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Synthesis and use of affinity matrices for epigenetic protein studies

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Master thesis, Molecular Technologies

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INTRODUCTION

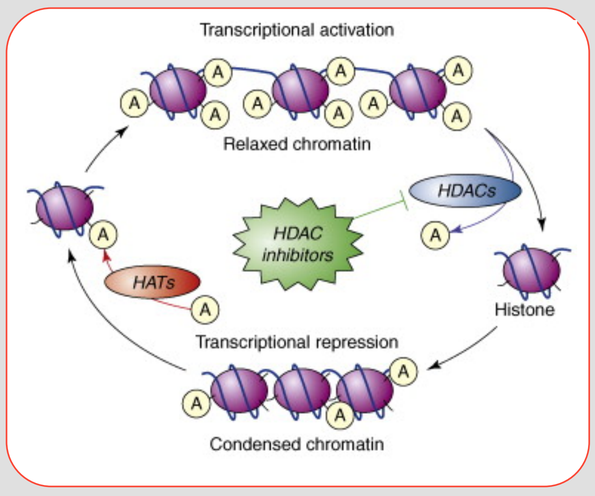

Fig- 1: Chromatin structure is determined by histone acetylation. Adapted from [2].

Changes in gene expression, e.g. by chromatin remodeling, are governed by post-translational modifications of histones, with acetylation being a major regulatory mechanisms. [1]

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate this process and HDACs have emerged as important drug targets as dysregulation of HDACs is characteristic of several human diseases.

Challenges in drug discovery

- lack of drug selectivity
- missing physiological context
- use of recombinant protein or protein fragments
- *in vitro* studies
- absence of regulatory subunits

Chemoproteomics

- *in vivo* studies
- studies with endogenous protein or in tissues
- studies in physiological environment
- preservation of post-translational modifications

CONCEPT

Principle
- affinity matrix chromatography
- HDAC enrichment of cells or tissues lysates
- rely on solid supported linkable HDAC inhibitors
- SAHA-based matrix efficient in HDAC binding of class I and class IIb [3]

Aims
- generation of solid supported linkable HDAC inhibitors
- evaluation of matrix-HDAC binding efficiency by pulldown assay and mass spectrometry readout

Objectives
- generation of new SAHA analogue affinity matrices
- by variation of linker, connecting unit (CU) and ZBG moiety

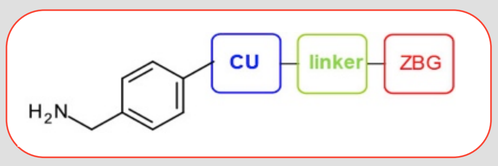

Fig. 2: Illustration of SAHA analogue.

- functionalization of preactivated sepharose beads with newly synthesized probes
- HDAC affinity enrichment of cell lysates

RESULTS

Five newly synthesized affinity probes were evaluated in pulldown assays

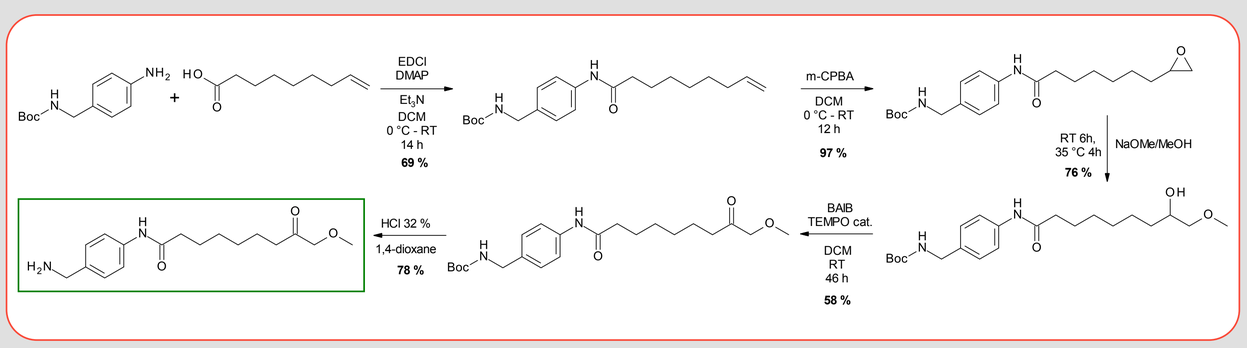

Fig. 3: Exemplary illustration of a synthetic route to affinity probes.

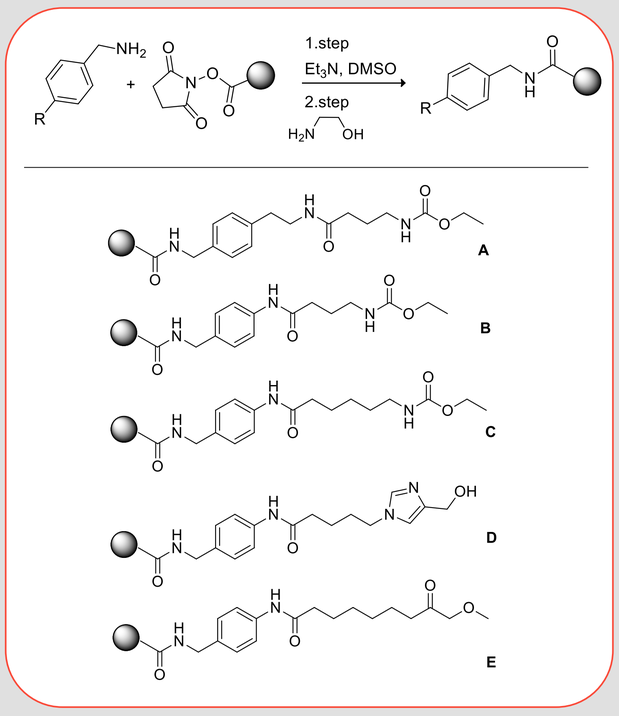

Fig. 4: Overview syntesized SAHA-analogues.

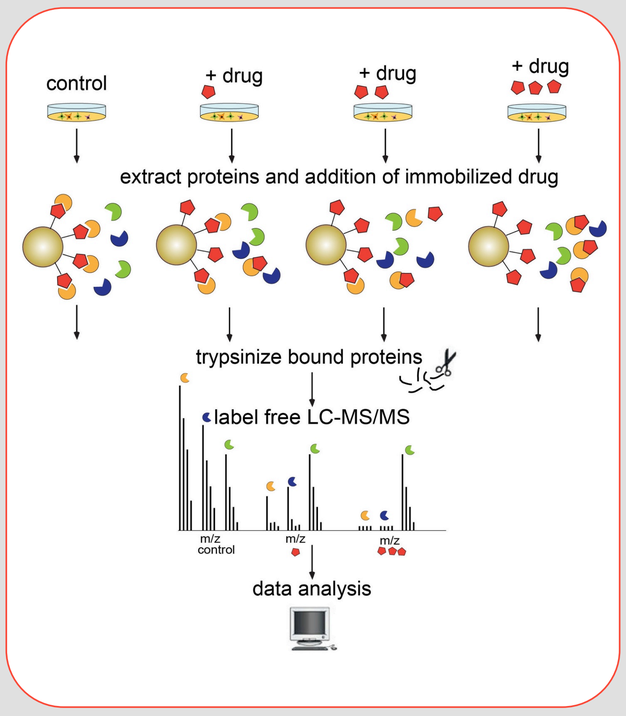

Fig. 5: Selectivity profiling of newly synthesized matrices.

- compounds A, B and C show low or now binding affinity towards HDACs
- compounds D and E are promising baits for HDAC enrichment
- further evaluation is ongoing

***Santacruzamate* is not active in the zinc binding pocket of HDACs**

- *santacruzamate* was reported to be a highly potent and selective inhibitor against the isoform HDAC2. [4]

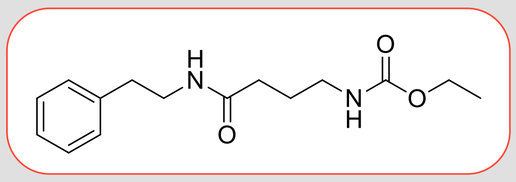

Fig. 6: Structure of *santacruzamate*

- MS readout allowed to generate a dose-response curve and to calculate the half maximal inhibitory concentration (IC_{50})

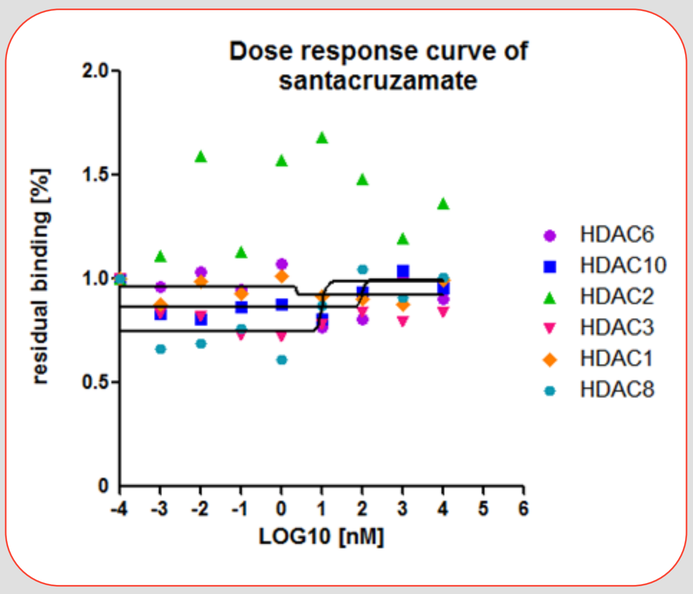

Fig. 7: Dose response curve of synthetic *santacruzamate*.

- profiling of *santacruzamate* with SAHA-beads revealed no inhibitory effect against all HDACs captured by SAHA

CONCLUSION

- five affinity probes evaluated for HDAC binding
- *santacruzamate* is not active in the zinc binding pocket of HDACs
- two affinity probes show promising data
- best probe will be further used for profiling of 64 cancer cell lines for HDAC abundance

REFERENCES

[1] Haberland, M et al., 2009, Nat Rev Genet
[2] Chuang, D et.al., 2009 Trends Neurosci
[3] Bantscheff, M et al., 2011, Nat Bio Tech
[4] Pavlik, CM et al., 2013, J. Nat Prod

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