

# **Polymer–assisted, multi-step solution phase synthesis and biological screening of histone deacetylase inhibitors**

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*Org. Biomol. Chem.*, **2004** 2, 611-620

## **Overview**

What are histone deacetylase inhibitors?

Polymer assisted solution phase synthesis

Library

Biological results

## What are Histones?

Histones -proteins that play a part in the regulation of transcription by helping to condense DNA into its compact form as chromosomes

Transcription is one of the steps involved in the production of proteins from DNA

### DNA packaging problem

Human chromosome  $10^7$  to  $10^8$  DNA base pairs

DNA stretched out is 8 cm

Human cell 20  $\mu$ m

Nucleus 5  $\mu$ m

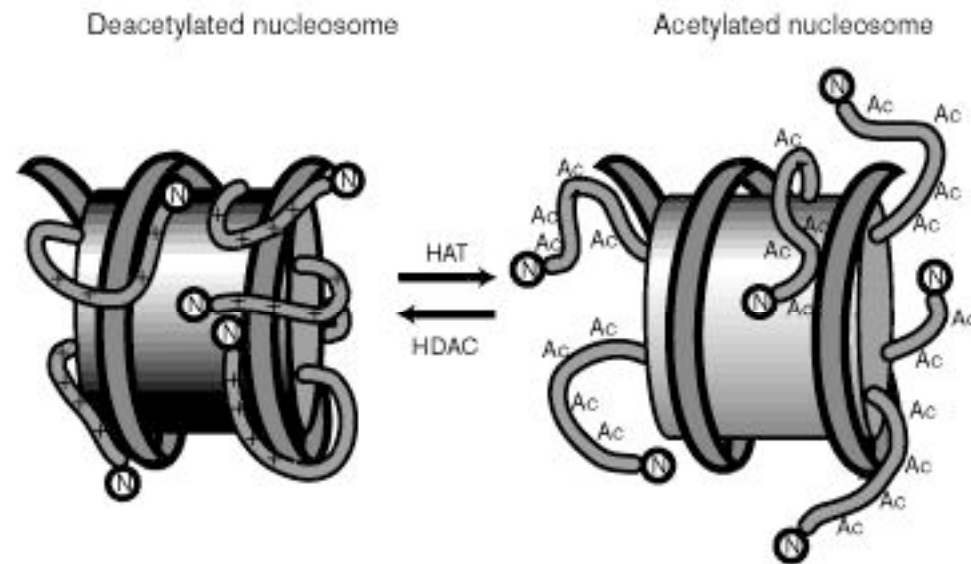
DNA is condensed by wrapping around protein spools called **nucleosomes**.

Nucleosomes can form further higher order structures allowing the further compaction of DNA

Nucleosomes are composed of an octamer of core histones

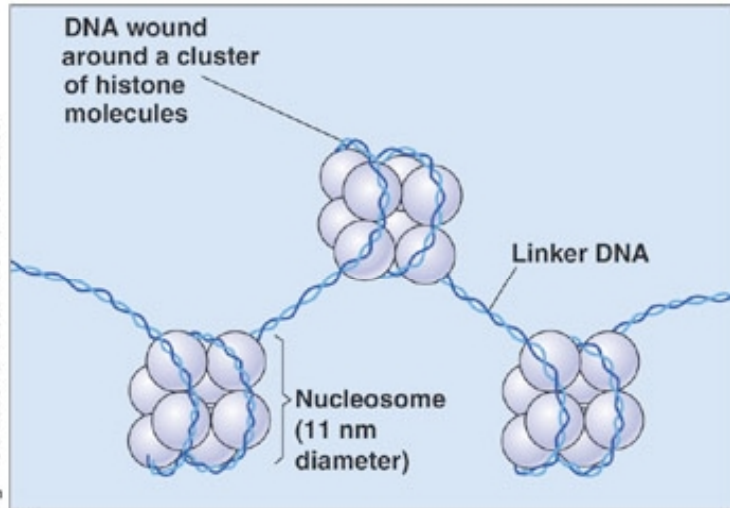
Five major classes of histones H1 H<sub>2</sub>A H2 B1 H3 H4

All have a large proportion of positively charged residues (Arg and Lys)

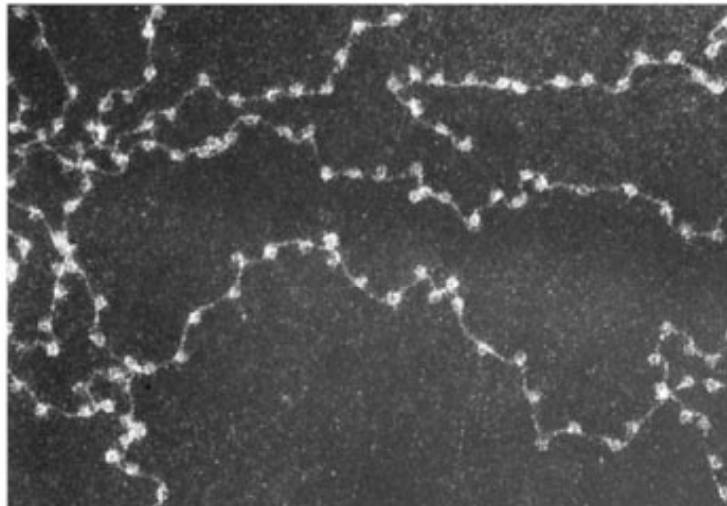


Current Opinion in Chemical Biology

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(a)



(b)

100 nm

In order for transcription to occur molecules known as transcription factors have to bind to specific binding sites on the DNA

COMPACT DNA-DIFFICULT FOR OTHER PROTEINS TO ACCESS IT  
TRANSCRIPTION OCCURS INFREQUENTLY

Binding of histones to DNA is controlled by enzymes

**histone acetylase (HAT)**  
**histone deacetylase (HDAC)**

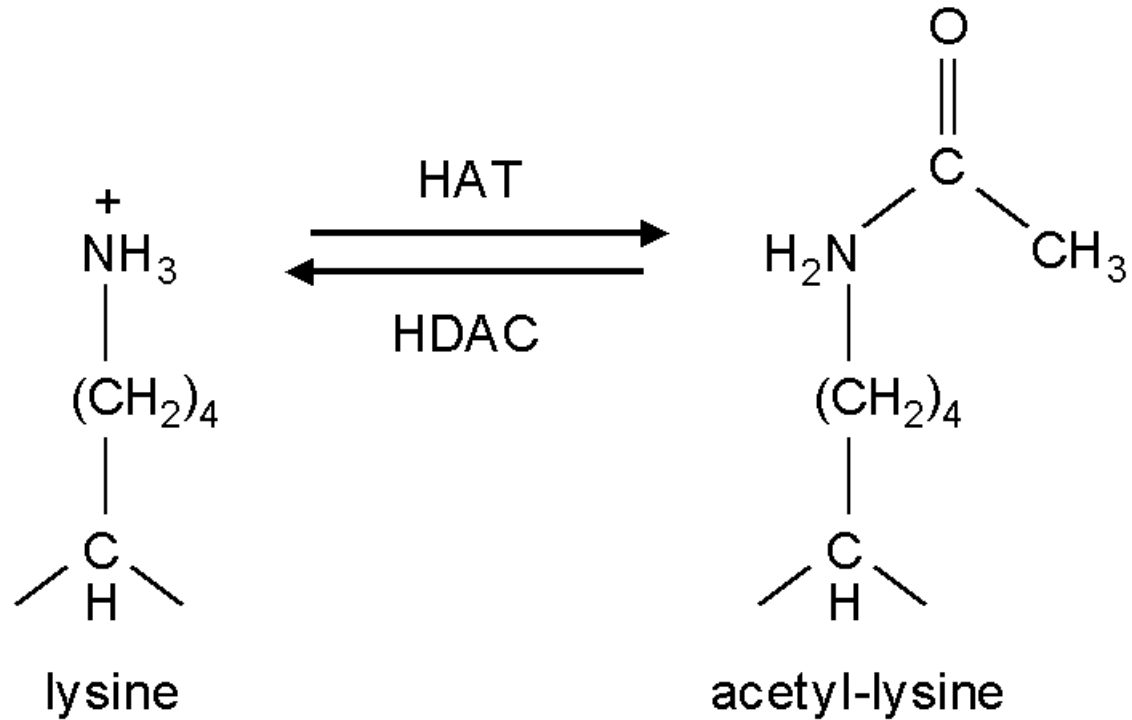
Histone proteins are acetylated and deacylated in a controlled fashion to alter the structure of chromatin

The  $\epsilon$ -amino groups of lysine residues of histones H3 and H4 are acetylated by cytoplasmic histone acetylase (HAT)

Acetylation neutralizes the positively charged lysine residues weakening the interaction with DNA

This disruption opens up the nucleosome for transcription

# Histone acetylation/deacetylation



**An imbalance in the level of histone acetylation has been associated with malignant diseases**

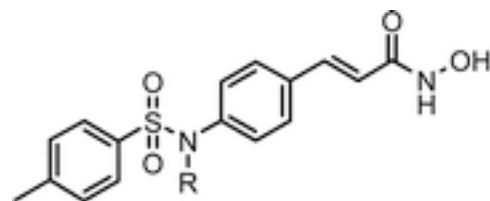
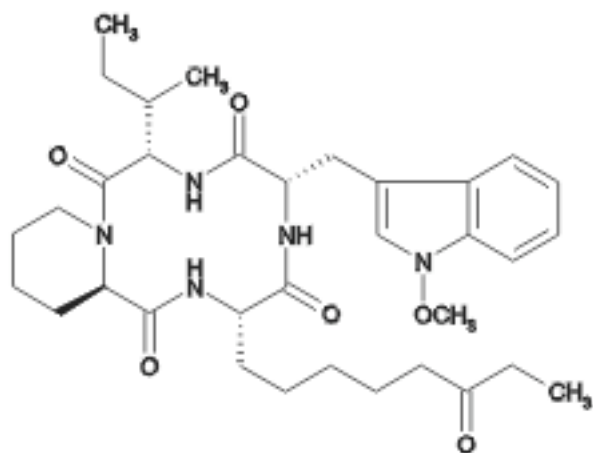
**Also observed to result in inhibition of angiogenesis**

**Potential new anti-cancer agent**

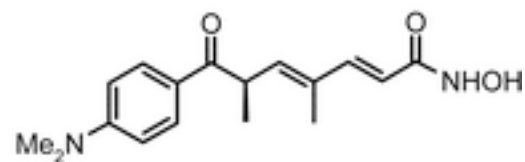
**Also promise as novel anti-protozoal and anti-viral agents**



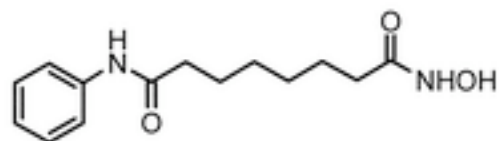
**HDAc inhibitors include**



1a: R=H  
1b: R=Me



2; Trichostatin A (TSA)



3; SAHA

**Apicidin**

**Trichostatin A (TSA) natural product**

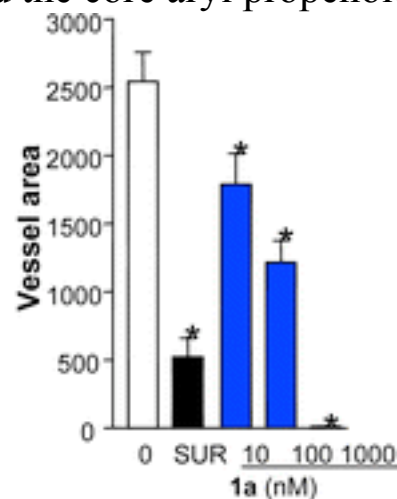
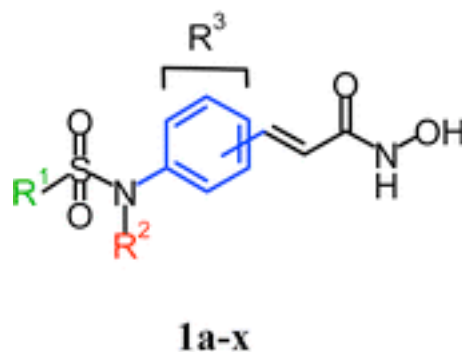
**Suberoylanilide hydroxamic acid (SAHA) now in Phase I clinical trials**

## Mode of action

Hydroxamic acid functionality in TSA coordinates to a zinc cation that is held by several hydrogen bonds in the active site of HDLP (histone-deacetylase-like-protein), a bacterial homologue of eukaryotic HDACs.

The polyene chain in TSA acts as a spacer group that occupies a lipophilic channel leading from the zinc binding domain of the enzyme to a region occupied by the aromatic capping group.

- (i) Prepare a focused array based upon **1a** that retained the core aryl propenoic hydroxamic



acid functionality but incorporated **three points of diversity** (i) sulfonamide *N*-methylation, (ii) regioisomerism about the aniline, and (iii) different substituents on the terminal benzenesulfonamide.

## Aim

- Develop a multi-step synthesis that did not require aqueous work-ups
- Using only a sequence of immobilised reagents and scavenger resins
- Use the same solvent for all transformations would further simplify reaction work-up and establish a route that would subsequently be amenable *to* full automation

*Ley* reported a communication for the **first fully automated multi-step polymer assisted solution phase synthesis** *Org. Biomol. Chem.* **2003**, 1, 2419-242

# Polymer assisted solution phase synthesis (PASP)

## Solid phase synthesis

Long reaction development and optimization times  
Extra linker steps

Use large excesses to drive reactions to completion  
Easily automated

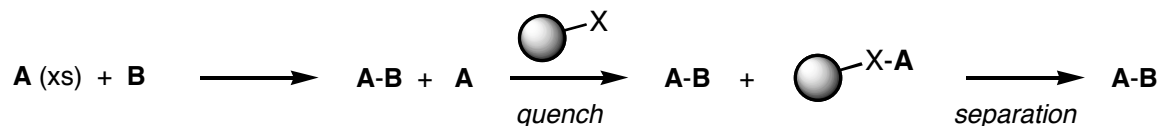
## Solution Phase Synthesis

Large literature of reactions to choose from

Drawback is the isolation and purification of individual products when multi-step synthesis is required

## PASP combines the advantages of solid phase with those of solution phase

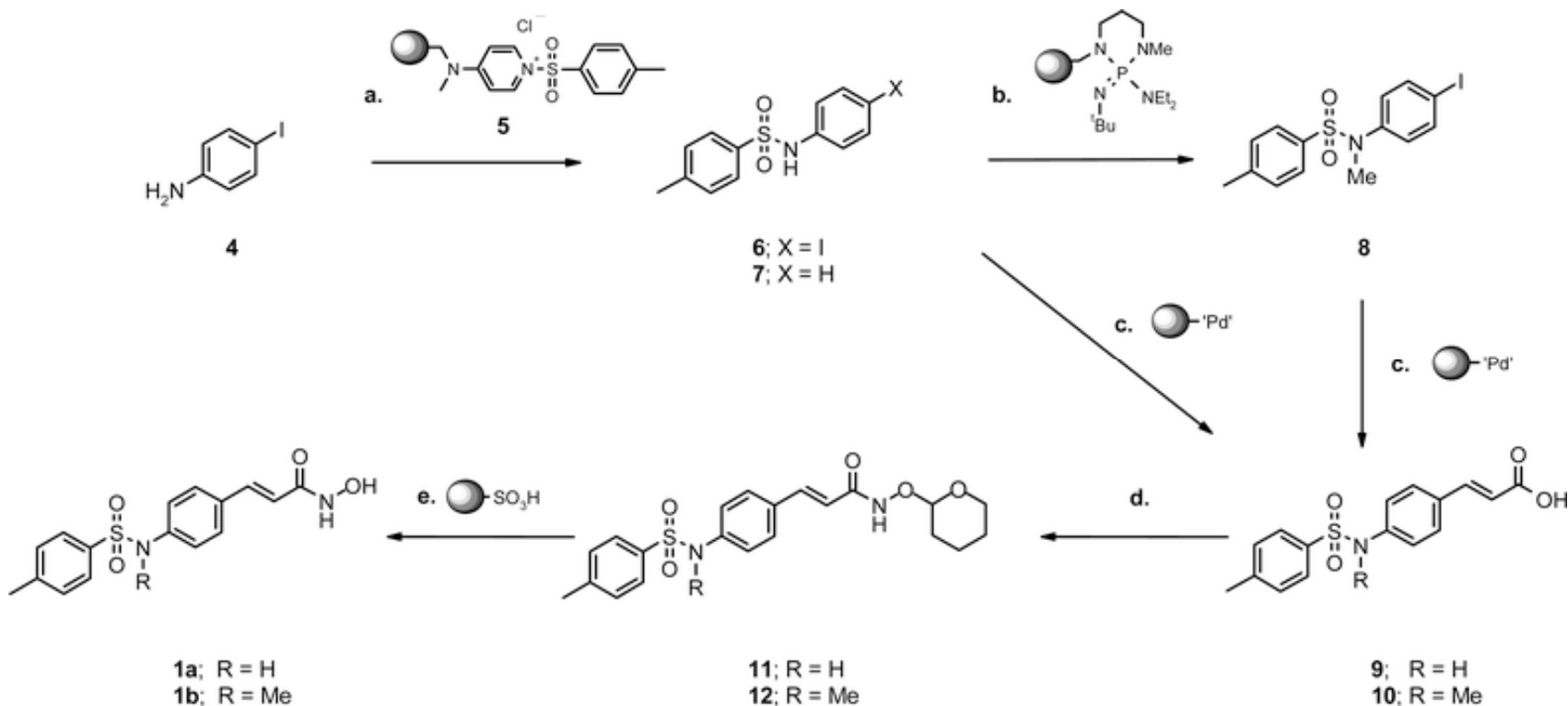
Excess reagents can be used  
By-product can be removed by filtration  
polymer supported reagents and scavengers



- reduces reaction work-ups to a series of simple filtrations and facilitates in line purification by solid phase extraction and ‘catch and release’ techniques
- suited for rapid multi-step synthesis of focused array compounds

### **Library Development**

- Initially developed a suitable polymer-assisted procedure for each step of the synthesis
- combined these protocols to prepare a sample of **1a** in a flow-through manner, without isolation or chromatographic purification of any intermediates.
- Finally, the 24 array **1a-x** was assembled in a similar way by parallel polymer-assisted multi-step synthesis.



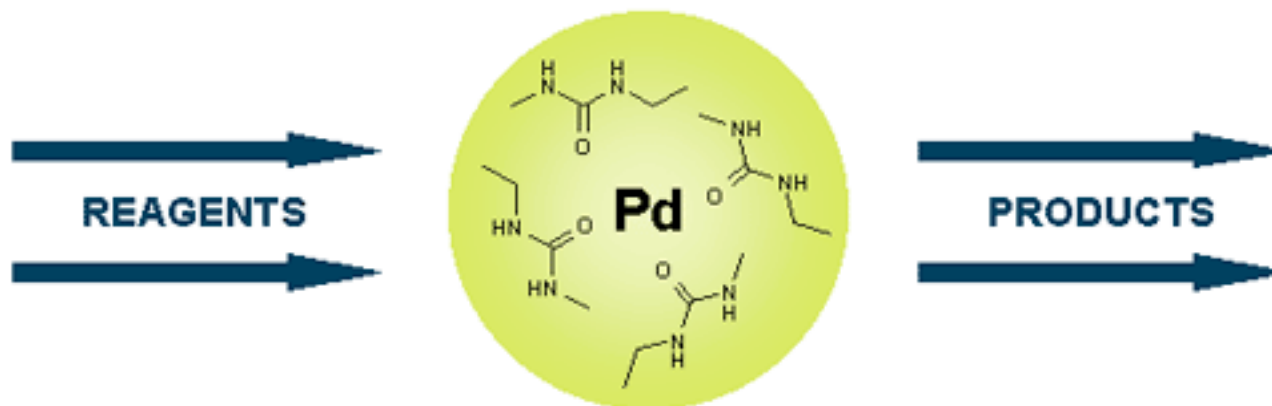
*Reagents and conditions:* a)(i). DMF, 50 °C, 1 h, Å~2; (ii). SCX SPE purification; b) MeI, DMF, rt, 18 h; c) acrylic acid, triethylamine, DMF, 90 °C, 18 h; d)(i). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF, rt, 20 min; (ii). hydroxylamine-OTHP, 50 °C, 18 h; (iii). NH<sub>2</sub> SPE purification; e) Amberlyst H-15, MeOH, rt, 2h

# Immobilized source of palladium

Extremely low Pd levels in products

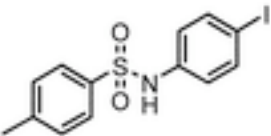
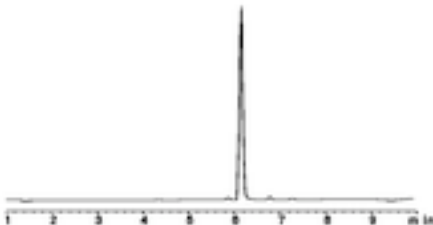
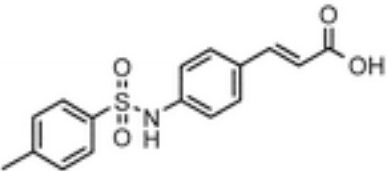
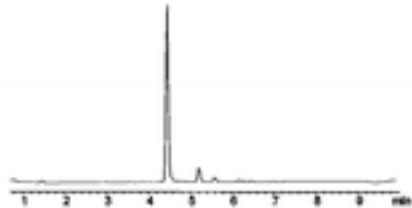
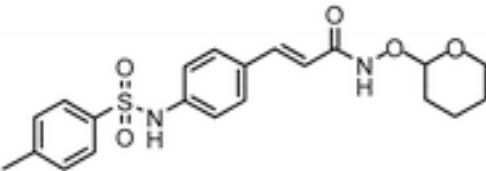
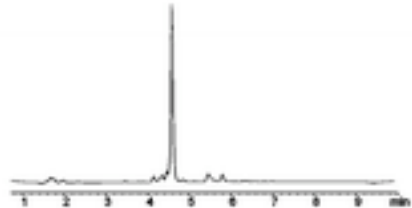
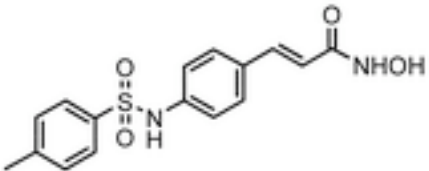
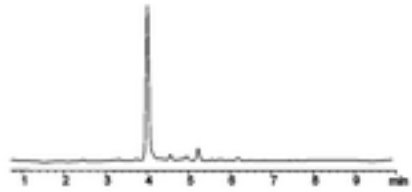
- \* Catalyst recovery by simple filtration
- \* Catalyst can be recycled
- \* No phosphine ligands required (but can be custom made if needed)

## polyurea matrix



[www.avecia.com](http://www.avecia.com)

Purity Profile for flow through reaction sequence

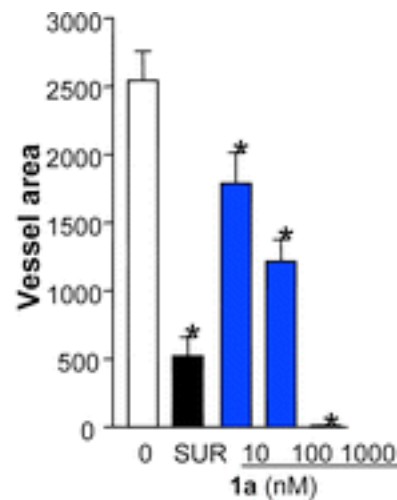
Compound	HPLC trace <sup>a</sup>	
6		
9		
11		
1a		

<sup>a</sup> Crude HPLC traces measured at 254 nm plotting absorbance vs time.

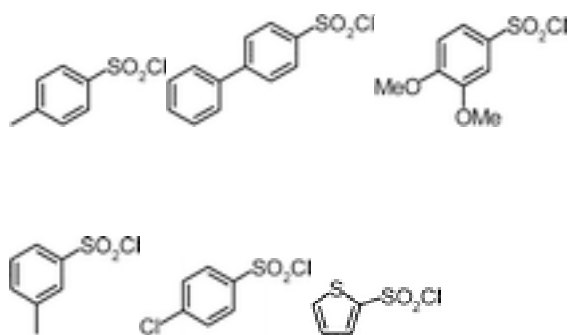




1a-x

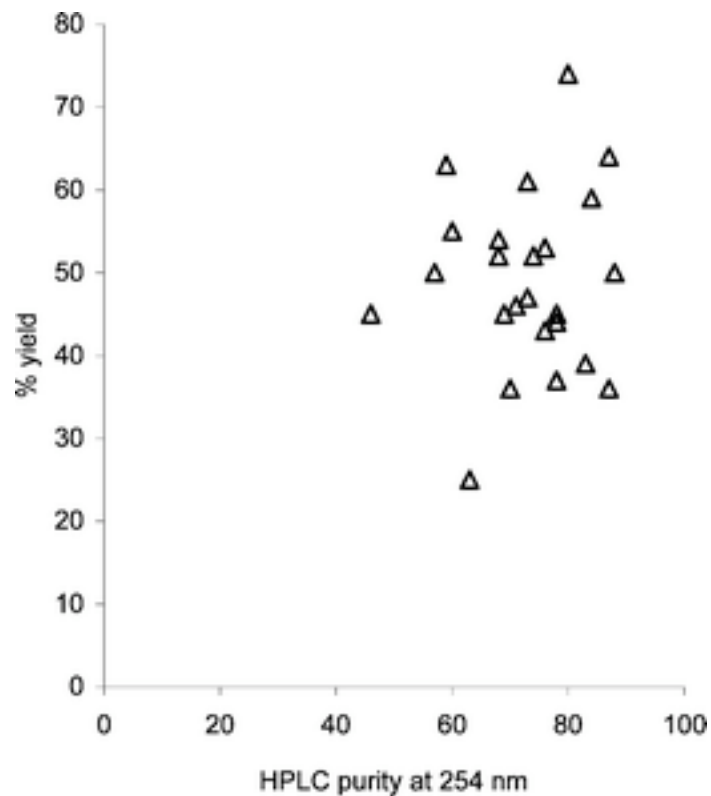


R<sub>1</sub>-SO<sub>2</sub>Cl:



R<sub>2</sub>: H Me

I-R<sub>3</sub>-NH<sub>2</sub>: Monomer sets chosen for combinatorial array 1a-x



**Greenhouse Parallel reaction block for heating and stirring  
All other reactions performed in fritted plastic filter tubes on a laboratory shaker**

**All the desired 24 products were obtained in reasonable yields  
with acceptable purities**

**Prior to screening a single auto-preparative purification step was performed**

**Purities in excess of 95%**

## **Biology**

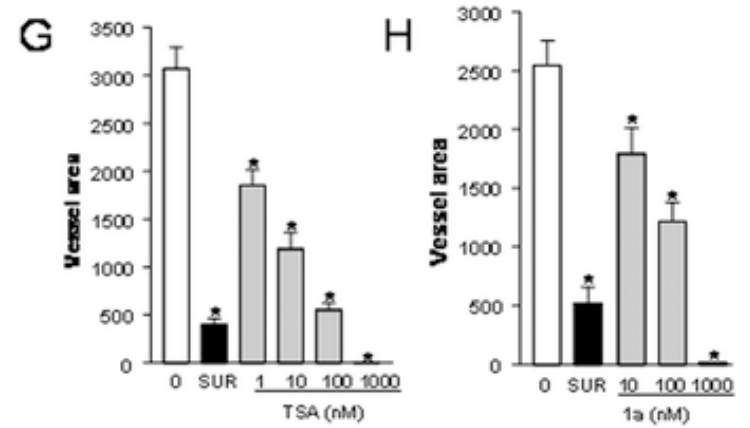
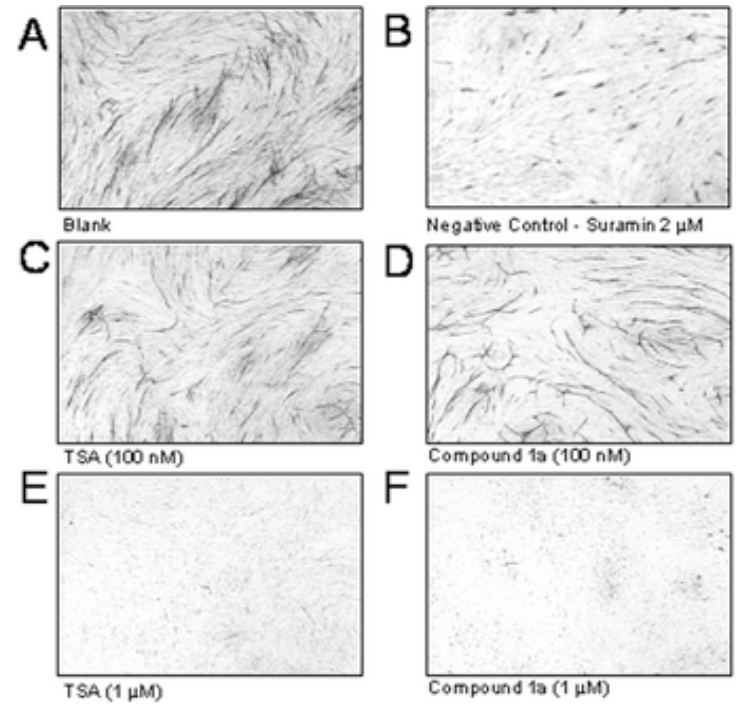
(i) *In vitro* biological activity measured in a primary HDAC enzyme inhibitory assay using HeLa cell nuclear extract

[deacylated substrate]  $\mu$  (1 / enzyme inhibitory activity)

(ii) The ability of the compounds to inhibit (human umbilical vein endothelial cell) HUVEC proliferation was also determined

The number of viable cells remaining after incubation with the inhibitor was measured spectrophotometrically after 48 h

(iii) Endothelial cells play a central role in angiogenesis-essential for the growth of tumours  
The most active analogues were also measured for their ability to inhibit tubule formation in a HUVEC –fibroblast co-culture which is an *in vitro* model of angiogenesis



**All compounds were effective in  
reducing total vessel area**

**Compounds 1a and 1c were essentially equipotent  
and effective at sub-micromolar concentrations**

**TSA is 10 fold more potent  
Effective below 10 nM**

## **Conclusions**

Constructed an array of hydroxamic acids using a parallel multi-step polymer assisted solution phase synthesis

A number of these compounds were found to have useful biological activity

PASP is useful for natural product synthesis

