Structure-based drug discovery in bromodomains

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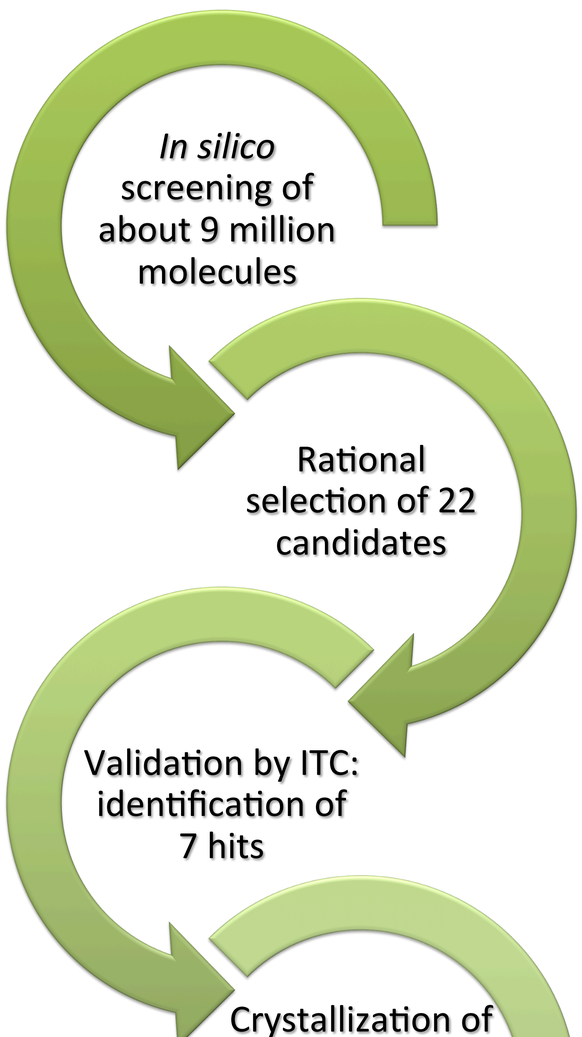
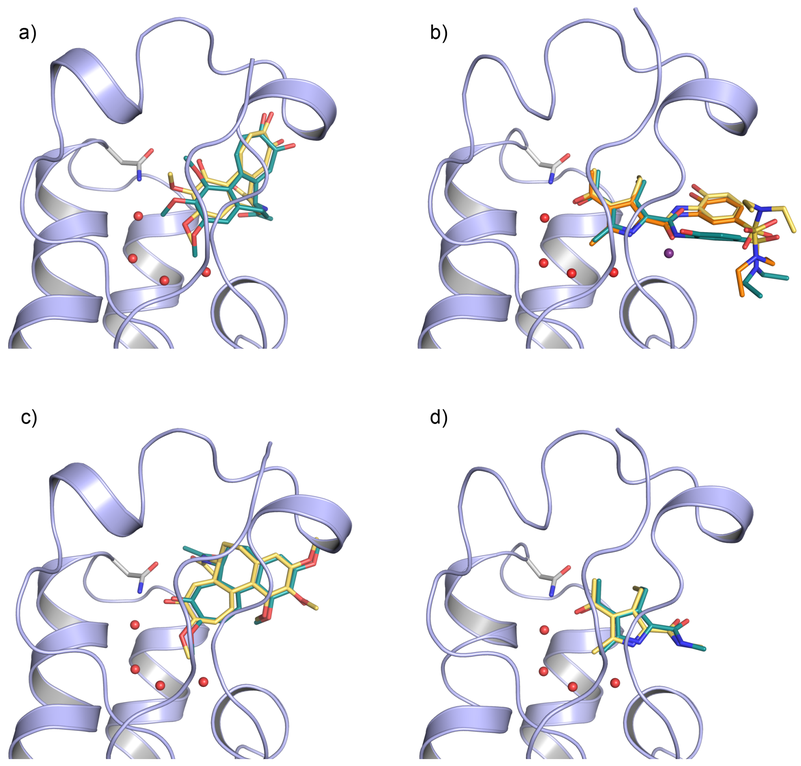
Bromodomains are epigenetic mark 'readers' that specifically recognize ε-*N*-acetylated lysine residues. Their potential as therapeutic targets has attracted much attention due to their implication as regulators of disease-relevant gene expression. We carried out a structure-based drug discovery project focusing on BRD4, a bromodomain of the BET subfamily that has been recently characterized as a key determinant in several types of cancer.

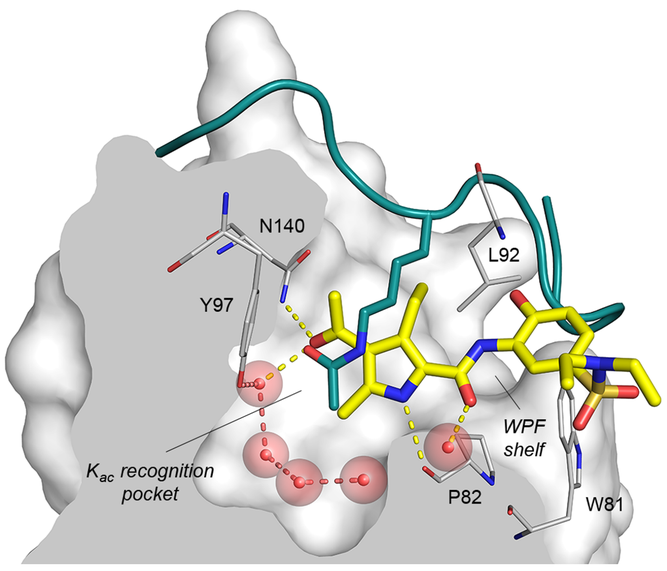
Hit identification





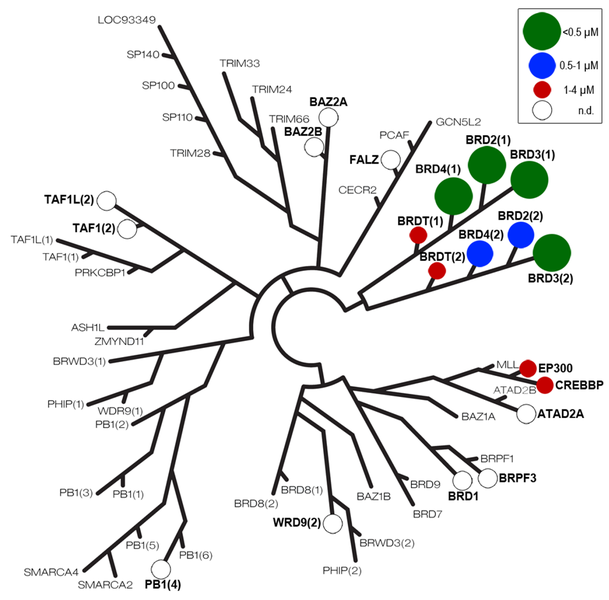
Docking models (turquoise) have accurately predicted the crystallographic binding mode (pale yellow) for identified hits: a) XD1, colchiceine; b) XD14; c) XD25, colchicine; and d) XD46.

XD14 (yellow) mimics the recognition of an acetylated lysine peptide (green) by BRD4(1) exploiting key hydrogen-bond interactions. Five conserved water molecules surround the 4-acyl pyrrole core, and the aryl sulfonamide moiety engages in CH–π interactions with W81 and L92 ("WL trap").



Bromodomain profiling

BROMO*scan*® shows that XD14 selectively inhibits the BET family, CREBBP, and EP300.



	BRD2(1)	BRD2(2)	BRD3(1)	BRD3(2)	CREBBP
K_D (nM)	170	830	380	490	1600

	BRD4(1)	BRD4(2)	BRDT(1)	BRDT(2)	EP300
K_D (nM)	160	850	1200	3700	2600

Cell proliferation assay

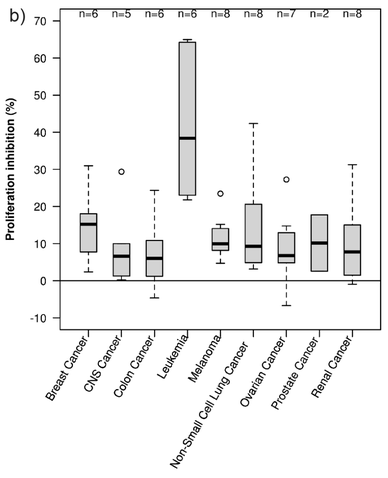
The NCI60 Human Tumor Cell line Anticancer Drug Screen indicates that XD14 selectively inhibits the growth of specific leukemia cell lines.

a)

	Cell line	GI (%)
Breast cancer	T-47D	31.0
	HS 578T	18.0
	BT-549	15.3
	MCF7	15.1
	MDA-MB-468	7.7
	MDA-MB-231/ATCC	2.4
Colon cancer	KM12	24.3
	HCT-116	10.9
	SW-620	7.3
	HCT-15	4.7
	HT29	1.2
	COLO 205	-4.7
Leukemia	HL-60(TB)	65.0
	SR	64.2
	K-562	42.0
	MOLT-4	34.8
	CCRF-CEM	23.0
	RPMI-8226	21.8
Melanoma	UACC-62	23.5
	M14	15.2
	SK-MEL-5	12.9
	UACC-257	10.3
	MDA-MB-435	9.6
	SK-MEL-2	9.1
	SK-MEL-28	7.2
	MALME-3M	4.7
Prostate cancer	PC-3	17.8
	DU-145	2.6

	Cell line	GI (%)
CNS cancer	SNB-75	29.4
	SF-295	10.0
	U251	6.6
	SNB-19	1.3
	SF-268	0.2
Non-small cell lung cancer	NCI-H522	42.4
	NCI-H322M	25.4
	HOP-92	15.8
	NCI-H226	9.7
	NCI-H23	8.9
	HOP-62	5.7
	A549/ATCC	4.1
	NCI-H460	3.2
Ovarian cancer	IGROV1	27.3
	OVCAR-4	14.8
	OVCAR-3	11.2
	OVCAR-8	6.7
	SK-OV-3	6.4
	NCI/ADR-RES	3.2
	OVCAR-5	-6.7
Renal cancer	UO-31	31.3
	786-0	15.6
	A498	14.5
	SN12C	9.4
	CAKI-1	6.1
	ACHN	3.6
	TK-10	-0.7
	RXF 393	-1.0

b)



Conclusions

Structure-based virtual screening of about 9 million small molecules has yielded 7 novel scaffolds inhibiting the epigenetic target BRD4, including the potent 4-acyl pyrrole derivative XD14 (K_D = 237 nM). This promising hit compound inhibits the epigenetic target by mimicking the natural substrate, has selectivity for the BET bromodomain family, and inhibits cell proliferation of leukemia cell lines. Currently, *in vivo* activity of XD14 is being assessed and XD46 is being used as starting point for a fragment-based screening approach.

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SFB 992 Medical Epigenetics

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Prinjha RK, Witherington J, Lee K, Trends Pharmacol. Sci. (2012), 33(3): 146-53.

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