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

Silvio Massa,* Antonello Mai,*† Gianluca Sbardella,‡ Monica Esposito,‡ Rino Ragno,§ Peter Loidl,¶ and Gerald Brosch,*†

Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, via Aldo Moro, 53100 Siena, Italy, Dipartimento di Studi Farmaceutici, Università degli Studi di Roma “La Sapienza”, P.le A. Moro 5, 00185 Roma, Italy, Dipartimento di Studi Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università degli Studi di Roma “La Sapienza”, P.le A. Moro 5, 00185 Roma, Italy, and Department of Microbiology, University of Innsbruck, Medical School, Fritz-Pregl-Strasse 3, 6020 Innsbruck, Austria

Received March 9, 2001

Abstract: Novel 3-(4-aryloyl-2-pyrrolidinyl)-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 pKᵢ values are in good agreement with VALIDATE predicted pKᵢ values of new derivatives. All compounds 7a–h show HDAC inhibitory activity in the micromolar range, with 7e as the most potent derivative (IC₅₀ = 1.9 μM). The influence of the 4'-substituent in the aryl moiety is not significant for the inhibitory activity, as all compounds 7a–g show IC₅₀ 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 hypoxacetylation has been found to be clearly associated with transcriptionally activated or hypoacetylated 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 (13), trichostatin A (TSA) (14), suberyl-anilide hydroxamic acid (SAHA) (3), the cyclic tetrapeptides trapoxin (4), HC-toxin (5,7), and apicidin (6) 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-aryloyl-1-methyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides 7a–e as antifungal, antibacterial, and antiviral agents (25,26). Compounds 7a–e are characterized by an arylpyrrole 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.

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 (entry code 1cr) 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) and conformational searches (MCCMM 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 was then applied to predict the inhibitory activities (expressed as pKi values) of compounds 7a–e. A previous VALIDATE model without the HDLP/2 complex was used, and the pKi values of 2 and 3 were also recalculated to assess the general applicability of such QSAR methodology to the HDLP coordinates (Table 1).
Since the predicted $pK_i$ values of 7a-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-hydroxypropenamide 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.

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-1H-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-1H-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...
normal Friedel–Crafts conditions with the proper aryl chloride (Scheme 2).

Results and Discussion. The pyrrole derivatives 7a–j have been evaluated for their ability to inhibit HDAC as published previously 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. HD2, which was characterized in detail, 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.

The results, expressed as percent of inhibition at fixed dose and IC50 (50% inhibitory concentration) values, are summarized in Table 2.

The pKi values of compounds 7a–h, calculated from the corresponding IC50 values in comparison with the pKi/IC50 ratio of 2 (K1 = 0.0043 μM, IC50 = 0.0072 μM), are in good agreement with the VALIDATE predicted pKi values. 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. Compound 7e is the most active derivative (IC50 = 1.9 μM), showing a 500-fold higher inhibitory potency than sodium butyrate (1). In comparison with

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>X</th>
<th>Y</th>
<th>% inhib^b</th>
<th>IC50 ± SD (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>H</td>
<td>CH=</td>
<td>OH</td>
<td>86</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>7b</td>
<td>Cl</td>
<td>CH=</td>
<td>OH</td>
<td>87</td>
<td>2.4 ± 0.07</td>
</tr>
<tr>
<td>7c</td>
<td>F</td>
<td>CH=</td>
<td>OH</td>
<td>84</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>7d</td>
<td>NO2</td>
<td>CH=</td>
<td>OH</td>
<td>78</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>7e</td>
<td>CH3</td>
<td>CH=</td>
<td>OH</td>
<td>86.4</td>
<td>1.9 ± 0.06</td>
</tr>
<tr>
<td>7f</td>
<td>OCH3</td>
<td>CH=</td>
<td>OH</td>
<td>75.2</td>
<td>2.9 ± 0.06</td>
</tr>
<tr>
<td>7g</td>
<td>N(CH3)2</td>
<td>CH=</td>
<td>OH</td>
<td>88</td>
<td>2.4 ± 0.07</td>
</tr>
<tr>
<td>7h</td>
<td>H</td>
<td>CH2=</td>
<td>CH2</td>
<td>OH</td>
<td>45</td>
</tr>
<tr>
<td>7i</td>
<td>H</td>
<td>CH=</td>
<td>NH2</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>7j</td>
<td>H</td>
<td>CH=</td>
<td>CH2CH2-</td>
<td>OH</td>
<td>0.9</td>
</tr>
</tbody>
</table>

sodium butyrate (1) 35
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

Data represent mean values of at least three separate experiments. ^Percent of HDAC activity inhibition at 30 μM. °Percent of HDAC activity inhibition at 5 mM.

TSA (2), SAHA (3), trapoxin (4), and HC-toxin (5), 7e exhibits 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 IC50 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 hydroxamic acid residue by a hydrazide or 2-hydroxyethylamidine moiety (compounds 7i and 7j, respectively) produces a loss of inhibitory activity.

Acknowledgment. The authors are grateful to Prof. Marino Artico for the helpful critical discussion. This work was supported in part by the Austrian Academy of Sciences (to G.B.).

Supporting Information Available: Melting points, 1H NMR spectra, and IR spectra for compounds 7f–j are reported.
This material is available free of charge via the Internet at http://pubs.acs.org.

References


