Discovery of (Aryloxopropenyl)pyrrolyl Hydroxyamides as Selective Inhibitors of Class IIa Histone Deacetylase Homologue HD1-A

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Received August 4, 2003

Abstract: Chemical manipulations performed on aroyl pyrrolyl hydroxyamides, a new class of HDAC inhibitors previously reported by us, led to (aryloxopropenyl)pyrrolyl hydroxyamides **3a**−**g**. Such compounds, showing better inhibitory activity against maize HD1-A than HD1-B (two homologues of mammalian class IIa and I HDACs, respectively), are the first class of IIa-selective inhibitors (fold selectivity: 7−78). They could be useful as tools for probing the biology of these enzymes and eventually as new anticancer agents with low toxicity.

Introduction. Histone deacetylases (HDACs) are the catalytic subunits of multiprotein complexes responsible for deacetylation of histone and nonhistone proteins. Lysine acetylation, i.e., the transfer of an acetyl moiety from acetyl-coenzyme A to the ϵ-amino group of a specific lysine residue, has emerged as the major form of posttranslational modification of histones and other proteins and has been correlated with transcription, chromatin assembly, DNA repair, and recombinational events.[1−6] Histone acetylation in vivo is a dynamic, reversible process governed by the opposite actions of histone acetyltransferases (HATs) and HDACs. Aberrant acetylation of histone tails, emerging from either HAT mutations or abnormal recruitment of HDACs, has been clearly linked to carcinogenesis.[7−9] Three families of deacetylases have been identified in eukaryotes so far: class I, class II, and class III HDACs, according to their homology with *Saccharomyces cerevisiae* histone deacetylase RPD3 (HDAC1−3, 8, 11, class I), HDA1 (HDAC4−7, 9, 10, class II), or Sir2 (SIRT1−7 (sirtuins), class III).[10]

Another deacetylase, maize HD2,[11] is structurally quite different from mammalian HDACs and has been attributed to a class of its own. Nevertheless, maize HD2 has shown to be a good predictive model for the behavior of class I mammalian HDACs with various series of HDAC inhibitors.[12−16] On the basis of sequence homology among their deacetylase domains, class II can be divided into two subclasses, namely IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and IIb (HDAC6, containing as unique feature two homologous deacetylase domains, and HDAC10, more similar to HDAC6 than to HDAC4−7).[17,18]

Class II HDACs differ from class I HDACs depending on their tissue expression, subcellular localization, and biological roles. Class I HDACs are ubiquitously expressed, whereas class II enzymes display tissue-specific expression in humans and mice (i.e., human HDAC4 is the most abundant in skeletal muscle, modest in brain, heart, and ovary, but not detectable in liver, lung, spleen, and placenta, and HDAC5 is expressed in mouse heart, brain, liver, and skeletal muscle, but not in spleen).[17,18]

In contrast to class I HDACs which are mainly nuclear enzymes, under different cellular conditions class II HDACs localize either to the cell nucleus or to the cytoplasm, depending on their phosphorylation extent and subsequent binding of 14−3−3 proteins. For example, HDAC4 is actively shuttled between the nucleus and the cytoplasm in vivo, and phosphorylation and overexpression of 14−3−3 proteins promote cytoplasmic accumulation of HDAC4.[18]

Class I HDACs are well-known transcriptional corepressors. Emerging evidences indicates that class IIa members play similar roles.

Particularly, class IIa HDACs interact with one or more DNA-binding transcription factors including MEF2, BCL6, PLZF, and TR2; with transcriptional corepressors such as N-CoR, SMRT, BCoR, and CtBP; and with the methyllysine-binding protein HP1.[17,18,20] Among these interactions, the most studied is that with MEF2. Class IIa HDACs inhibit MEF2-dependent transcription in reporter gene assay and regulate myogenesis in muscle differentiation models in vitro.[17,18] Moreover, in HDAC9-lacking mice the development of age-dependent cardiac hypertrophy has been observed, strongly implicating class IIa members as important regulators of myogenesis.[21]

Several classes of compounds able to inhibit HDACs and to cause transformed cells growth arrest, differentiation, and/or apoptosis have been identified (illustrative HDAC inhibitors structures are drawn in Chart 1).[22] Several HDAC inhibitors are in clinical trials for the treatment of both solid tumors and hematological malignances.[23−27]

There is evidence for differences in sensitivity of different members of class I and class II HDACs to different inhibitors, but no compound strongly selective for one or another HDACs has been reported yet. Few HDAC inhibitors are known to be selective for class I HDACs (Table 1). In this year a small molecule, tubacin, which has been discovered through a multidimensional, chemical genetic screen of 7392 molecules, has been described as specific α-tubulin deacetylation inhibitor in mammalian cells (Table 1).[28−31] Between the two HDAC6 catalytic domains, tubacin selectively binds only that with tubulin deacetylase activity, without affecting histone acetylation, gene expression, or cell-cycle progression.[31] No references are reported about class IIa HDACs selective inhibitors. Nevertheless, class IIa-specific inhibitors may have great therapeutic potential

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10.1021/jm034167p CCC: $25.00
Published on Web 10/15/2003

Chart 1. Structures of Known Histone Deacetylase Inhibitors

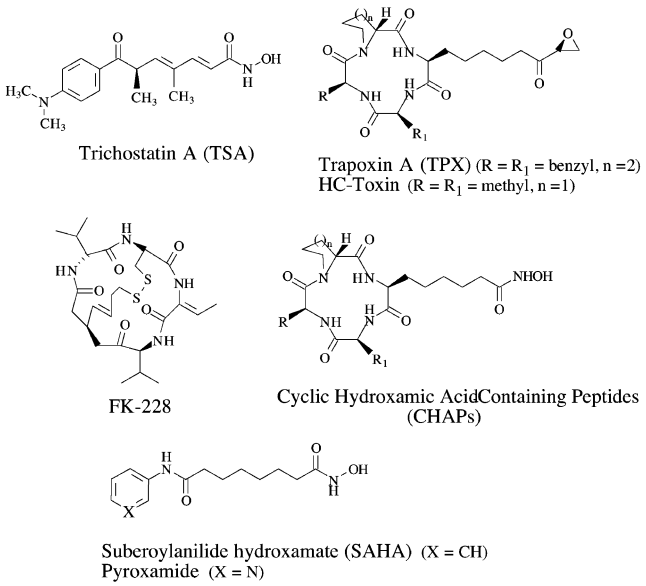
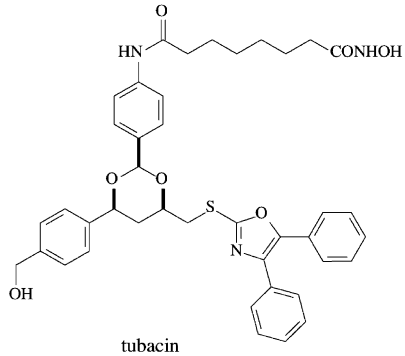


Table 1. Literature-Reported Selective HDAC Inhibitors



	IC_{50}[a] (nM)/EC_{50}[b] (μM)			
compd	HDAC1/ *Ac-histone*[b]	HDAC4	HDAC6/ *Ac-tubulin*[b]	ref
TSA	6.0 ± 2.5	38 ± 4	8.6 ± 1.4	22e
TPX A	0.82 ± 0.29		524 ± 240	22e
TPX B	0.11 ± 0.01	0.30 ± 0.03	360 ± 160	22e
CHAP1	1.9 ± 0.5	2.7 ± 1.3	19 ± 3	22e
FK-228	36 ± 16	510 ± 340	14000 ± 3100	32
tubacin	*217 ± 96*		*2.9 ± 0.9*	29

[a] Enzyme inhibiting assays. [b] Induction of acetylated histone and acetylated tubulin in mammalian cells.

as anticancer agents, being more discriminating and less toxic than no selective HDAC inhibitors. Moreover, they would be powerful tools for probing the biology of class IIa enzymes.

Recently, we reported a novel series of hydroxamate compounds as a new class of synthetic HDAC inhibitors, namely 3-(4-aroyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides (APHAs).[12−14] Chemical modifications performed on 3-(4-benzoyl-1-methyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamide (**1**) (Figure 1) led us to obtain a novel lead, *N*-hydroxy-3-(1-methyl-4-(phenylacetyl)-1*H*-pyrrol-2-yl)-2-propenamide **2** (Figure 1, route A), which was 38-fold more potent than **1** in inhibiting maize HD2 activity.[14]

Pursuing our researches in the anti-HDAC field, we performed further chemical manipulations on **1**, particularly by inserting an hydrophobic spacer (i.e., an ethylene link) between the C_4-pyrrole position and the carbonyl group of the benzoyl moiety (compounds **3a**−**g**, Figure 1, route B).

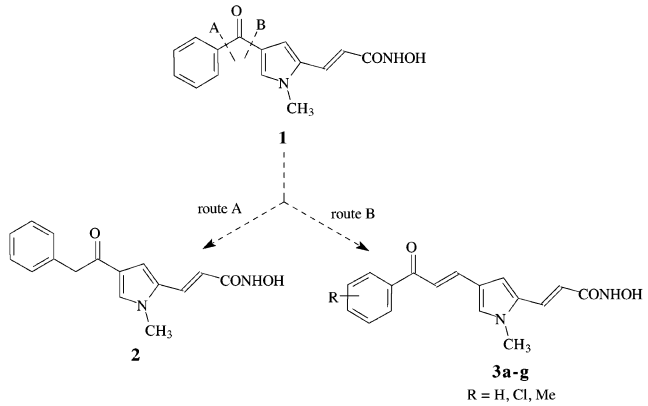
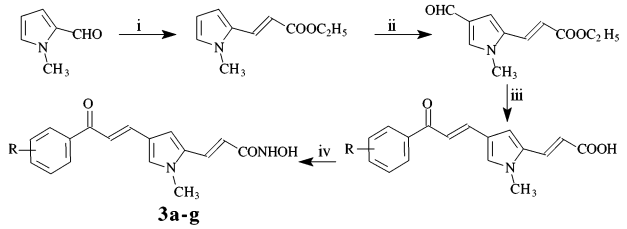


Figure 1. Aroyl pyrrolyl hydroxyamides (APHAs): Two different routes to optimize the lead compound.

Scheme 1[a]



[a] i, $(C_2H_5O)_2OPCH_2COOC_2H_5$ K_2CO_3, C_2H_5OH, 80 °C; ii, $(COCl)_2$, DMF, dichloroethane, rt; iii, (un)substituted acetophenone, KOH, C_2H_5OH, H_2O, rt; iv, (1) $ClCOOC_2H_5$, $(C_2H_5)_3N$, THF, 0 °C; (2) NH_2OH.

Chemistry. The synthesis of derivatives **3a**−**g** is illustrated in Scheme 1. Formylation reaction performed on ethyl 1-methyl-1*H*-pyrrole-2-propenoate according to the Vilsmeier−Haack procedure yielded the ethyl 4-formyl-1-methyl-1*H*-pyrrole-2-propenoate as the sole product, the reaction being regioselective at the C_4 position of pyrrole. Condensation of such an intermediate with the appropriate acetophenones in alkaline medium afforded the 3-[4-(3-aryl-3-oxopropen-1-yl)-1-methyl-1*H*-2-pyrrolyl]-2-propenoic acids, which were in turn converted into the corresponding *N*-hydroxyamides with ethyl chloroformate and hydroxylamine.

Results and Discussion. The pyrrole derivatives **3a**−**g** were evaluated for their ability to inhibit HDAC activity using maize HD2.[33−37] The majority of test compounds exhibited inhibiting activity against such an enzyme at micromolar concentrations (**3b**−**d**,**f**,**g**; IC_{50} values: 5.2−25.2), with **3a**,**e** being active at 0.27 μM (Table 2).

Thus, we tested **3a**−**g** against the maize histone deacetylase HD1-B (homologue of mammalian class I HDACs)[36] and HD1-A (homologue of mammalian class IIa HDACs),[36] with the aim to explore the effect of title derivatives on class I and class IIa homologous enzymes. Additionally, TSA and SAHA were tested as reference drugs.

The results, expressed as IC_{50} (50% inhibitory concentration) values, are reported in Table 3. The unsubstituted compound **3a** showed good inhibiting activity against both HD1-B and HD1-A, lacking in class selectivity. Importantly, the insertion of a chlorine atom

Table 2. Maize HD2 Inhibitory Activities of **3a**−**g**[a]

compd	R	IC_{50}, μM
3a	H	0.28 ± 0.01
3b	2-Cl	5.2 ± 0.2
3c	3-Cl	24.2 ± 1.2
3d	4-Cl	25.2 ± 1.3
3e	2-Me	0.27 ± 0.01
3f	3-Me	7.9 ± 0.24
3g	4-Me	10.2 ± 0.41
TSA[b]		7.2 ± 0.3[b]
SAHA		0.05 ± 0.001

[a] Data represent mean values of at least three separate experiments. [b] Data in nM concentrations.

Table 3. HD1-B and HD1-A Inhibitory Activities of **3a**−**g**[a]

		IC_{50}, μM		fold selectivity	
compd	R	HD1-B	HD1-A	class I	class IIa
3a	H	0.3 ± 0.02	0.2 ± 0.01		
3b	2-Cl	1.6 ± 0.08	0.05 ± 0.003		32
3c	3-Cl	31.4 ± 1.3	0.4 ± 0.02		78
3d	4-Cl	29.4 ± 1.2	18.6 ± 1.1		
3e	2-Me	0.4 ± 0.02	0.06 ± 0.002		7
3f	3-Me	2.5 ± 0.07	0.2 ± 0.007		12
3g	4-Me	4.8 ± 0.3	0.5 ± 0.02		10
TSA[b]		0.4 ± 0.01[b]	0.8 ± 0.03[b]	2	
SAHA		0.03 ± 0.001	0.2 ± 0.009	7	

[a] Data represent mean values of at least three separate experiments. [b] Data in nM concentrations.

or a methyl group at each position of the benzene ring caused a dramatic change in the inhibiting effect of pyrrole derivatives on the two deacetylases. Particularly, 2-substituted compounds **3b**,**e** were the most potent against both the enzymes, being the anti-HD1-A activities higher than those against HD1-B. Thus, **3b**,**e** showed a class IIa selectivity ($IC_{50}^{HD1-B}/IC_{50}^{HD1-A}$ ratio) of 32 and 7, respectively (Table 3). Introduction of the chlorine atom (or the methyl group) at the 3-position of the benzene ring afforded pyrrole derivatives (**3c**,**f**) which were inactive (**3c**) or low active (**3f**) against HD1-B, but retained inhibitory activity against HD1-A. Such compounds were the most class IIa selective among the two (Cl and Me) series, with values of fold selectivity of 78 (**3c**) or 12 (**3f**) (Table 3). Benzene C_4-chlorine substitution (compound **3d**) abated both the anti-HD1-B and HD1-A activities of pyrrole derivatives, while the C_4-methyl compound **3g** was less active than its 2- and 3-substituted counterparts **3e** and **3f** against both deacetylases, still retaining a submicromolar IC_{50} value against class IIa homologue HD1-A (Table 3).

Further synthetic and molecular modeling studies are in progress to optimize both the potency and class IIa selectivity of such 3-[4-(3-aryl-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-*N*-hydroxy-2-propenamides.

Acknowledgment. This work was supported by grants of "Progetto Finalizzato Ministero della Salute 2002" (A.M.), "AIRC proposal 2003" (A.M.), and the Austrian Science Foundation P13209 (G.B.) and P13620 (P.L.).

Supporting Information Available: Experimental procedures and chemical and physical data for compounds **3a**−**g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM034167P