas and isoxazoles are good acetyl lysine mimetics.
- Selective chemical probes are possible.

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