3-(4-Aroyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides, a New Class of Synthetic Histone Deacetylase Inhibitors

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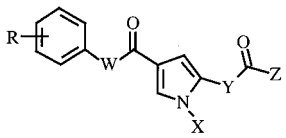
Abstract: Novel 3-(4-aroyl-2-pyrrolyl)-*N*-hydroxy-2-propenamides are disclosed as a new class of histone deacetylase (HDAC) inhibitors. Three-dimensional structure-based drug design and conformational analyses into the histone deacetylase-like protein (HDLP) catalytic core suggested the synthesis and biological evaluation of compounds **7a**−**h**. Experimental pK_i values are in good agreement with VALIDATE predicted pK_i values of new derivatives. All compounds **7a**−**h** show HDAC inhibitory activity in the micromolar range, with **7e** as the most potent derivative (IC_{50} = 1.9 μM). The influence of the 4′-substituent in the aroyl moiety is not significant for the inhibitory activity, as all compounds **7a**−**g** show IC_{50} values between 1.9 and 3.9 μM. Otherwise, the unsaturated chain linking the pyrrole ring to the hydroxamic acid group is clearly important for the anti-HDAC activity, the saturated analogue **7h** being 10-fold less active than the unsaturated counterpart **7a**.

Introduction. Acetylation of nuclear histones plays a crucial role in gene expression, since histone hyperacetylation or hypoacetylation has been found to be clearly associated with transcriptionally activated or inactive genes.[1−4] Enzymatic complexes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) have been identified as transcriptional coactivators and transcriptional corepressors, respectively.[5−8]

Recently, a link between oncogene-mediated suppression of transcription and recruitment of HDAC into a nuclear complex has been established.[9−12] On the other hand, compounds acting as HDAC inhibitors such as sodium butyrate **1**,[13] trichostatin A (TSA) **2**,[14] suberoylanilide hydroxamic acid (SAHA) **3**,[15] the cyclic tetrapeptides trapoxin **4**,[16] HC-toxin **5**,[17] and apicidin **6**[18] (Chart 1) and synthetic compounds[19−21] have been reported to induce differentiation of several cancer cell lines and suppress cell proliferation. Moreover, some of them have been shown to have potent antitumor effect in vivo in tumor-bearing animals.[22,23]

Crystal structures of a bacterial HDAC homologue (histone deacetylase-like protein, HDLP) bound to **2** and **3** have been reported.[24] On the basis of the X-ray coordinates of these complexes, the design of new compounds able to bind the deacetylase core could be made by computational procedures.

Previously we described the synthesis and biological evaluation of some 3-(4-aroyl-1-methyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides **7a**−**e** as antifungal, antibacterial, and antiviral agents.[25,26] Compounds **7a**−**e** are characterized by an aroylpyrrole moiety connected to a hydroxamic acid group by an unsaturated chain. These chemical features, resembling some structural elements exhibited by **2** and/or **3**, prompted us to investigate new compounds of general formula **7** as HDAC inhibitors. The present communication reports preliminary data on 3D structure-based drug design, synthesis, biological evaluation, and structure−activity relationships (SARs) of compounds **7a**−**j**.



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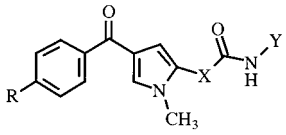

7a-j

7a R = H, X = CH=CH, Y = OH
7b R = Cl, X = CH=CH, Y = OH
7c R = F, X = CH=CH, Y = OH
7d R = NO_2, X = CH=CH, Y = OH
7e R = CH_3, X = CH=CH, Y = OH
7f R = OCH_3, X = CH=CH, Y = OH
7g R = $N(CH_3)_2$, X = CH=CH, Y = OH
7h R = H, X = CH_2-CH_2, Y = OH
7i R = H, X = CH=CH, Y = NH_2
7j R = H, X = CH=CH, Y = CH_2CH_2OH

Three-Dimensional Structure-Based Drug Design and Molecular Modeling Studies. The crystal structure of **2** extracted from the HDLP/**2** complex filed in the Brookhaven Protein Data Bank[27] (entry code 1c3r) was used as a template to build the 3D structures of **7a**−**e**. Following replacement of **2** with the new modeled compounds in the HDLP catalytic core, five new complexes were furnished which were then refined by geometry optimization (MACROMODEL 6.5, all atoms Amber force field)[28−30] and conformational searches (MCMM routine). Figure 1 shows the global minimum obtained from the calculations on the HDLP/**7a** complex. The binding conformation of the ligand shows a "SAHA-like" disposition of the ketone group (Figure 2), different from that shown by its starting conformation modeled directly from **2** ("TSA-like" disposition).

A 3D QSAR model using the VALIDATE paradigm[31−34] was then applied to predict the inhibitory activities (expressed as pK_i values) of compounds **7a**−**e**. A previous VALIDATE model without the HDLP/**2** complex was used, and the pK_i values of **2** and **3** were also recalculated to assess the general applicability of such QSAR methodology to the HDLP coordinates (Table 1).

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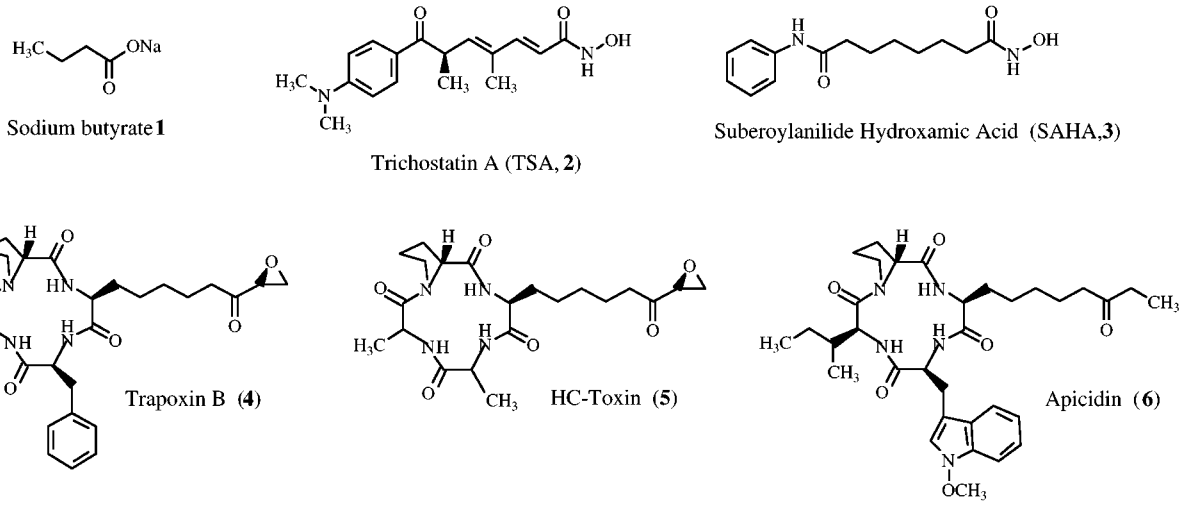
Chart 1. Structures of Known Histone Deacetylase Inhibitors



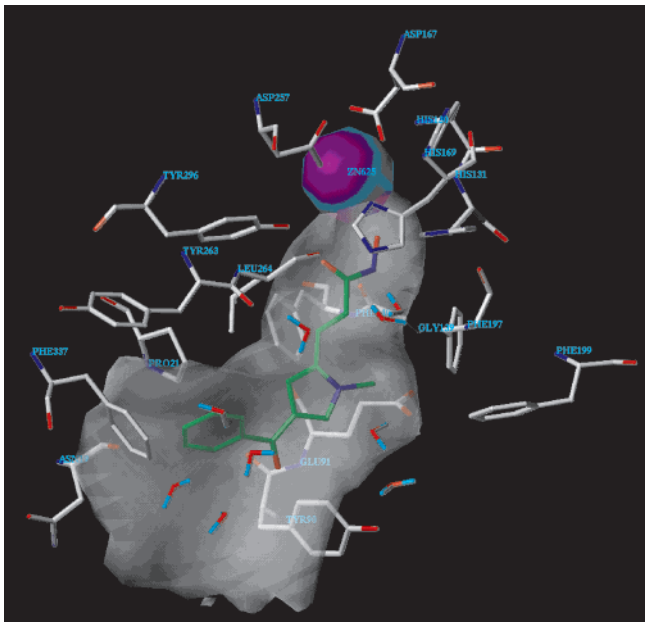


Figure 1. Model of **7a** docked into the HDLP catalytic core.

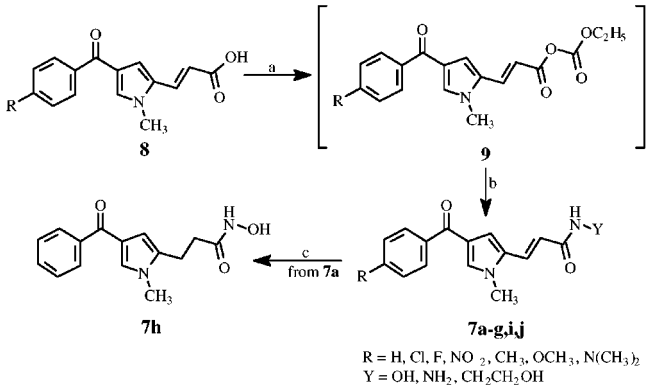
Table 1. VALIDATE[a] Predicted and Experimental Anti-HDAC Activities of **2**, **3**, and **7a**−**h**

				pK_i	
compd	R	X	Y	predicted	exprmt[b]
7a	H	CH=CH	OH	6.52	5.64
7b	Cl	CH=CH	OH	7.06	5.84
7c	F	CH=CH	OH	6.75	5.64
7d	NO_2	CH=CH	OH	6.02	5.63
7e	CH_3	CH=CH	OH	7.02	5.95
7f	OCH_3	CH=CH	OH	6.92	5.76
7g	$N(CH_3)_2$	CH=CH	OH	6.89	5.84
7h	H	CH_2−CH_2	OH	6.62	8.36
2 (TSA)				8.66	8.36
3 (SAHA)				6.27	6.88

[a] A modified procedure with an extended training set of 86 complexes was used. [b] Experimental pK_i values of **7a**−**h** were obtained by the corresponding IC_{50} values (Table 2) in comparison with the pK_i and IC_{50} values of TSA (**2**) (see ref 41).

Since the predicted pK_i values of **7a**−**e** complexed with HDLP were in the low (or sub-) micromolar range, we have designed and modeled into the HDLP catalytic core new aroylpyrrole-*N*-hydroxypropenamides **7f**−**h** exhibiting, when compared to the lead compound **7a**, various substituents in the benzene ring (**7f**,**g**) or a saturated *N*-hydroxypropanamide in the place of the *N*-hydroxy-2-propenamide chain (**7h**). Predicted pK_i data of **7f**−**h** complexed with HDLP, again in the submicromolar range, suggested that we prepare the new derivatives and test them as HDAC inhibitors. Moreover, the hydrazido and 2-hydroxyethylamido analogues of **7a** (**7i** and **7j**, respectively) have been prepared.

Scheme 1[a]



[a] a: ClCOOEt, Et_3N, THF, 0 °C. b: NH_2OH, NH_2NH_2, or $NH_2CH_2CH_2OH$, MeOH, rt. c: H_2, Pd/C, rt.

Chemistry. The synthesis of the new derivatives **7f**−**j** was accomplished by a one-step reaction, under neutral conditions, of 3-(4-aroyl-1-methyl-1*H*-pyrrol-2-yl)-2-propenoic acids **8** with hydroxylamine, hydrazine, and 2-hydroxyethylamine to give the hydroxamides **7a**−**g**, the hydrazide **7i**, and the 2-hydroxyethylamide **7j**, respectively, via ethoxycarbonyl anhydrides **9** (Scheme 1). The saturated hydroxamide **7h** has been prepared by catalytic reduction of **7a** with hydrogen and Pd/C (Scheme 1).

The pyrrolylpropenoic acids **8** were easily prepared by a Wittig−Horner reaction between 4-aroyl-1-methyl-1*H*-pyrrole-2-carboxaldehydes (**10**) and triethyl phosphonoacetate in the presence of potassium carbonate, followed by alkaline hydrolysis of the resulting ethyl pyrroleacrylates (**11**). The aldehydes **10** were obtained by acylating the Vilsmeier−Haack intermediate, formed from 1-methylpyrrole, DMF, and oxalyl chloride, under

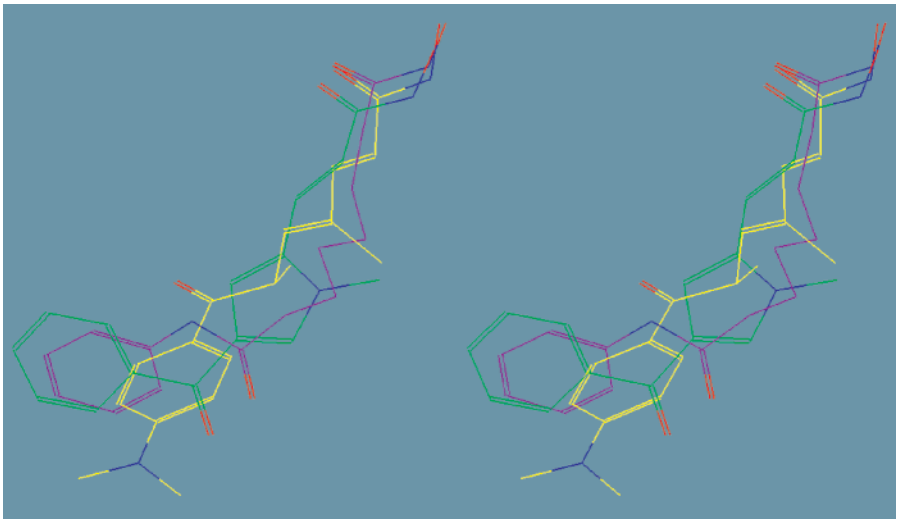
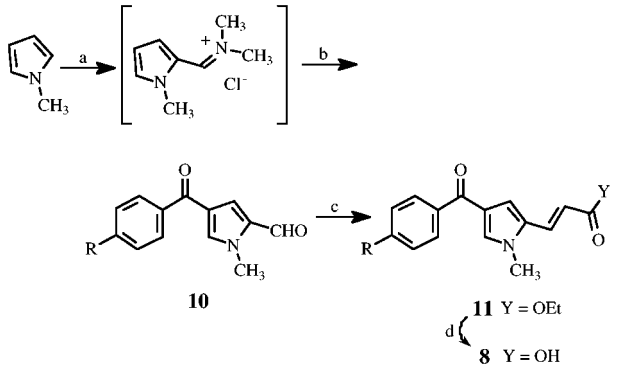


Figure 2. Proposed binding conformation of **7a** (green) overlapped with TSA (**2**, yellow) and SAHA (**3**, purple).

Scheme 2[a]



[a] a: $(COCl)_2$, DMF, dichloroethane, 0 °C. b: (1) R-Ph-COCl, $AlCl_3$, rt; (2) NaOH 50%, rt. c: $(EtO)_2POCH_2COOEt$, K_2CO_3, EtOH, 80 °C. d: KOH, EtOH, H_2O, 70 °C.

normal Friedel–Crafts conditions with the proper aroyl chloride (Scheme 2).[25]

Results and Discussion. The pyrrole derivatives **7a**–**j** have been evaluated for their ability to inhibit HDAC as published previously[35] in comparison with sodium butyrate (**1**), TSA (**2**), SAHA (**3**), trapoxin (**4**), and HC-toxin (**5**) tested as reference drugs.

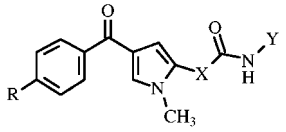
The maize histone deacetylase HD2 was used as the enzyme source.[36] HD2, which was characterized in detail,[37,39] was shown to have an in vitro enzyme activity comparable to those of HDACs from other sources, such as fungi and vertebrates, using our HDAC assay.[35,40]

The results, expressed as percent of inhibition at fixed dose and IC_{50} (50% inhibitory concentration) values, are summarized in Table 2.

The pK_i values of compounds **7a**–**h**, calculated from the corresponding IC_{50} values in comparison with the pK_i/IC_{50} ratio of **2** ($K_i = 0.0043$ μM, $IC_{50} = 0.0072$ μM),[41] are in good agreement with the VALIDATE predicted pK_i values.[42] Inhibitory activities of **7a**–**g** are only 2- to 12-fold weaker than their predicted values (Table 1).

All compounds **7a**–**h** are endowed with HDAC inhibitory activity in the micromolar range, confirming that the hydroxamic acid group is crucial for an anti-HDAC effect.[43] Compound **7e** is the most active derivative (IC_{50} = 1.9 μM), showing a 500-fold higher inhibitory potency than sodium butyrate (**1**). In comparison with

Table 2. Anti-HDAC Activity of Compounds **7a**–**j**



compd	R	X	Y	% inhbtn[b]	$IC_{50} \pm SD$ (μM)
7a	H	CH=CH	OH	86	3.8 ± 0.1
7b	Cl	CH=CH	OH	87	2.4 ± 0.07
7c	F	CH=CH	OH	84	3.8 ± 0.1
7d	NO_2	CH=CH	OH	78	3.9 ± 0.2
7e	CH_3	CH=CH	OH	86.4	1.9 ± 0.06
7f	OCH_3	CH=CH	OH	75.2	2.9 ± 0.06
7g	$N(CH_3)_2$	CH=CH	OH	88	2.4 ± 0.07
7h	H	CH_2–CH_2	OH	45	36.8 ± 1.1
7i	H	CH=CH	NH_2	0.2	
7j	H	CH=CH	CH_2CH_2-OH	0.9	
sodium butyrate (**1**)				35[c]	
TSA (**2**)					0.0072 ± 0.0003
SAHA (**3**)					0.05 ± 0.0015
trapoxin (**4**)					0.01 ± 0.0003
HC-toxin (**5**)					0.11 ± 0.0044

[a] Data represent mean values of at least three separate experiments. [b] Percent of HDAC activity inhibition at 30 μM. [c] Percent of HDAC activity inhibition at 5 mM.

TSA (**2**), SAHA (**3**), trapoxin (**4**), and HC-toxin (**5**), **7e** exibits a 264-, 38-, 190-, and 17-fold lower potency, respectively. The influence of the 4′-substituent in the benzoyl portion is not significant for the biochemical effect, as all compounds **7a**–**g** show IC_{50} values between 1.9 and 3.9 μM. Otherwise, the unsaturated chain linking the pyrrole ring to the hydroxamic acid residue is clearly important for the anti-HDAC activity, the saturated analogue **7h** being 10-fold less active than the unsaturated counterpart **7a**. Replacement of the hydroxamide function with a hydrazide or 2-hydroxyethylamide moiety (compounds **7i** and **7j**, respectively) produces a loss of inhibitory activity.

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Supporting Information Available: Melting points, ^1H NMR spectra, and IR spectra for compounds **7f**–**j** are reported.

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(36) For this purpose, HD2-activity was extensively purified by anion exchange chromatography (Q-Sepharose), affinity chromatography (Heparin-Sepharose, Histone-Agarose), and size exclusion chromatography (Superdex S200) as described elsewhere.[37,38]
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(42) The sole exception is represented by **7h**, which shows an experimental pK_i value lower by up to 2 orders of magnitude compared to that predicted by the VALIDATE approach.
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