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Design and synthesis of aryl ether and sulfone hydroxamic acids as potent histone deacetylase (HDAC) inhibitors

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ABSTRACT

A series of novel hydroxamic acid based histone deacetylases (HDAC) inhibitors with aryl ether and aryl sulfone residues at the terminus of a substituted, unsaturated 5-carbon spacer moiety have been synthesized for the first time and evaluated. Compounds with *meta*- and *para*-substitution on the aryl ring of ether hydroxamic acids **19c**, **20c**, **19e**, **19f** and **19g** are potent HDAC inhibitors with activities at low nanomolar levels.

Histone deacetylase (HDAC) enzymes are critically important in the functional regulation of gene transcription as well as chromatin structure remodeling and have become an emerging target in the search for new anticancer drugs.[1] Currently, there are 18 HDAC known in humans which are classified into four main phylogenetic classes. Of these known HDAC enzymes, it is commonly accepted that class I HDAC1, HDAC2, HDAC3 and HDAC8 serve a critical function in cell proliferation and survival. The primary function of HDAC's is the deacetylation of terminal amines present in the lysine residues found in the core of histones. This deacetylation results in free amino groups which are capable of strongly binding with DNA and results in the formation of chromatin structures which effectively block gene transcription. The antibiotic trichostatin A, (TSA), is a potent inhibitor of HDACs and is postulated to result in the induction of p21, a cyclin-dependent kinase inhibitor, which is responsible for cell cycle arrest, differentiation, and apoptosis in several types of cancer cells.[2] Several natural products including trapoxin, herbimycin, depudecin, apicidin, FR 901228 and a few synthetic congeners such as suberoylanilide hydroxamic acid (SAHA), MS-275, VX-563, Scriptaid and Oxamflatin have been identified as HDAC inhibitors (Fig. 1) and are reported to revert the morphological changes following the transformation of cells in culture.[3,4] While the hydroxamic acid containing compounds have been shown to have better activity in comparison to their non-hydroxamic analogs, they typically have poor metabolic stability.[5] It is promising to develop potentially superior HDAC inhibitors based on TSA or SAHA since detailed crystallographic data of their binding interactions with the active binding site of a bacterial HDAC homologue (HDLP) have been reported.[6a] Furthermore, both

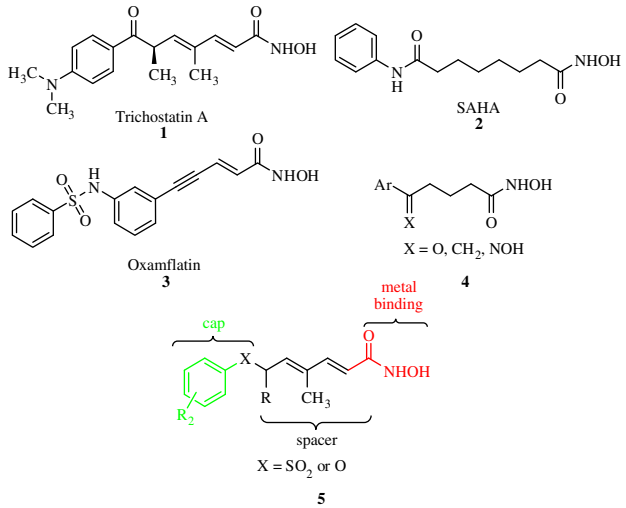

Figure 1.

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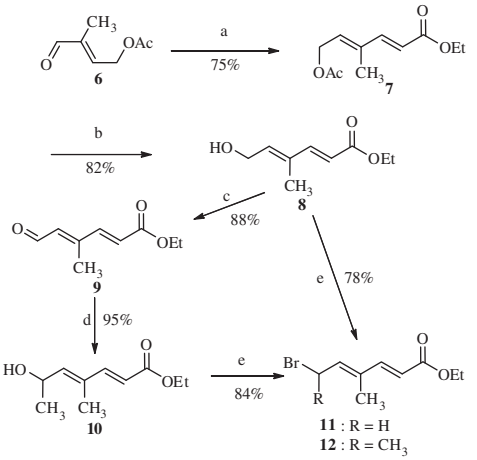
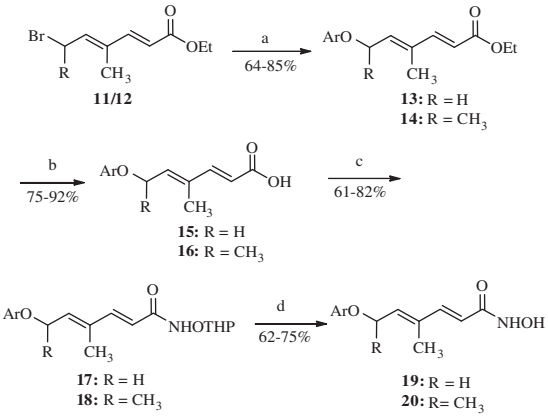


Scheme 1. Reagents and conditions: (a) NaH, THF, $(EtO)_2P(O)CH_2CO_2Et$; (b) K_2CO_3, EtOH; (c) MnO_2, CH_2Cl_2, 10 h, reflux; (d) 3.0 M CH_3MgBr in Et_2O, 4 h, 0 °C; (e) PBr_3, THF.



Scheme 2. Reagents and conditions: (a) ArOH, Cs_2CO_3, THF; (b) LiOH, EtOH, H_2O, rt, 8 h; (c) NH_2OTHP, EDCI, HOBt, THF, rt, 8 h; (d) MP-SO_3H, MeOH, rt, 3 h.

TSA and SAHA have been identified as potent and specific HDAC inhibitors and specifically SAHA has been approved by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL).

The active site of an HDAC enzyme consists of a tubular pocket leading to a zinc(II) and two aspargine–histidine charge relay systems; class I and II HDACs share this common feature of a zinc(II) species present at the enzymatic catalytic site. Consequently, reversible HDAC inhibitors reported to date have a chelating group that forms a complex with the zinc(II) and a hydrophobic spacer that fits into the tubular pocket. Therefore, options for the structural modification of the chelating group and the hydrophobic spacer are limited.[6] Several groups have reported the synthesis and bioassay of novel HDAC inhibitors based on TSA with varying chain length, aryl substitution, and aryl-chain connection with functionality that includes ketones, alkenes and oximes, etc. Of these analogs, those which possess a hydroxamic acid metal binding group have shown HDAC inhibition IC_{50} in the low nanomolar range.[7]

These studies led us to initiate structural modifications of the terminal residue ('cap') while retaining the hydroxamic acid group. It is postulated that structural features of the molecule in the 'cap' region interact at the entrance of the HDAC pocket and provides opportunities to discover potent and possibly even selective HDAC inhibitors.

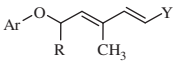
We now report our results on the design, synthesis and HDAC inhibitory data for a series of the TSA analogs which contain either an aryl ether or sulfone functionality as the 'capping' group, a branched chain diene spacer linker and carboxylic or hydroxamic acid terminal group capable of binding to the zinc residue contained within the active site of HDAC enzymes.

Synthesis of analogs started from commercially available (*E*)-3-methyl-4-oxobut-2-enyl acetate **6** and the preparation of alcohols **8**[8] and **10**[3a] is illustrated in Scheme 1. Specifically, aldehyde **6** undergoes a Horner–Wadsworth–Emmons olefination with triethyl phosphoroacetate and NaH in THF to give *trans*-diene **7** in moderate yield. Acetate deprotection cleanly gave primary alcohol **8**. Incorporation of the second branched methyl to the diene backbone was achieved by a two-step process; oxidization with manganese dioxide to give aldehyde **9** followed by methylmagnesium bromide addition afforded secondary alcohol **10**. Both alcohols **8** and **10** were treated with PBr_3 to yield bromides **11** and **12**.

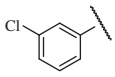
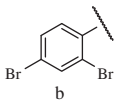
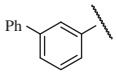
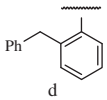
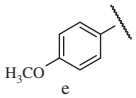
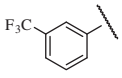
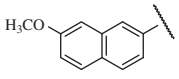
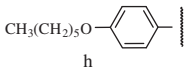
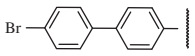
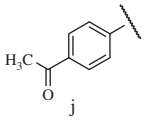
Aryl ethers **15/16**, and **19/20** were prepared from bromides **11** and **12** in four steps by the procedure outlined in Scheme 2. Reaction of **11** and **12** with substituted phenols in the presence of cesium carbonate gave aryl ethers **13** and **14** in good yields. These were directly saponified with lithium hydroxide giving carboxylic acids **15** and **16** in moderate yields. Subsequent coupling with THP-protected hydroxylamine (NH_2OTHP) in the presence of EDCI and HOBT gave protected hydroxamic acids **17** and **18**. THP deprotection using solid phase macroporous sulfonic acid resin (MP-TsOH) in methanol provided the aryl ether hydroxamic acids **19** and **20** in good yields.[7a,9] The HDAC inhibition activity of the compounds reported herein were assayed using a peptide substrate and mixture of HDACs, predominantly HDAC1 and HDAC2[10] and the in vitro IC_{50} values of the various derivatives were obtained. Bioassay results for aryl ethers **15/16** (carboxylic acids) and **19/20** (hydroxamic acids) with various 'cap' substitution are presented in Table 1. The first notable observation is the lack of inhibition activity for all of the carboxylic acid derivatives (**15**/**16**) tested; all exhibiting greater than >1 μm HDAC IC_{50} activity; conversely, nearly all hydroxamic acid derivatives (**19**/**20**) showed promising inhibition results. Incorporation of *m*-substituted aryloxy moieties proved successful in achieving activity for a number of analogs presumably thru interactions at the hydrophobic region of the HDAC pocket (Fig. 1). Our initial lead compound, 3-chlorophenyl **19a**, showed moderate HDAC inhibitory activity (IC_{50} = 188 nM). Further probing of the hydrophobic pocket interaction with 3-phenylaryloxy **19c** gave a fivefold increase in activity (IC_{50} = 41 nM), and a further threefold increase in activity was obtained with the 7-methoxynaphthyl analog **19g** (IC_{50} = 20 nM). Activity was retained for **20c** which incorporated a second methyl group on the diene spacer portion of the molecule (IC_{50} = 55 nM). The most potent compound tested in this series of analogs incorporated a 3-trifluoromethylphenoxy group **19f**, displaying an HDAC inhibitory activity of IC_{50} = 18 nM.

Substitution of the aryloxy group in the *para* position reduced activity; 4-bromophenyl-4-phenoxy **20i** showed a fourfold decrease in activity (IC_{50} = 162 nM) compared with the similar 3-phenyl analog **19c**. Both 2,4-dibromoaryloxy **19b** and 4-*n*-hexylaryloxy **20h** analogs significantly lost activity with nearly micromolar activity IC_{50} = 890 nM and 1100 nM, respectively. HDAC inhibition was mostly dependent on the substitution on the aryl ring and it changed only minimally with methyl substitution on diene chain as demonstrated for the 3-biphenyl analogs **19c** and **20c** both showing similar inhibitory activity for the methyl and unsubstituted diene chain (**19c**, 41 nM; **20c**, 55 nM). These results indicated that for this series of compounds, arene substitution at the *meta*- and/or *para*-positions is preferred for good inhibition activity. Similar results have previously been reported that support the observation for the preference of *para*-substituted aryl 'cap' groups.[11]

Table 1
In vitro inhibitory activities for aryloxy analogs at histone deacetylase[10]



15: Y = CO_2H, R = H **19**: Y = CONHOH, R = H
16: Y = CO_2H, R = CH_3 **20**: Y = CONHOH, R = CH_3

HDAC Inhibition IC_{50}[10] (M)				
Y = CO_2H		Ar	Y = CONHOH	
R = H (**15**)	R = CH_3 (**16**)		R = H (**19**)	R = CH_3 (**20**)
	>1	 a	0.188	
	>1	 b	0.890	
>1	>1	 c	0.041	0.055
	>50	 d	1.200	
>1		 e	0.068	
	>50	 f	0.018	
		 g	0.020	
	>1	 h		1.100
		 i		0.162
		 j		>50
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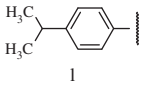
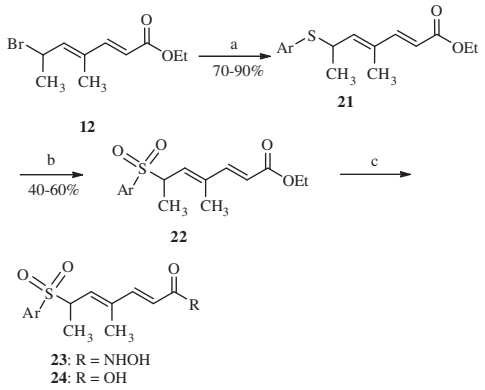
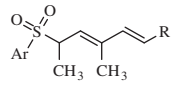
>1	>50	 l		
		Tricostatin A **1**		0.003

Recently the Marson group[12] reported SAR data for examples of aryl sulfide and sulfoxide analogs at 'cap' region of the SAHA core **2** with both fully saturated and diene unsubstituted backbone 'spacer' linker, with interesting HDAC inhibition activity but did not provide enough data to draw an accurate conclusion on whether sulfones would be active. Having prepared the key intermediate **12** we decided to further probe the 'cap' region of our core molecule by replacement of the aryloxy group with a series of arylsulfone derivatives. While the sulfone is a significantly different

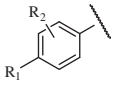
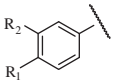
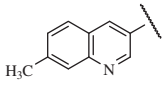
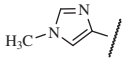
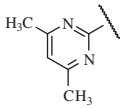


Scheme 3. Reagents and conditions: (a) ArSH, NaH, THF; (b) *m*CPBA, CH_2Cl_2, 0 °C, 8 h; (c) KOH, $NH_2OH{\cdot}HCl$, rt, 8 h.

Table 2
Inhibitory activities for sulfone hydroxamic acid and sulfone carboxylic acids at histone deacetylase[10]



23: R = CO_2H
24: R = CONHOH

HDAC Inhibition IC_{50}[10] (M)		
R = CO_2H **23**	Ar	R = CONHOH **24**
>1.0	 R_1 = Br, Cl, F; R_2 = H R_1 = CH_3; R_2 = 3-CH_3, 2-CH_3	
	 R_1 = Br, CH_3, OCH_3, NAc; R_2 = H R_1 = H; R_2 = OCH_3 R_1 = Cl; R_2 = Cl	>1.0
		>1.0
		>1.0
		>1.0

capping group compared to the aryloxy group, we hoped that the activity seen as a result of incorporating a hydroxamic acid metal binding group would allow for some tractable SAR data. Specifically bromide **12** was used to incorporate the desired aryl diversity elements by reaction with a variety of thiophenols to generate sulfides **21** that were converted to the target aryl sulfones by oxidation using *m*-chloroperbenzoic acid (Scheme 3) giving esters **22**. Finally, one pot hydroxamination of the resultant esters afforded the sulfone hydroxamic acids **23** in low to moderate yields along with isolated acids **24** as by product. Unfortunately all of the arylsulfonates **23** as well as the analogous carboxylic acid derivatives **24** had HDAC inhibitory activity IC_{50} >1 μm. (Table 2)

Molecular modeling: In order to better understand the results for compounds **19**/**20** we built a model of TSA in HDAC-2. Recently, an

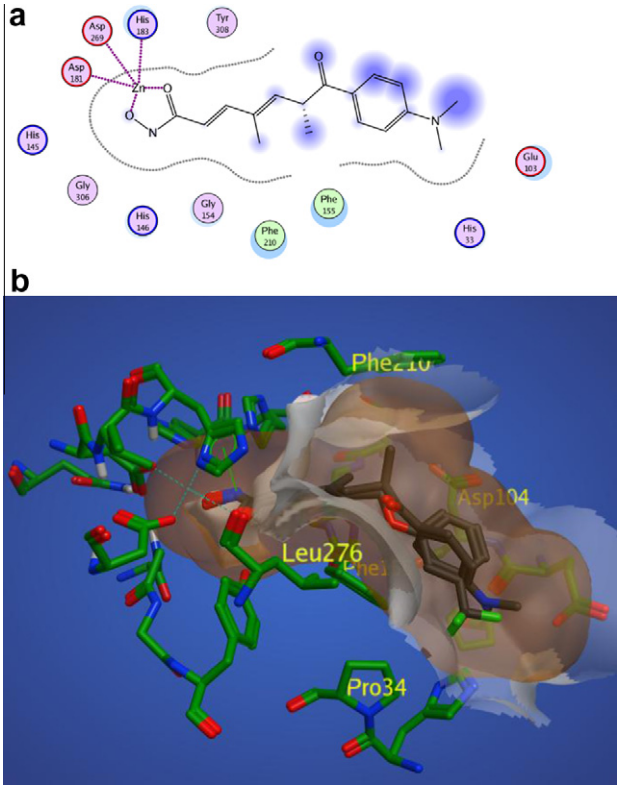


Figure 2. Model of HDAC2 and TSA. (a) Interaction diagram for the HDAC2 model with TSA. (b) 3D image of the HDAC2:TSA and HDAC2:**19f**.complexes. A brown surface surrounds the TSA ligand and a white surface outlines the binding pocket. Images generated using MOE software.[14]

HDAC-2:inhibitor structure was disclosed (pdb code: 3max) and this structure was used as a starting point for the protein structure. Details of the modeling are described in Ref. 13. Figure 2 illustrates the final model for the TSA molecule and **19f**. The zinc binding site remains nearly static over the course of the simulation along with the diene portion of TSA. Phe210, Phe155 and Leu 276 form a hydrophobic pocket for the ketone/aryl-oxy linker. The variability observed in the SAR is consistent in the changes from **19** (R = H) to **20** (R = Me) since the closest point of contact is Phe210 according to this model. The dimethyl amino group of TSA and the CF_3 group of **19f** fill similar areas of the solvent exposed region and illustrates that substantial variation is tolerated at this position in the SAR of **19** and **20**. The phenyl group prefers to pack edge-on against relatively rigid hydrophobic pocket formed by Leu276, Pro34 and Phe155 side chains with the opposite edge solvent exposed. These observations lead us to an explanation of the strong preference of the ether linkage over the sulfone. Asp104 is relatively free to move away from the phenyl group and ketone of TSA. In **19** and **20**, the linking group is small and freely rotating and therefore allows the inhibitors to pack pendant aromatic moieties in the hydrophilic face of the channel leading to the solvent exposed region. However, we hypothesize that the larger sulfone linker of **23** interacts unfavorably with Leu276 and potentially with Asp104 and therefore sulfonyl-containing compounds are unlikely to adapt their conformations to the hydrophobic pocket of the channel. Previous work[12] indicates that sulfonyl groups may be permitted in this region of the binding site when these compounds contain a more flexible, saturated spacer permitting more significant accommodation of the sulfonyl-aromatic cap.

In summary, we have designed and synthesized a series of sulfone and aryl ether based TSA analogs, and evaluated their inhibitory effect on HDACs. We have shown that the potency depends on the substitution pattern on the arene ring. We have also observed the multifold increase in activity with the replacement of large arylsulfone group with the corresponding aryl ether. SAR results with the aryl ether hydroxamic acids are consistent with the hypothesis that these HDAC inhibitors operate as mimetics of the natural product TSA. These small molecule aryl ether hydroxamic acids may be useful as tools for biological research and as orally bioavailable anticancer drugs. Currently, further detailed SAR studies and the next stage of evaluation are underway.

Acknowledgments

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