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Amide Analogues of TSA: Synthesis, Binding Mode Analysis and HDAC Inhibition

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Abstract—The synthesis of new amide type histone deacetylase inhibitors is described, having an (*R*)-methyl substituent and a diene or saturated structure of the chain linking the hydroxamic acid and dimethylaminobenzoyl groups. The saturated compound shows stronger HDAC inhibition than the unsaturated analogue. Molecular modeling suggests that the flexibility of the linker chain is important for an optimal orientation of the dimethylaminobenzoyl group in the enzyme.

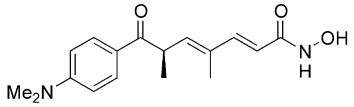
Histone deacetylase (HDAC) inhibitors have recently attracted considerable interest because of their therapeutic potential for the treatment of cell proliferative diseases.[1,2]

The natural product Trichostatin A (TSA) **1** has been identified as a potent and specific HDAC inhibitor[3] and its phase 1 biotransformation has been elucidated.[4] Structurally simpler TSA analogues have been described such as SAHA **2**,[5] the amide analogues **3**[6] and arylketones **4**.[7,8] These analogues are generally less potent than TSA. A major structural difference is the presence in TSA of an unsaturated chain linking the important hydroxamate function and the aromatic ring, and the presence of an (*R*)-methyl group. The absolute configuration of the chiral center has been shown to be important since the enantiomer (*S*)-TSA is inactive.[3,9]

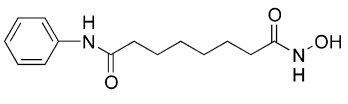
We now report our results on the synthesis and HDAC inhibition of amide analogues having a (*R*)-methyl group and a diene unsaturated chain **9** or a saturated chain **10**. The binding mode of these new analogues to HDLP has been investigated by molecular modeling.

A synthetic route towards the unsaturated amide is outlined in Scheme 1. The (*R*)-stereochemistry of the chiral center originated from D-alanine.

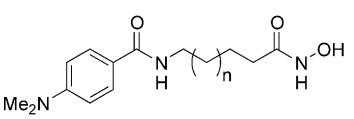
Reduction of the *N*,*O*-dimethylhydroxamate **5** to aldehyde **6**,[10] followed by a Wittig–Horner olefination using



TSA **1**



SAHA **2**



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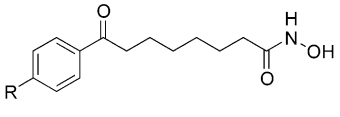

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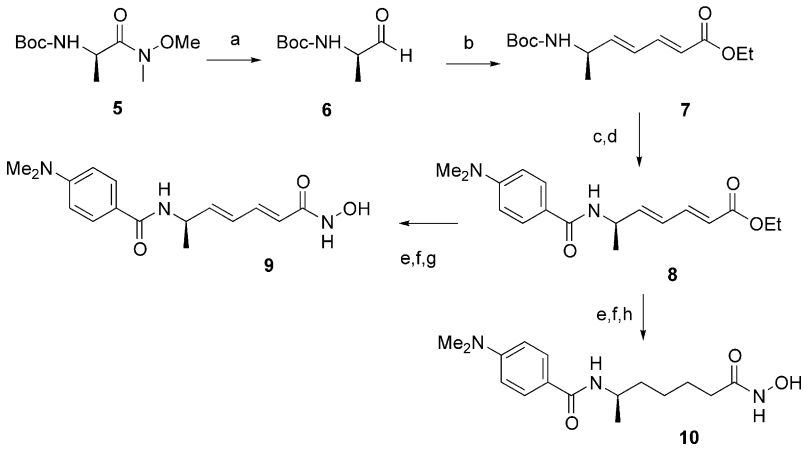
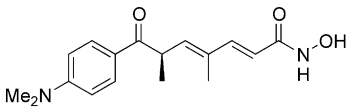
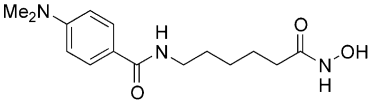
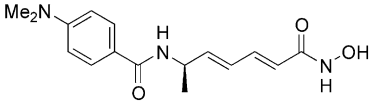
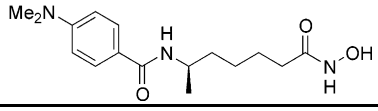


Scheme 1. Conditions: (a) $LiAlH_4$; (b) $(EtO)_2POCH_2CH{=}CHCOOEt$, LiOH; (c) TFA; (d) 4-dimethylaminobenzoic acid, TBTU, NEt_3; (e) 5 N HCl, acetone, reflux, overnight; (f) $NH_2OBn{\cdot}HCl$, TBTU, *N*-methylmorpholine; (g) HF_{liq}, anisole, 0 °C, 1 h; (h) H_2, Pd/C.

triethyl-4-phosphocrotonate[11,12] afforded the unsaturated ester **7** with high (>95%) E stereoselectivity, in 63% yield calculated from **5**. The enantiomeric purity (90% ee) was determined after Boc-deprotection, and derivatisation with Marfey's reagent,[13] followed by LC–MS analysis. The peak of the minor isomer was identified after synthesis of the reference epimeric compound starting from L-alanine.

After removal of the Boc-nitrogen protection, 4-dimethylaminobenzoic acid was coupled to the amine. In contrast to previous reports,[14] the use of BOP-Cl as coupling reagent resulted in low yields (44%). Using TBTU with a preactivation time of the dimethylaminobenzoic acid of 2 h, gave a 84% yield of **8**. The ester was hydrolysed in acidic conditions (70%), and the resulting carboxylic acid was converted to the *O*-benzyl protected hydroxamate to using TBTU as coupling reagent (95%). All attempts to selectivity remove the *O*-benzyl protection by hydrogenolysis failed, but resulted in the saturated analogue **10**. The hydroxamic acid **9** was obtained after treatment with anhydrous liquid hydrogen fluoride (76%) and HPLC purification.

Table 1. HDAC inhibition data

Compd	Structure	HDAC IC_{50} (nM)
1 (TSA)		12±1[a]
3 ($n=2$)		152±15
9		12,187±1384
10		359±92

[a]Data from ref 20.

HDAC inhibition by analogues **9** and **10** was determined using [^{3}H]-acetate prelabeled chicken reticulocyte core histones as enzyme substrate and HDAC from rat hepatocyte lysate as enzyme source.[14,15] The liberated [^{3}H]-acetate was quantified by scintillation counting and the results are shown in Table 1.

As a reference compound the amide **3** ($n=2$), which is one of the most potent analogues of this type of compounds,[16] was used. The inhibition data indicate that the introduction of unsaturation in the chain resulting in **9** has a negative effect on the potency of the compound. Saturation of the chain to **10** restores the inhibitory capacity of **10** to a level similar to that of reference compound **3** ($n=2$). Compound **10** has an identical backbone structure as **3** ($n=2$), but carries an (*R*)-methyl substituent as in TSA **1**. These results therefore indicate a negligible effect of this substituent on the potency. The decreased potency of (*S*)-TSA **1**, should therefore be ascribed to an unfavourable interaction of the methyl group in this enantiomer with the enzyme.

In order to study the differences in potency of these compounds, their binding mode in the catalytic site of the HDLP protein was studied by molecular modeling. We have developed a model of the active site of HDLP, based on the published structure of the TSA/HDLP complex.[17] The model consists essentially of all amino acid residues within a radius of 4.5 Å around TSA. This model allowed the ab initio minimization of TSA **1** in this active site model at the HF 3-21G(*) level resulting in an excellent agreement between the calculated binding conformation and the observed TSA/HDLP complex.[18] Since the residues that make up the active site are conserved in the HDAC family,[17] the results of this model can be extrapolated to other HDAC types, including rat HDAC.[19] The same active site model was used to model the interactions with the analogues **9** and **10**, using the MMFF94 forcefield. The hydroxamic acid was fixed at the position found in the X-ray structure, and a systematic conformational search was conducted

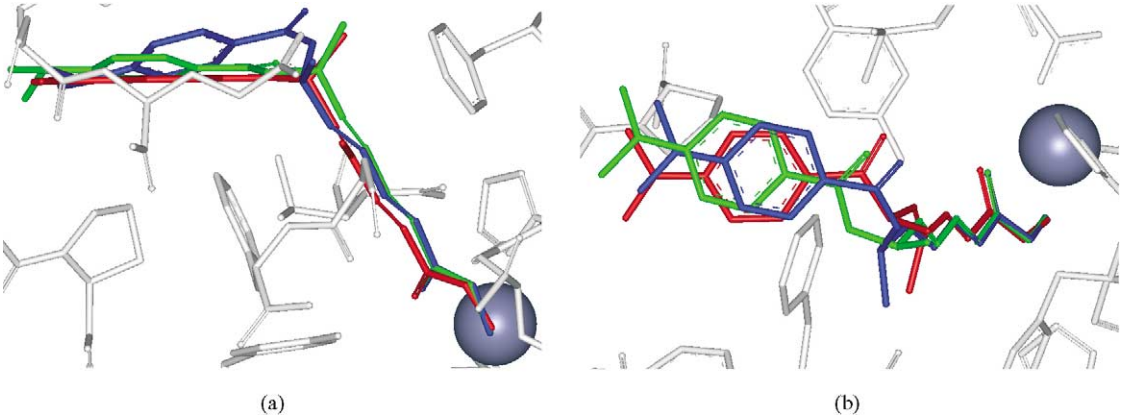


Figure 1. Superposition of the low energy conformations of **9** (blue and green) and TSA (red) in the active site model of HDLP; (a) side view, (b) top view.

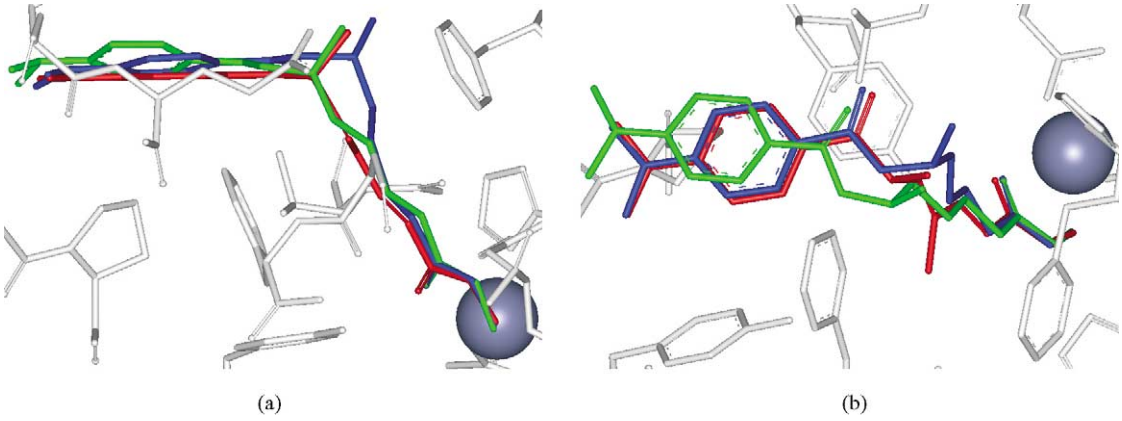


Figure 2. Superposition of the low energy conformations of **10** (blue and green) and TSA (red) in the active site model of HDLP; (a) side view, (b) top view.

over all rotatable bonds. The energy of each generated conformation was calculated. An in-house routine was used to find all local minima in the resulting energy landscape, and the conformations found by this procedure within 20 kcal/mol from the absolute minimum were further minimized. After minimization, two low energy conformations within 4 kcal/mol were found for analogue **9** bound in the active site. Compared to the bound conformation of TSA (red), the unsaturated chain has an almost identical orientation in the tube-like cleft in the enzyme (Fig. 1a). However it appears that the amide bond region of both conformers of **9** is significantly displaced from the corresponding ketone part in TSA. Consequently, the dimethylaminobenzoyl group also shifted (Fig. 1b), apparently resulting in less efficient interactions with the enzyme's surface. For the saturated analogue **10**, two relevant low energy conformers were obtained within 4 kcal/mol. In this case, as could be expected, considerable more deviations in the orientation of the saturated chain versus the unsaturated chain in TSA were observed (Fig. 2a). In contrast, the more flexible saturated chain allows an orientation of the dimethylaminobenzamide part corresponding more closely to that in TSA (Fig. 2b). Therefore, in these structures, it is apparent that the optimal orientation of the 'capping' region of HDAC inhibitors contributes significantly to their potency, and that the contribution of π–π interactions in the tube-like cleft, if important, is difficult to evaluate. Similarly, the effect of the introduction of an (*R*)-methyl substituent in the amide analogues is minimal, either due to an imperfect orientation compared to that in TSA, or due to a lack of interaction with the enzyme.

In conclusion, the introduction of the (*R*)-methyl group and of the diene function, which are present in TSA **1**, into the amide bond analogues does not result in an increased HDAC inhibitory potency. The optimal interaction of the aromatic end group with the enzyme is crucial for this potency, and is influenced by differences in the flexibility of the spacer chain. Although these conclusions are derived from a histon deacetylase-like protein, the similarity in the active site residues in rat and human HDAC's allows speculation that they are also valid for these types and the results are therefore valuable for the further design of potent analogues.

Acknowledgements

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