Novel histone deacetylase (HDAC) inhibitors as anti-cancer and anti-dementia agents

(OTT1392)

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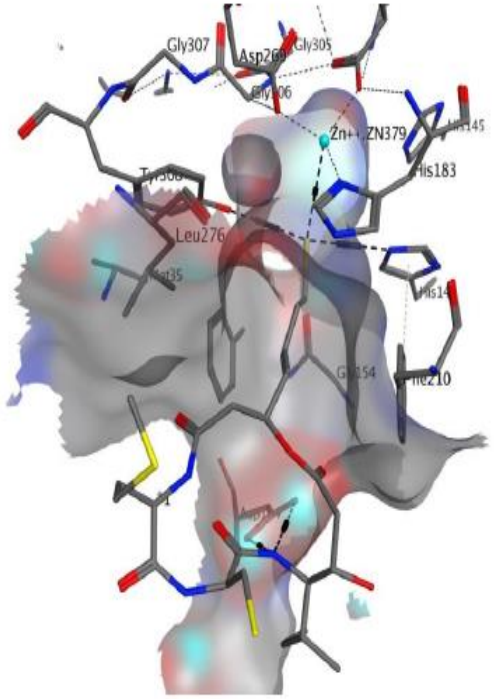
HDAC Inhibitors

- Histone deacetylase inhibitors (HDAC inhibitors, HDIs) are a class of compounds that interfere with the function of histone deacetylase

- HDIs have a long history of use in psychiatry and neurology as mood stabilizers and anti-epileptics

- More recently they are being investigated as possible treatments for cancers, parasitic and inflammatory diseases



Shortfalls of current drugs

Problems:

- Current HDAC inhibitors used in cancer are toxic with many side effects to patients
- Current drugs lack specificity and affect several HDAC types
- Poor solubility

Solution:

- The inventors have discovered novel small molecules with less toxicity, better solubility, and better specificity toward specific HDAC types
- New analogs show promising effects on cervical cancer, breast cancer, colon cancer, and renal cancer cell lines with GI50 values in single to sub µM range
- The compounds are easy to synthesize and inexpensive

Market, Intellectual Property, and Partnering

Market

- The global market for histone deacetylase inhibitors (HDIs) was valued at $223.2 million in 2012 and was estimated at $361.8 million for 2013
- The market is expected to grow to $954.3 million by 2018
- To date, there are just two approved HDIs -- vorinostat (Zolinza) from Merck & Co. and romidepsin (Istodax) from Celgene Corporation

Intellectual Property

- A PCT application was filed in October 2015

Partnering

- This technology is part of an active and ongoing research program and is seeking partners for development of the final product
- It is available for developmental research support/licensing under either exclusive or non-exclusive terms

HDAC classification

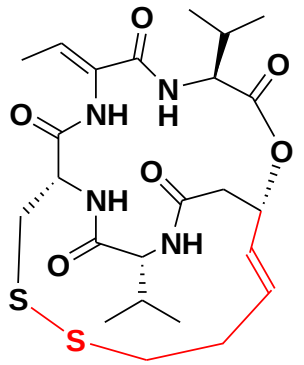
18 known human histone deacetylases are classified into four groups

Zn^{2+} cationic dependent proteins				NAD^{+} dependent proteins
Class I	**Class II**		**Class IV**	**Class III**
	Class IIa	Class IIb		
HDAC 1	HDAC 6	HDAC 4	HDAC 11	SIRT 1
HDAC 2	HDAC 10	HDAC 5		SIRT 2
HDAC 3		HDAC 7		SIRT 3
HDAC 8		HDAC 9		SIRT 4
				SIRT 5
				SIRT 6
				SIRT 7

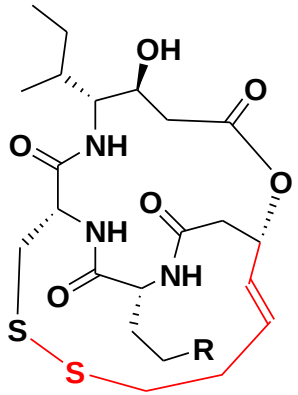
Zn dependent HDACs in Cancers

Group	Tumor Implication	Tumor expression[a]
Class I (type RPD3)		
HDAC1	Possible prognostic indicator for lung and breast cancers. Over expressed in prostate cancers (hormone-refractory), gastric, and colorectal.	++
HDAC2	Over expressed in colorectal and gastric cancers. Loss of antigen presenting cells in colorectal cancers gave HDAC2 over expression.	++
HDAC3	Over expressed in lung cancers and several solid tumors	++
HDAC8	Knock down inhibits cell growth in several human tumor cells.	++
Class II (type HAD1)		
HDAC4	Unknown	++
HDAC5	Down regulated in colon cancers and acute myeloid leukemia	- -
HDAC6	Ambiguous prognostic in breast cancer	

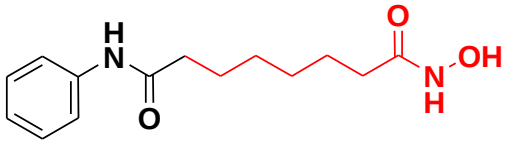
Natural HDACs



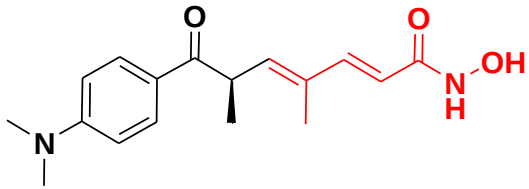
FK228



TDP-A (R=SCH_3)
TDP-B (R=CH_2CH_3)

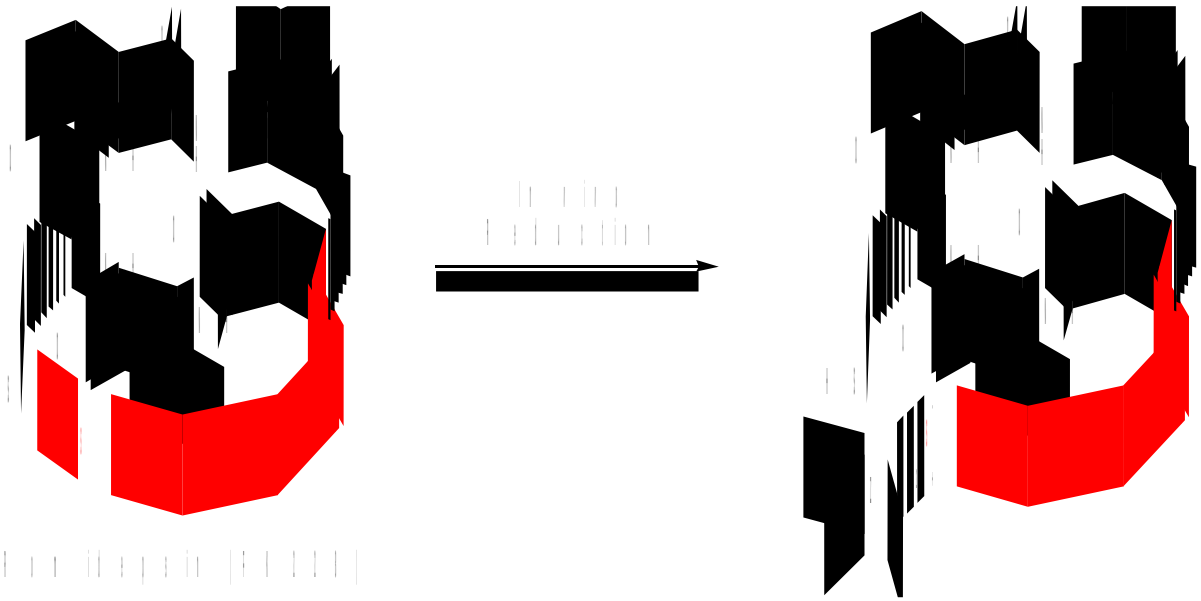


SAHA



Trichostatin A, TSA

Mechanism of Inhibition



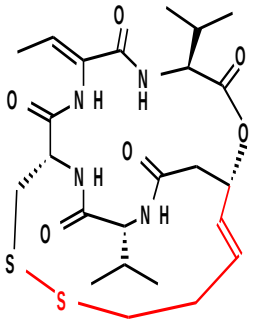
Limitations of TDP and Romedepsin

Advantage

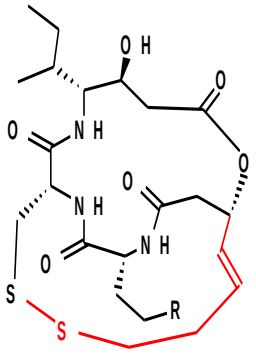
- Usually Potent, moderately isoform-selective

Disadvantages

- Poor solubility
- Excessive cytotoxicity

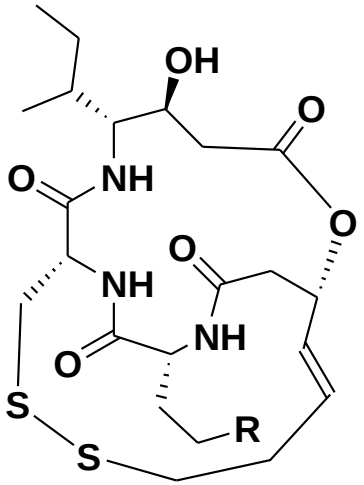


FK228



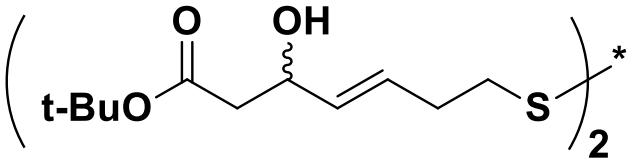
TDP-A (R = SCH$_3$)
TDP-B (R = CH$_2$CH$_3$)

Synthetic protocol for new HDAC inhibitor



small fragments desgined from natural products

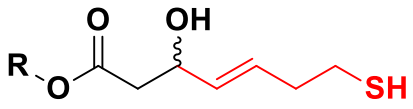
Subjected to in vivo and vitro testing



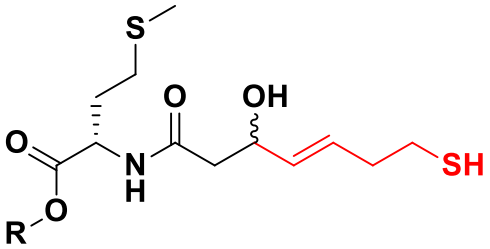
Cpd1'

Thailandepsin A, TDP-A (R=SCH_3)
Thailandepsin B, TDP-B (R=CH_2CH_3)

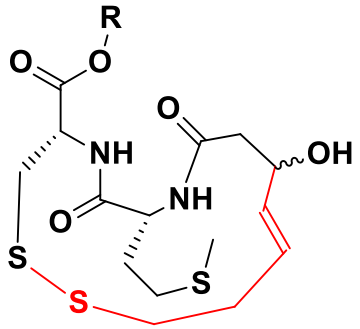
Derivatives prepared in the SAR study



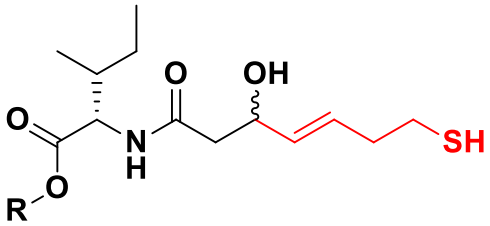
Cpd1 R=H
Cpd1' R=t-butyl



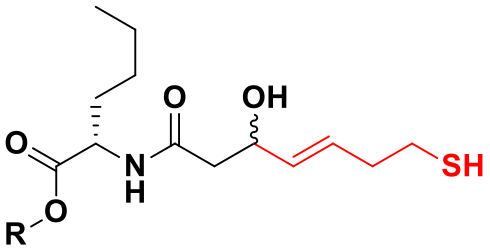
Cpd2 R=H
Cpd2' R=CH_3



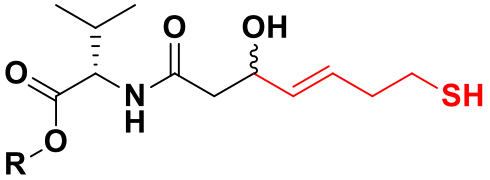
Cpd3 R=H
Cpd3' R=CH_3



Cpd4 R=H
Cpd4' R=CH_3



Cpd5 R=H
Cpd5' R=CH_3



Cpd6 R=H
Cpd6' R=CH_3

Promising Activity *in-vitro* and *in-vivo*

Cpd	HDAC Inhibitory activity (IC_{50} in μM)						Antiproliferative activity (GI_{50} in μM)		
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC6	HeLa	HCT-116	RFX393
SAHA	0.021	0.069	0.170	7.04	116.73	0.179	2.8	2.7	2.5
FK228	0.004	0.018	0.019	1.48	2.37	0.508	0.001	0.001	0.001
Cpd1	0.023	0.088	0.819	62.85	>1000	>1000	0.6	0.3	0.2
Cpd1'	0.013	0.094	0.623	63.03	>1000	>1000	0.4	0.2	0.3
Cpd3	0.007	0.044	0.579	41.91	>1000	165.94	1.4	2.1	1.1
Cpd3'	0.004	0.025	0.540	34.21	~500	41.31	0.2	0.3	0.4
Cpd5	0.003	0.046	0.839	49.38	>1000	>1000	0.4	0.4	0.5

Novel compounds are much more selective than SAHA and FK228 and retain antiproliferative activity

In-vitro Activity Against Tumor Lines

Compound (GI_{50} in μM)	Ovarian SKOV-3	Prostate DU-145	Breast MDA-MB-231	Colon COLO-205
SAHA	1.5	2.0	1.5	0.5
Cpd1’	0.5	0.3	0.6	0.9
Cpd3’	0.5	0.6	0.5	0.8
Cpd5	0.7	0.6	0.5	0.9

Individual values were derived from the average of triplicate experiments

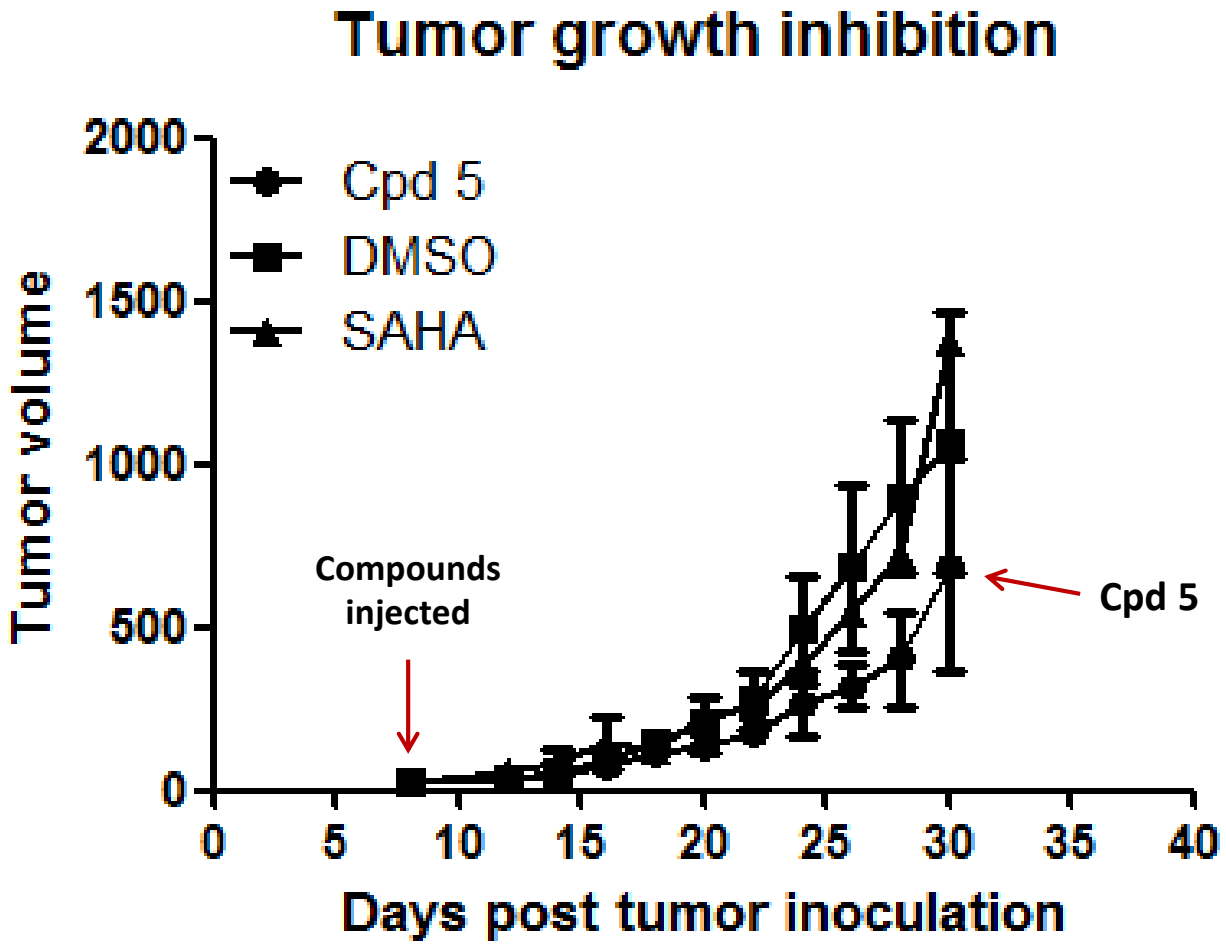
- The synthetic compounds were more active than the commercially available and FDA approved SAHA

Improved Solubility and MTD

Compound	Solubility	MTD
SAHA	Not soluble	>200
FK228	Partially soluble	3.125
Cpd1	Soluble	>200
Cpd1’	Mostly soluble	>200
Cpd3	Soluble	>200
Cpd3’	Partially soluble	>200
Cpd5	Mostly soluble	>200

10mg/ml cpd in 20% DMSO/saline and in healthy BALB/c mice, IP. Individual values were derived from the average of triplicate experiments with standard error within 20% margin.

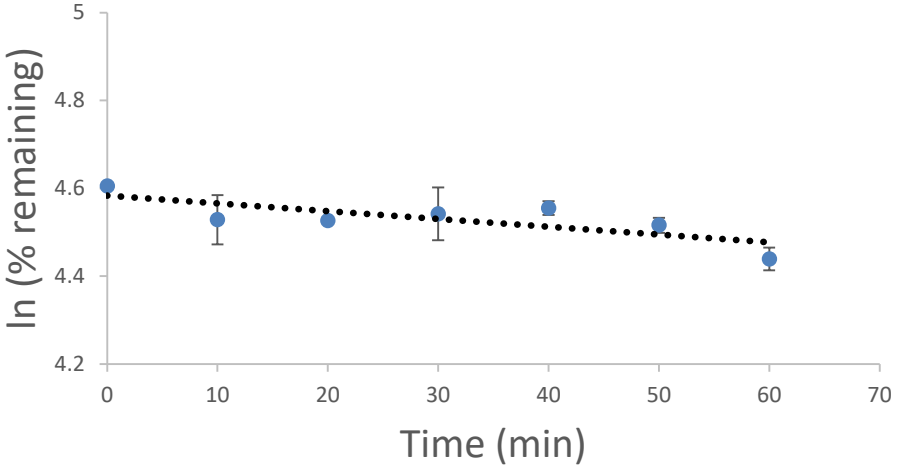
Cpd 5 inhibited tumor growth in mice



Cpd 5 is metabolically stable: Human

Human

Exp. 1

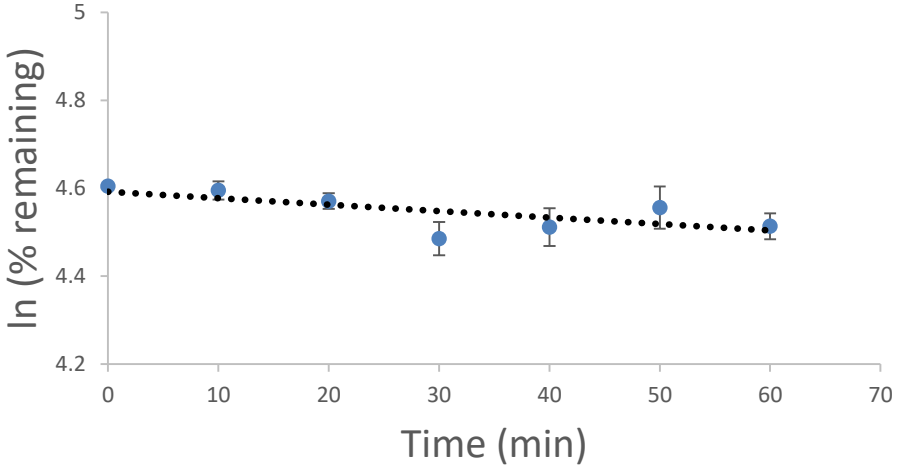


Metabolic parameters:

Half-life: 391 ± 114 min

% remaining at 60 min: 88 ± 0.36%

Exp. 2



Metabolic parameters:

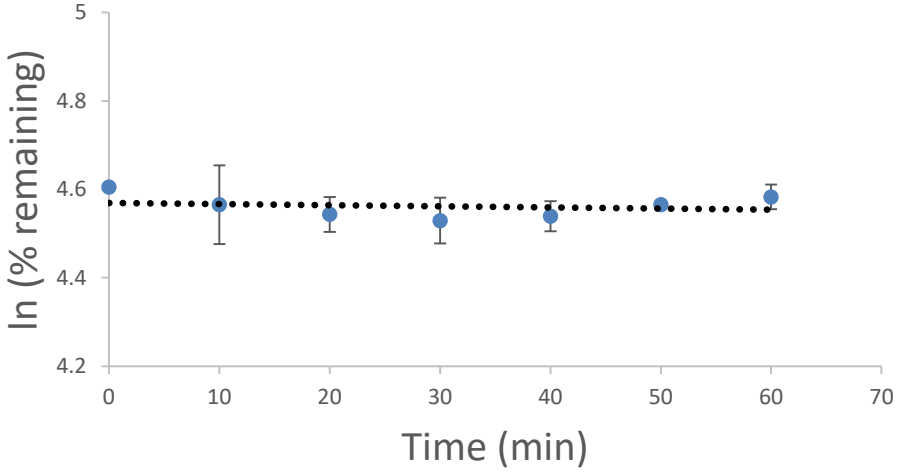
Half-life: 471 ± 162 min

% remaining at 60 min: 90.3 ± 0.36%

Cpd 5 is metabolically stable: Mice

Mouse

Exp. 1

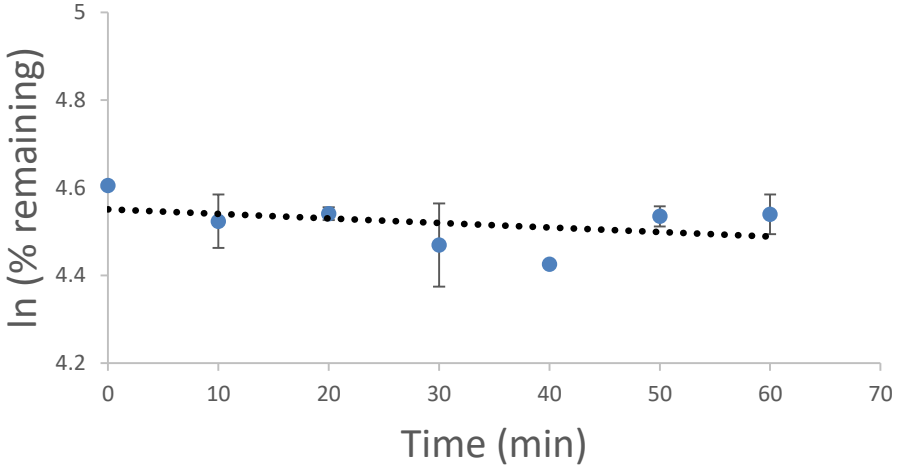


Metabolic parameters:

Half-life: 1514 ± 2057 min

% remaining at 60 min: 94.1 ± 0.47%

Exp. 2

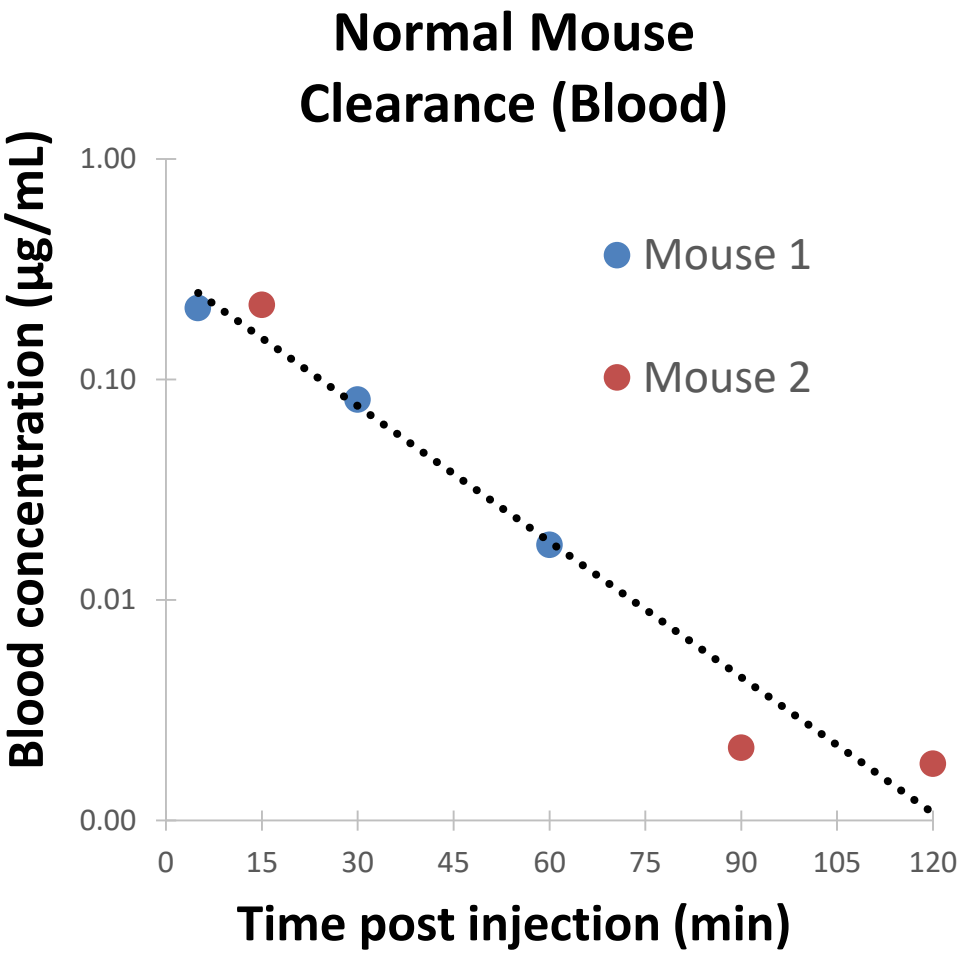


Metabolic parameters:

Half-life: 666 ± 500 min

% remaining at 60 min: 89 ± 0.51%

Cpd 5 is detectable in-vivo at 2 hrs



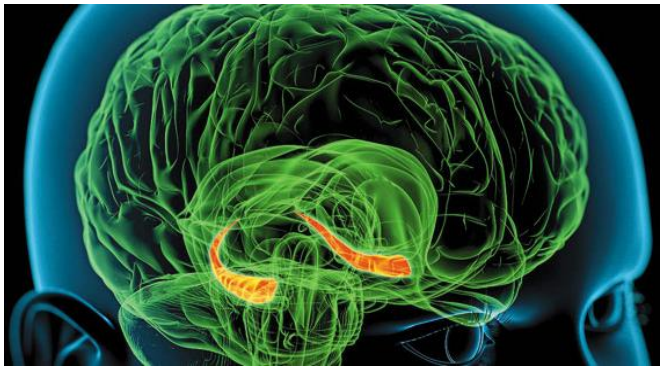
Time	Average Amount in Brain (normal mouse)
60 min	0.5433 ng
120 min	0.0707 ng

4T1 Tumor mice:

- Blood: similar clearance
- Presence of compound in brain **& tumor**
- Tumor data under analysis

HDAC Inhibitors and Memory Formation

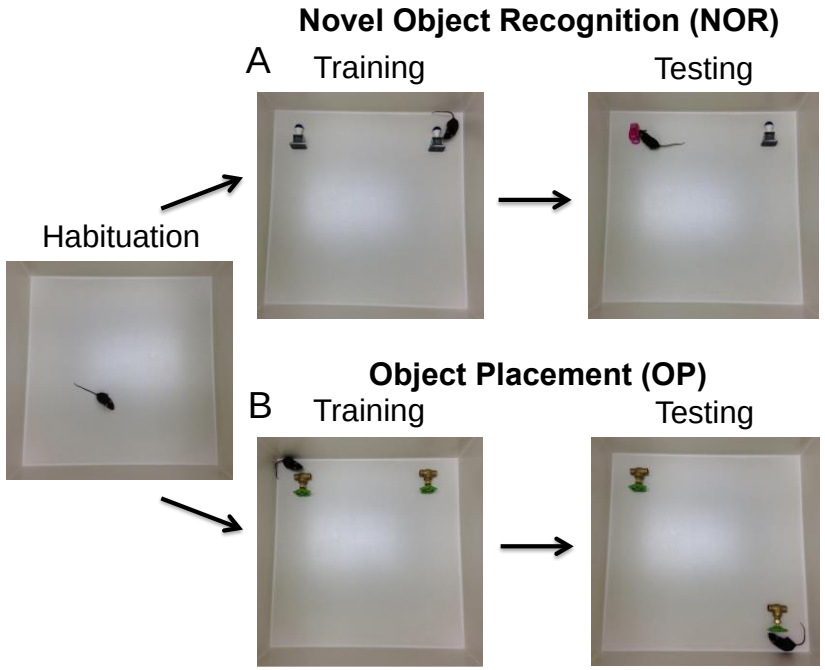
- Expression of HDAC2 and HDAC3 block neural plasticity and impair memory
- HDACi drugs (trichostatin-A, sodium butyrate):
  - Enhance neural plasticity in the hippocampus
    - Hippocampus=Brain region that deteriorates in aging and Alzheimer's disease
  - Make hippocampal memories last longer
  - Improve hippocampal memories in animal models of:
    - Age-related memory decline
    - Alzheimer's disease

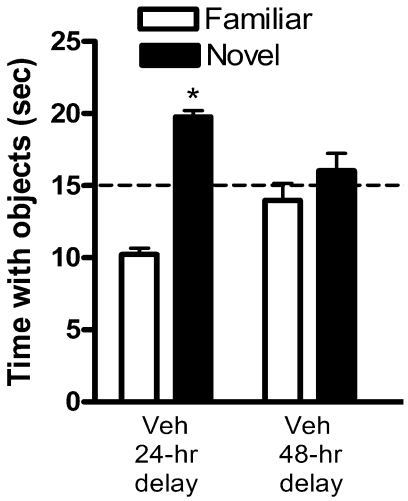


Market for Memory Enhancement

- The Alzheimer's disease market is currently estimated at $5B annually
- The global economic impact of Alzheimer's disease is shown by the worldwide cost of $640B
- The global sales of drugs to treat progressive dementia with other neurological abnormalities are expected to reach $900M by 2017
- Currently there are only a handful of memory-enhancing drugs on the market and there are no drugs that can cure Alzheimer's disease.

HDACi drugs enhance memory

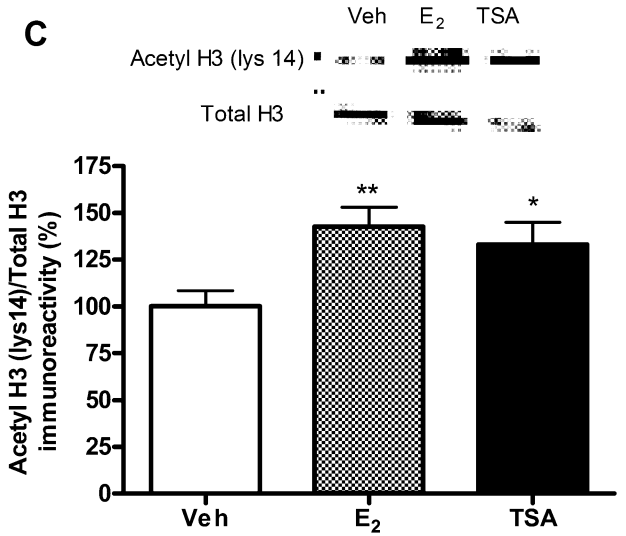


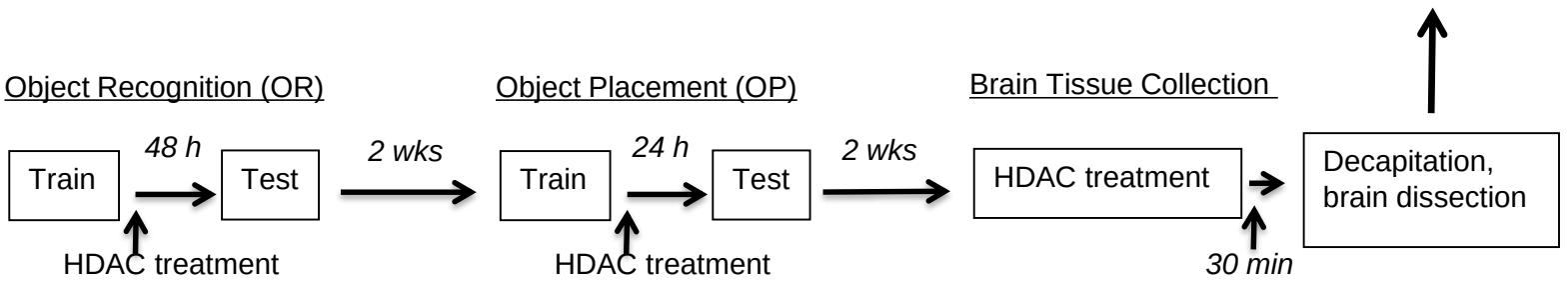


Zhao et al., 2010, *PNAS*, 107(12), 5605-5610

Memory Study design

- 10 week-old male C57BL/6 mice injected intraperitoneally
  - Vehicle
  - 3 doses of each compound
  - TSA (positive control)





Conclusion

- We have designed a set of compounds with the scaffold of the **FK228** and **TDP-A** natural products and discovered selective and potent HDAC1 and HDAC2 inhibitors.
- **Cpd5** is found to be metabolically stable and detectable in blood, brain and tumor.
- HDAC1 and HDAC2 selective inhibitors are thought to have considerable potential both for the development of novel cancer agents and also as memory enhancer.
- In vivo testing of selected designer fragments in animal models bearing human tumor xenografts are needed to determine the antitumor efficacy of these compounds and are currently underway.
- Effect of these inhibitors on hippocampal function and memory formation are also underway.

Next Steps

- Comprehensive pharmacokinetic studies
- Determine efficacy against human xenograft tumors in mice
- Comprehensive memory testing battery in males and females, including middle-aged and aged
- Test solubility and activity of compounds synthesized as salts to make them fully soluble

Novel histone deacetylase (HDAC) inhibitors as anti-cancer and anti-dementia agents

(OTT ID 1392)

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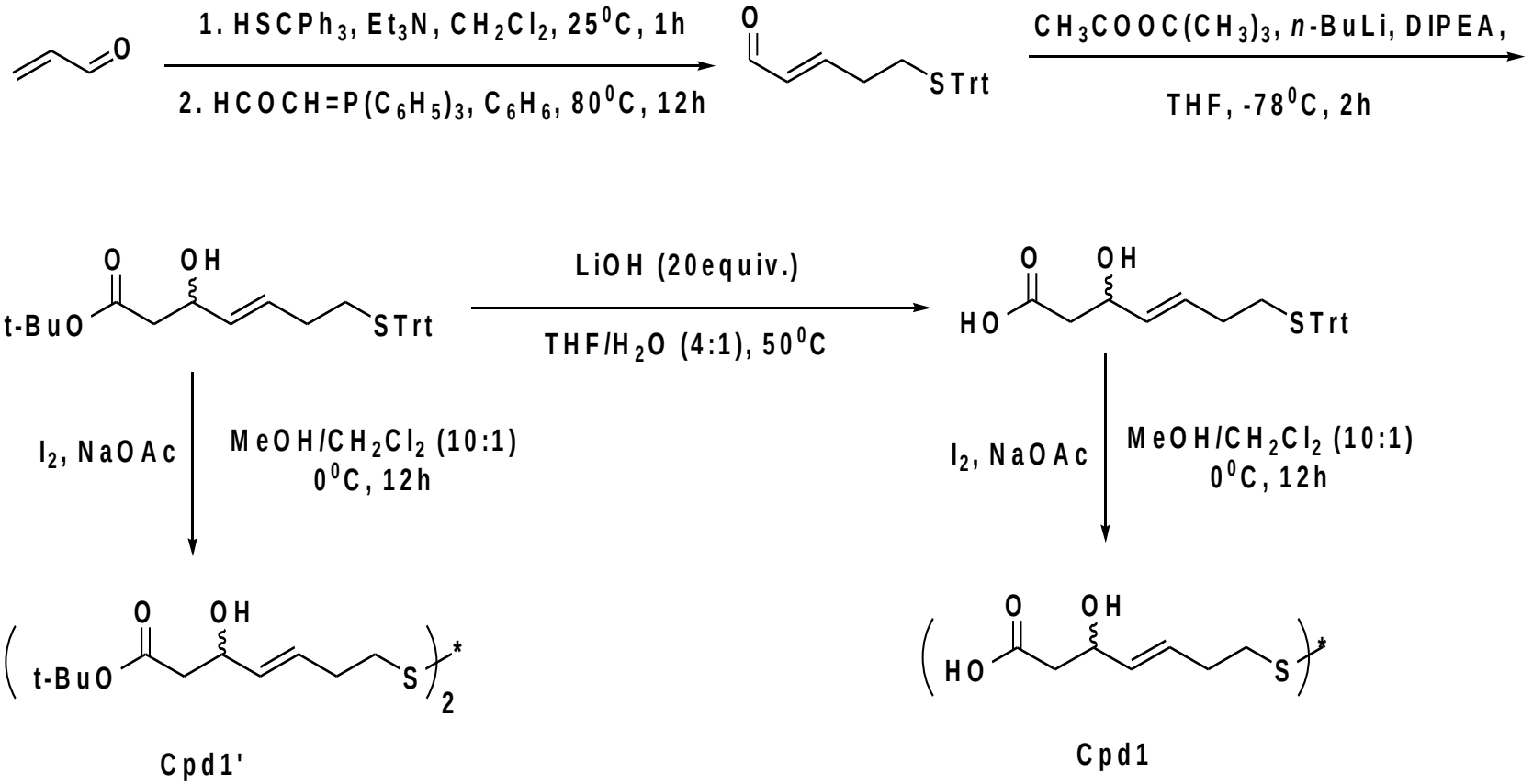
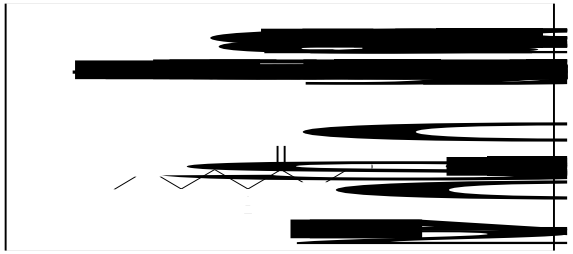
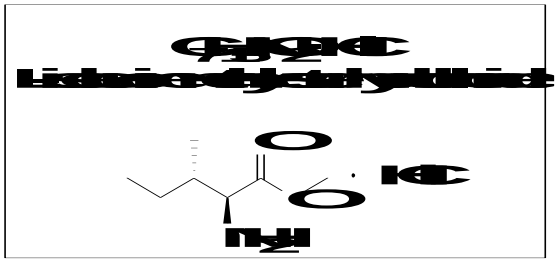
Synthesis of Cpd1 and Cpd1'



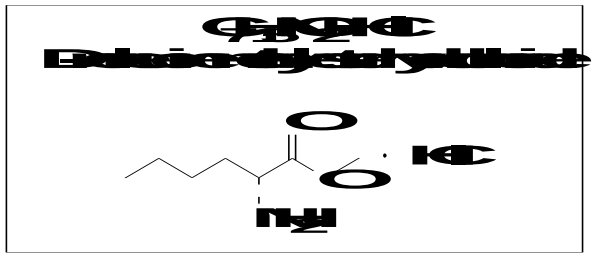
Synthesis of Cpd2 and Cpd3

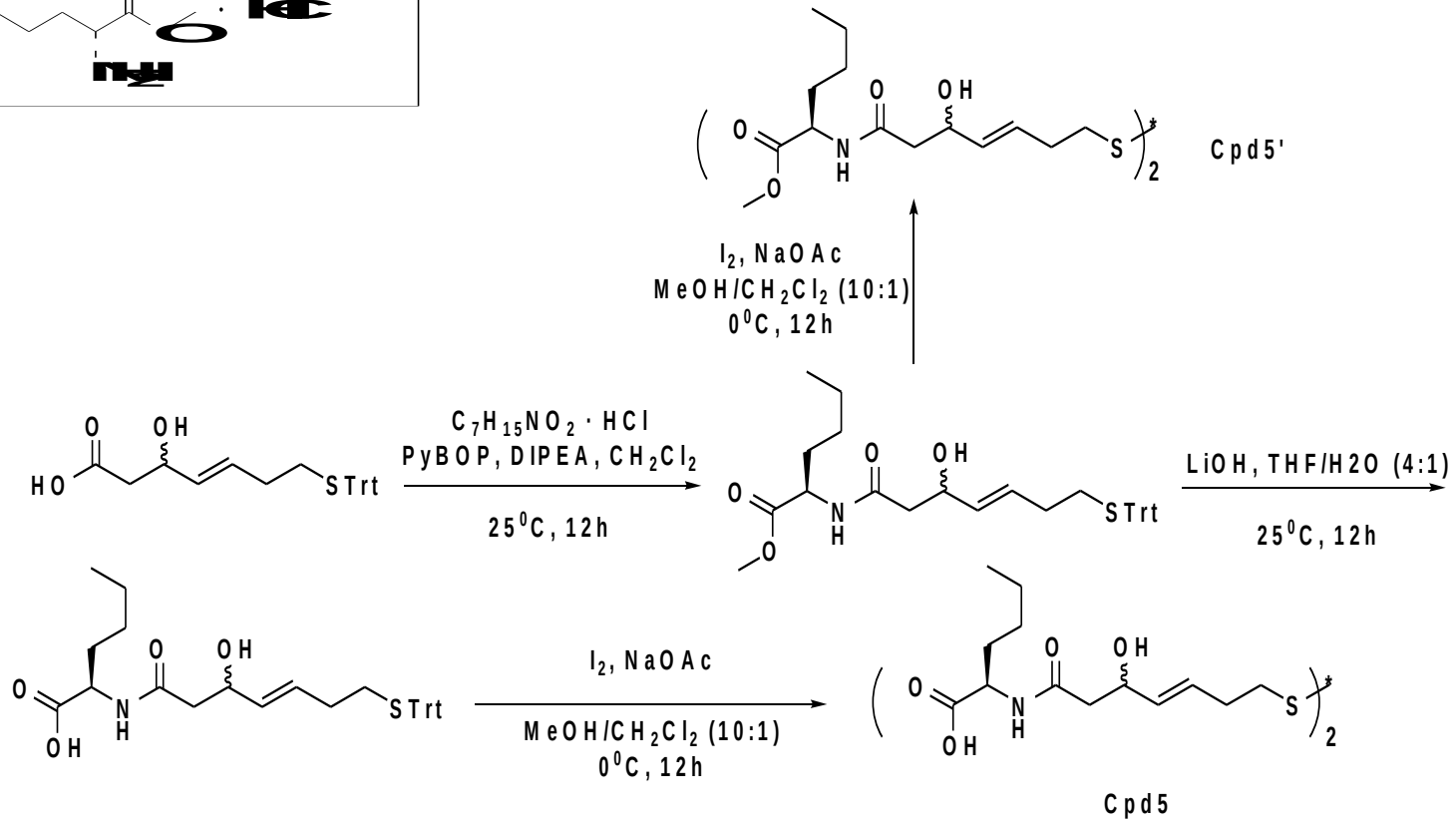


Synthesis of Cpd4 and Cpd4'



Synthesis of Cpd5 and Cpd5'





Synthesis of Cpd6 and Cpd6’