

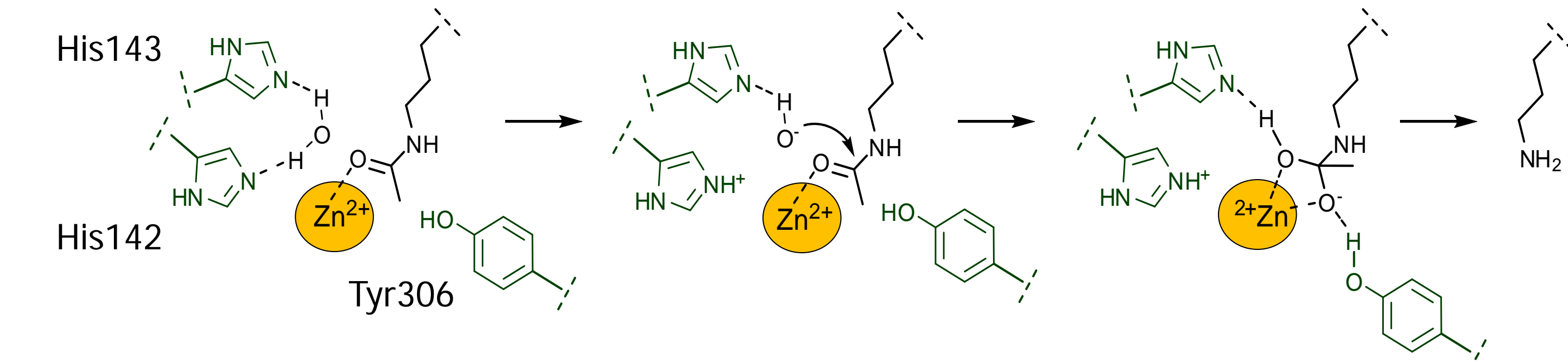
Introduction

HDAC4 is one of eleven metal-dependent histone deacetylase isoforms (HDACs) and has been recognized as a potential target for HD, based on the finding that the heterozygous knock-down of the HDAC4 gene (HDAC4^{+/-}) in R6/2 mice (HD disease model) resulted in a partially rescued HD phenotype (personal communication, Professor Gill Bates, King's College London). As HDAC4 adopts multiple biological functions, a critical question is whether blockage of its catalytic activity will replicate the gene knock-down data. To determine this, our objective is to develop potent and selective HDAC4 deacetylase inhibitors that exhibit pharmacokinetic profiles suitable for a proof-of-concept study in HD disease models.

HDAC enzymes can be subdivided into four major classes, class I, class II (a and b), class III and class IV enzymes. These classes comprise HDAC 1, 2, 3 and 8 (class I), HDAC 4, 5, 7 and 9 (class IIa), HDAC6 and 10 (class IIb), the NAD⁺ dependent sirtuins (class III) and HDAC11 (class IV).

All HDAC enzyme classes, except class IIa, have a conserved Tyr³⁰⁶ within their catalytic site (see Figure far right). This Tyr³⁰⁶ stabilizes a tetrahedral intermediate during amide hydrolysis through hydrogen bonding.

IC ₅₀ data (μM)				
CHDI	340043	315404	340039	339991
cat. HDAC4	0.04	0.02	0.25	0.33
cat. HDAC5	0.02 (0.5x)	0.01 (0.4x)	0.16 (0.6x)	0.15 (0.5x)
HDAC7	0.43 (12x)	0.08 (5x)	0.65 (3x)	0.07 (0.2x)
HDAC9	0.08 (2x)	0.02 (1x)	0.51 (2x)	0.12 (0.4x)
HDAC6	0.40 (11x)	0.28 (16x)	11.0 (43x)	4.92 (15x)
HDAC3/NCOR2	1.84 (53x)	0.38 (23x)	> 50	38.9 (118x)
HDAC8	0.30 (9x)	0.22 (13x)	43.0 (169x)	15.5 (47x)
Cell. TFA Lys	0.36	0.40	2.87	0.77
Cell. Ac Lys	2.49	0.85	> 50	> 50



The class IIa HDACs possess a His instead of a Tyr at this position, which can rotate away. As a result of this mutation, class IIa HDACs are about 1,000 fold less active deacetylases than class I family members¹.

Importantly, this His to Tyr amino acid change has been shown to open a putative pocket, which may be exploited in the development of selective class IIa inhibitors¹.

At the outset of this program, two compound classes with promising selectivity for class IIa HDAC enzymes have been reported, shown in the table (see left), with IC₅₀ data presented in μM and fold change from HDAC4 in brackets^{2,3}.

Computational chemistry

> HDAC4 flexibility and selectivity pocket

Structural data is available for the catalytic domains of HDAC2, HDAC4, HDAC7 and HDAC8 (occupied by ligands). The reported HDAC4 structures (a) have an open conformation; the loop shown in red is exposed to solvent^{4,5}. All the other structures, including the closely homologous HDAC7 (c), exhibit a closed-loop conformation. An apo structure of HDAC4 also adopts the closed conformation (CHDI, personal communication). To support the medicinal chemistry program, a closed-loop model of HDAC4 was generated based on information from the HDAC4 and HDAC7 structures.

Figure 1. a) HDAC4 open conformation (2VOJ structure³), b) HDAC4 closed-loop model with ligand (protein active site surface shown) and c) HDAC7 (3COY structure⁶). Mobile loops in red for clarity.

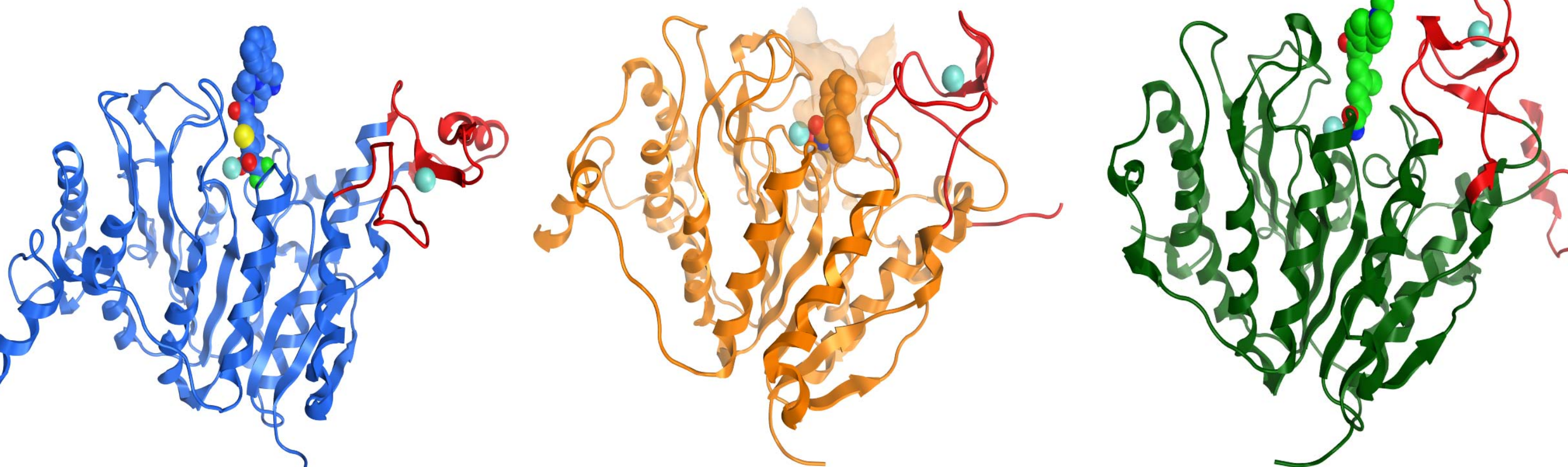


Figure 1. a) HDAC4 open conformation (2VOJ structure³), b) HDAC4 closed-loop model with ligand (protein active site surface shown) and c) HDAC7 (3COY structure⁶). Mobile loops in red for clarity.

- The lack of Tyr³⁰⁶ in the class IIa HDACs opens a pocket
- Compounds filling this pocket show good selectivity over class I and class IIb enzymes

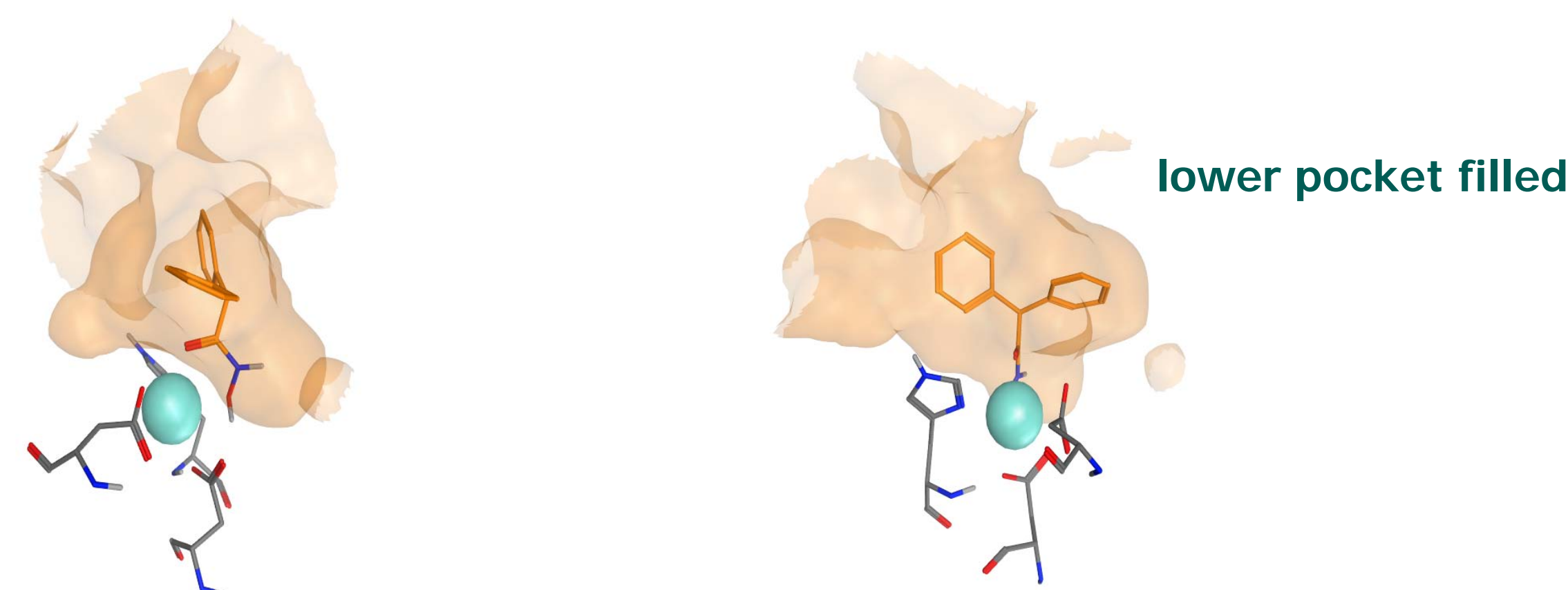
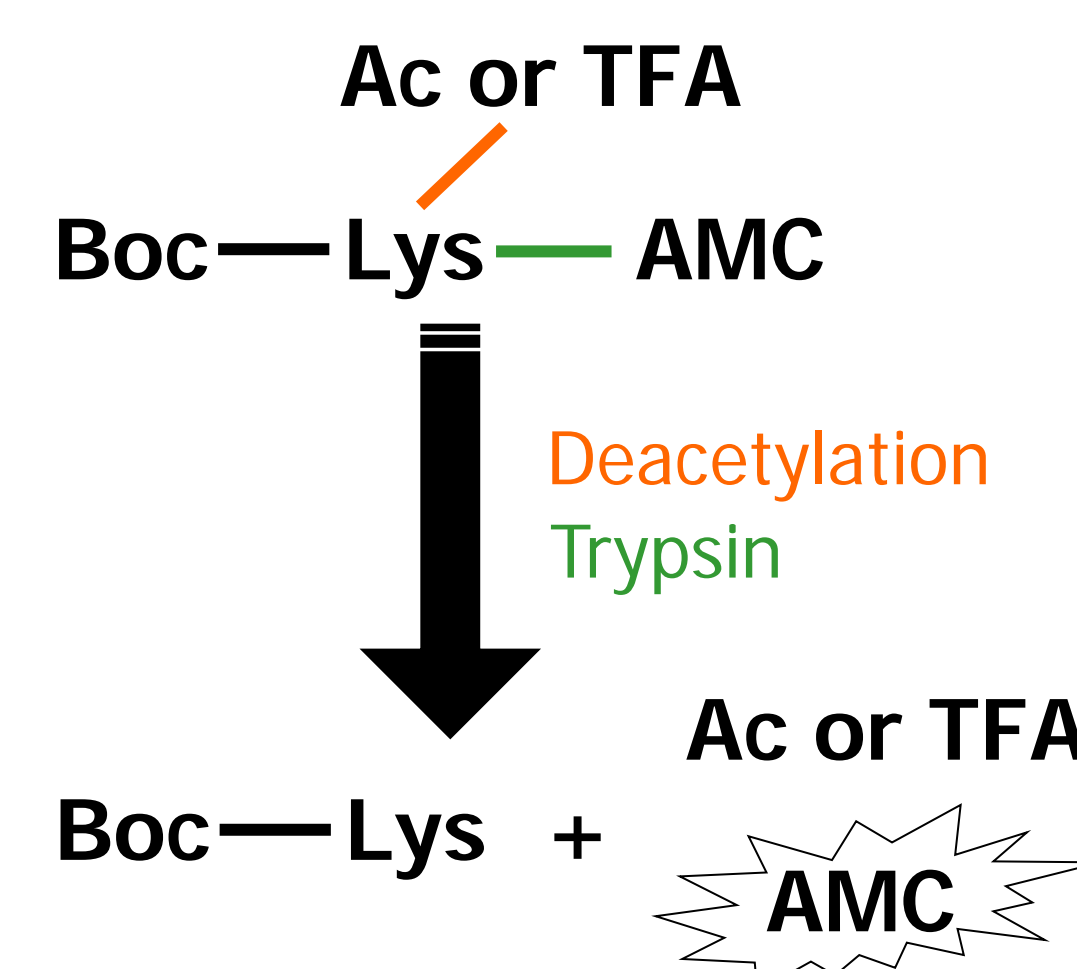


Figure 2. Modeled binding pose for compound CHDI-00340039 showing a bidentate chelation of the zinc ion and a filled lower pocket. Surface shown is the same as in Figure 1.

Biological assays

A Jurkat E6.1 cell based HDAC assay was transferred to BioFocus from Evotec. This assay measures the deacetylation of two acetylated substrates, Boc-Lys(Ac)-AMC and Boc-Lys(TFA)-AMC in Jurkat E6.1 cells. As illustrated in the figure below, an HDAC dependent step allows the liberation of the fluorophore AMC from the quenching effects of the Boc group. Liberated AMC is then detected by exciting at 355 nm and emission detection at 460 nm wavelength.



HDAC enzyme	HDAC class	Lys(Ac) substrate	Lys(TFA) substrate
cat. HDAC4	IIa		✓
cat. HDAC5	IIa		✓
HDAC7	IIa		✓
HDAC9	IIa		✓
HDAC6	IIb	✓	
HDAC1	I	✓	
HDAC2	I	✓	
HDAC3/NCOR2	I	✓	
HDAC8	I		✓

Figure 3. Schematic diagram (left) illustrating the deacetylation of the assay substrates and release of the fluorophore AMC. The HDAC selectivity of the Lys(Ac) and the Lys(TFA) substrates are shown in the table (right). The HDAC4 and HDAC5 enzymes used are catalytic domains, as denoted by cat. in the table, and not full-length enzymes. HDAC3 is complexed with human NCOR2(αα395-489) to retain deacetylase activity *in vitro*.

The assay described above was further developed to enable the measurement of HDAC activity in isolated human recombinant enzymes to assess compound selectivity. The substrates used were as illustrated in the table above, at or below K_m, ensuring assays were within the linear phase of the reaction.

Assay	K _m (μM)	S:B	Z'
cat. HDAC4	12.5	9.6	0.7
cat. HDAC5	8.0	11.7	0.7
HDAC7	14.0	10.9	0.8
HDAC9	25.0	6.9	0.7
HDAC6	7.2	7.6	0.7
HDAC3/NCOR2	120.0	6.7	0.7
HDAC8	8.0	27.2	0.8
E6.1 Jurkat (Ac)	n/a	7.3	0.8
E6.1 Jurkat (TFA)	n/a	19.4	0.8

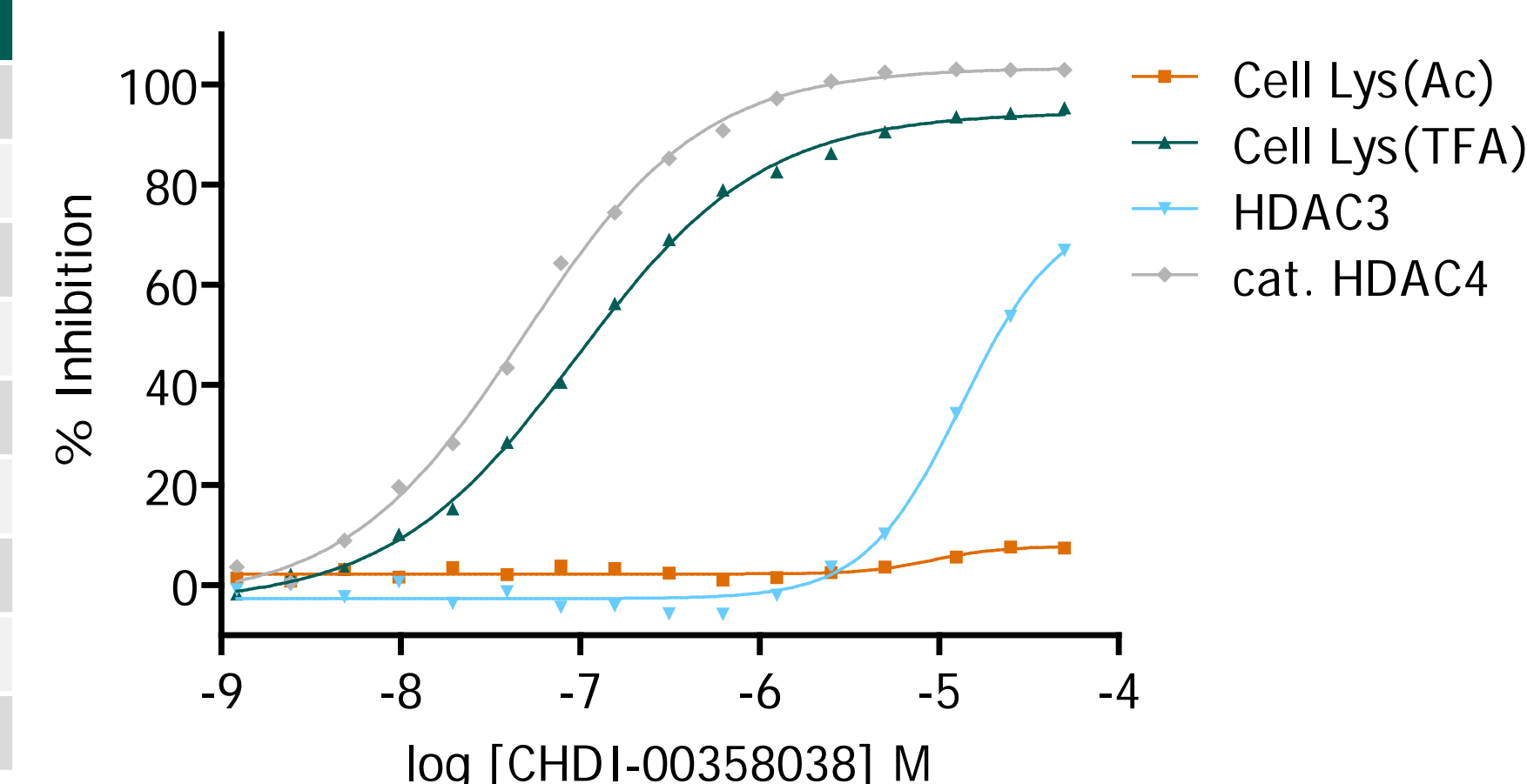


Figure 4. The table (left) shows the assay parameters for the recombinant enzyme assays as well as the two cell based assays. The graph (right) is an example of a lead molecule demonstrating selectivity in the cell based assays using either substrate and in the HDAC4 and HDAC3 biochemical assays.

Medicinal chemistry

> Overview of progress

sulfonamide caps	fluorides	ether caps	heteroaryl caps
<ul style="list-style-type: none"> • Sulfonamide orients unsubstituted phenyl into lower pocket • Good <i>in vitro</i> potency • High PSA • Pgp-mediated efflux • Low brain exposure 	<ul style="list-style-type: none"> • F reduces cell-shift • Pgp-mediated efflux persists 	<ul style="list-style-type: none"> • Good <i>in vitro</i> potency • No Pgp-mediated efflux • High metabolic turnover 	<ul style="list-style-type: none"> • Good <i>in vitro</i> potency • Low to moderate Pgp-mediated efflux • High permeability • Metabolically stable molecules identified

> Profile of selected lead molecule

Class IIa activity (μM)	Selectivity		ADME	
	cat. HDAC4	cat. HDAC5	HLM Cl _{int} (ml/min/kg BW)	MLM Cl _{int} (ml/min/kg BW)
0.049	0.023	HDAC6 61x	<36	109
0.026	0.040	HDAC3 514x		4.1
0.117		HDAC8 137x		280
		cell. TFA Lys >427x		

Conclusions

- Biology: Assays are in place to test *in vitro* activity and selectivity of compounds
- Computational chemistry: A model for the HDAC4 catalytic site has been constructed and is used for compound design
- Medicinal chemistry: Compounds have been optimized for *in vitro* activity and properties
- Challenges: - Establishment of an appropriate pharmacodynamic (PD) central and peripheral readout in R6/2 mice to verify target engagement - no endogenous substrate of HDAC4 has been identified to date
 - It is unknown how the catalytic activity influences other biological functions of HDAC4, i.e. N-terminal protein-protein interactions, transcriptional repression of MEF2. Assessment of MEF2 activity and protein binding interactions alongside HDAC4 catalytic activity will need to be assessed

References

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