Synthesis and Biological Evaluation of 2-, 3-, and 4-Acylaminocinnamyl-*N*-hydroxyamides as Novel Synthetic HDAC Inhibitors

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Abstract: A new series of 2-, 3-, and 4-acylaminocinnamyl-*N*-hydroxyamides **1-3** have been prepared, and their anti-HDAC (against maize HD2, HD1-B, and HD1-A enzymes) activities have been assessed. Cinnamyl-hydroxyamides bearing acylamino substituents at the C2 position of the benzene ring (compounds **1a-g**) showed very low HDAC inhibiting activities, with IC_{50} values in the high micromolar range. By shifting the same acylamino groups from C2 to C3 (compounds **2a-g**) as well as C4 (compounds **3a-f**) position of the benzene ring, a number of highly potent HDAC inhibitors have been obtained.

In the anti-HD2 assay **3c** (IC_{50} = 11 nM) was the most potent compound, being >11600-, 4.5-, and 10-fold more potent than sodium valproate, SAHA, and HC-toxin, respectively, and showing the same activity as trapoxin. HD1-B and HD1-A assays have been performed to screen the inhibitory action of **1-3** against mammalian class I (HD1-B) and class II (HD1-A) HDAC homologous enzymes. From the corresponding IC_{50} data, a selectivity ratio has been calculated. In general, compounds **1-3** showed no or little selectivity towards the class II homologue HD1-A, the most selective being **2a** with class II selectivity ratio = 4.3. About the inhibitory potency, the 4-(2-naphthoylamino)cinnamyl-*N*-hydroxyamide **3f** showed the highest inhibiting effect against the two enzymes ($IC_{50\text{-HD1-B}}$ = 36 nM; $IC_{50\text{-HD1-A}}$ = 42 nM).

Selected **2** and **3** compounds will be evaluated to determine their antiproliferative and cyto differentiating activities on HL-60 cells.

Key Words: Hydroxamic acid, histone deacetylase, chromatin remodelling, cinnamyl-hydroxyamide.

INTRODUCTION

Aberrant gene silencing of tumour suppressor genes is one of the key mechanisms involved in oncogenesis. Activation or repression of gene expression is established by recruitment to the chromatin structure of some enzymes, such as histone methyltransferases (HMTs), histone acetyl-transferases (HATs) and histone deacetylases (HDACs), that are able to modify the nucleosomal histone proteins [1] by covalent modifications. When HAT is bound to the chromatin, it locally acetylates the positively charged lysine residues in the N-terminal tails of the nuclear histones, resulting in a more open, transcriptionally active chromatin structure (euchromatin). The acetylation status of histones is a dynamic equilibrium: HATs acetylate whereas HDACs are responsible for the deacetylation of histone tails, resulting in a transcriptionally repressed chromatin state (hetero-chromatin). HDACs exist in large multi-protein complexes in the cells (*i.e.* Sin3, Mi-2/NuRD and Co-REST complexes) [2,3], and are classified into three classes. Class I (HDAC1-3,8,11) and II (HDAC4-7,9,10) HDACs remove the acetyl groups from lysine residues with a zinc ion-dependent mechanism, whereas class III HDACs comprises the sirtuins (SIRT1-7) and catalyse the deacetylation reaction through NAD^+ as a co-factor [4-6].

It is now well-documented that the aberrant transcription of genes regulating cellular differentiation, cell cycle, and apoptosis is due to altered expression or mutation of genes encoding HATs or HDACs. Surprisingly, only a small percentage (~2%) of mRNA transcripts are modulated by HDAC inhibitors, thus suggesting that genes regulated by HDAC inhibitors could exert a pleiotropic effect on key pathways involved in proliferation, apoptosis, tumour suppressors, DNA synthesis and repair, and protein turnover [7-11].

In the last ten years, a number of both natural and synthetic class I/II HDAC inhibitors have been reported as useful tools not only for the study of function of chromatin acetylation/deacetylation and gene expression, but also for the treatment of diverse forms of tumours. Growth inhibitory effects have been reported in virtually all transformed cell types, including cell lines arising from both haematological (leukaemias, lymphomas, and myelomas) and epithelial (such as breast, bladder, ovarian, prostate, and lung) tumours [12-15].

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The X-ray crystal structure of the catalytic core of an archaebacterial HDAC homologue (histone deacetylase-like protein, HDLP), reported in 1999 by Finnin *et al*. [16], revealed the mode by which the hydroxamic acid-based HDAC inhibitors trichostatin A (TSA) [17] and suberoylanilide hydroxamic acid (SAHA) [18] (Fig. (**1**)) bind to the pocket of the catalytic site of the enzyme (Fig. (**2**)). TSA and SAHA bind the deacetylase core by inserting their aliphatic chains into the HDLP pocket and by making multiple contacts to its tube-like hydrophobic portion. Particularly, their hydroxamic acid group reaches the polar bottom of the pocket, where it coordinates the zinc ion in a bidentate fashion (through CO and OH groups) and also contacts active-site residues (forming two hydrogen-bonds between its NH and OH groups and the two charge-relay systems His131/Asp166 and His132/Asp173, and another one between its CO and the Tyr297 hydroxyl group). Moreover, the hydroxamate function replaces the zinc-bound water molecule of the active structure with its OH group.

Starting from the X-ray crystallographic findings and the structure-activity relationship (SAR) of the various HDAC inhibitor classes reported to date [19], the structural features of known HDAC inhibitors can be summarised as depicted in Fig. (**3**). This pharmacophoric model consists in a cap (CAP) group, able to interact with the rim space at the entrance of the catalytic tunnel of the enzyme, linked to a hydrophobic spacer (HS) through a polar connecting unit (CU). At the end of the hydrophobic spacer, an enzyme-inhibiting group (EIG) assures the inhibition of the enzyme activity mainly by chelating the zinc ion near the bottom of the catalytic pocket [19,20].

The CAP is generally an extremely variable hydrophobic group, from a simple benzene ring to a more complex cyclic tetrapeptide; the CU is often a ketone, or amide, or sulfonamide group, the HS is comprised of linear or cyclic structures, either saturated or unsaturated, and to date, the most represented EIG is the hydroxamate function.

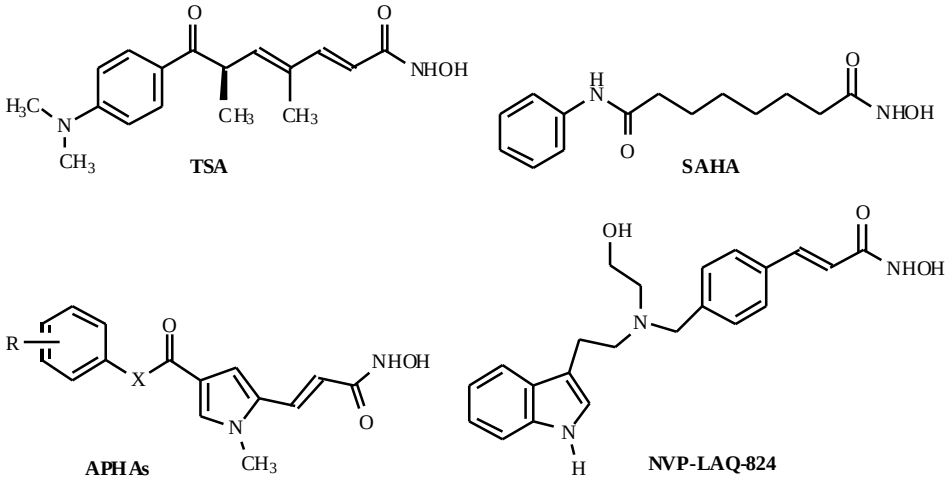


Fig. (1). Known HDAC inhibitors.

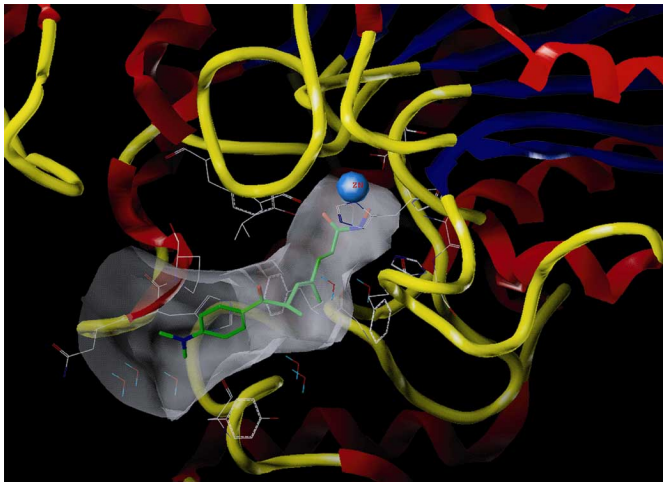


Fig. (2). Binding site structure of TSA co-crystallized into the HDLP catalytic pocket.

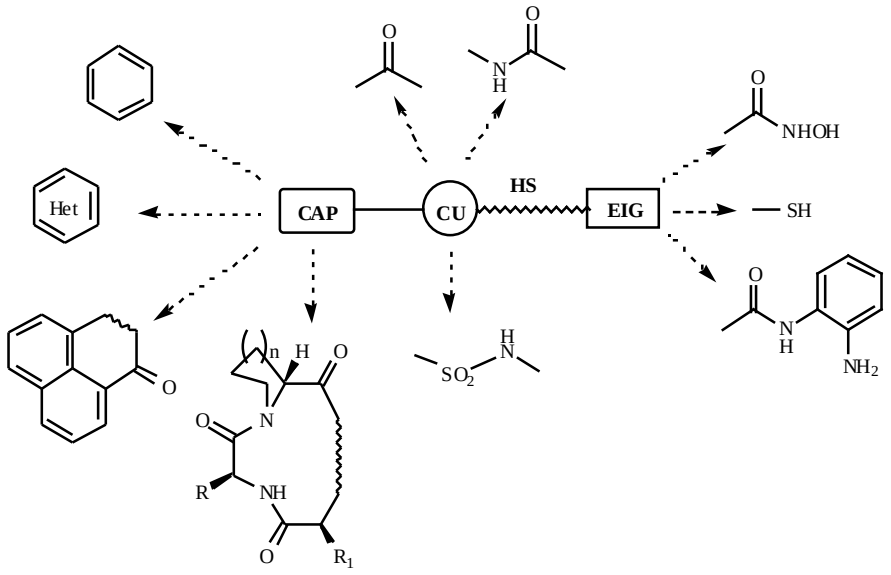


Fig. (3). Pharmacophore model for HDAC inhibition.

TSA and SAHA are two examples of compounds having a linear HS. As samples of HDAC inhibitors with a carbo/heterocyclic HS, we recently reported a number of aroyl-pyrrolyl-hydroxyamides (APHAs) endowed with submicromolar HDAC inhibiting activity [21-26] (Fig. (**1**)), and various cinnamyl-hydroxyamide containing compounds have been described by others [27-31]. Among them, NVP-LAQ-824 (Fig. (**1**)) is currently in phase I clinical trial as antitumor agent [32].

Pursuing our searches on HDAC inhibitors with a carbo/heterocycle as HS [21-26], we synthesised a new class of compounds having a cinnamyl-hydroxamate (HS plus EIG) function linked to 2-, 3-, or 4-acylamino moiety (CAP plus CU) (compounds **1-3**, Fig. (**4**)), and the new derivatives were tested against three maize enzymes with deacetylase activity, *i.e.* HD2 [33,34], HD1-B (homologue of mammalian class I HDACs) [35,36], and HD1-A (homologue of mammalian class II HDACs) [37,38].

CHEMISTRY

Ethyl 2-, 3-, and 4-aminocinnamates **4-6** [39-41] were treated with the appropriate acyl chloride in the presence of triethylamine to afford the amido-esters **7a-g**, **8a-g**, and **9a-g**, which were in turn hydrolysed in alkaline medium to the corresponding carboxylic acids **10a-g**, **11a-g**, and **12a-f**. Strangely, the hydrolysis under basic conditions (NaOH, KOH, LiOH) of the ethyl 4-(4-biphenylcarbonylamino) cinnamate **9g**, failed to give the corresponding cinnamic acid. Further conversion of **10-12** into the desired hydroxamates **1-3** has been accomplished by a one-pot, three step procedure involving (i) the formation of mixed anhydrides between **10-12** and ethyl chloroformate in the presence of triethylamine, (ii) the reaction of such activated anhydrides with *O*-(2-methoxy-2-propyl)hydroxylamine [42], and (iii) acidic hydrolysis of the *O*-(2-methoxy-2-propyl) hydroxamates with the Amberlyst® 15 ion-exchange resin (Scheme **1**). All compounds were purified by crystallisation.

Chemical and physical data of compounds **1-3** are listed in Table **1**. Chemical and physical data of the intermediate compounds **7-12** are reported in Table **2**.

RESULTS AND DISCUSSION

Compounds **1-3** have been evaluated for their inhibiting activities against three maize HDAC enzymes, namely HD2 [33,34], HD1-B [35,36], and HD1-A [37,38]. HD2 does not present any homology with mammalian class I/II HDAC enzymes, but its behaviour against HDAC inhibitors is similar to that of class I enzymes [22,23,26,43]. HD1-B and

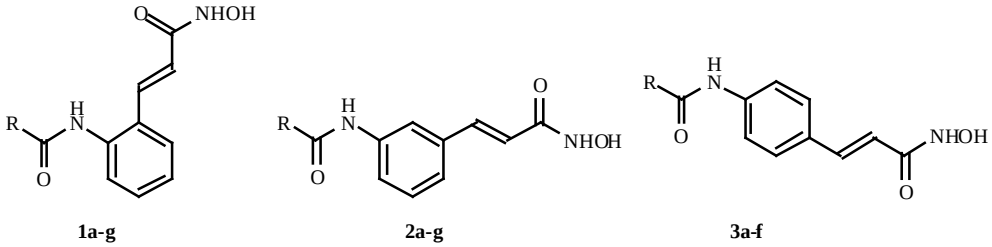
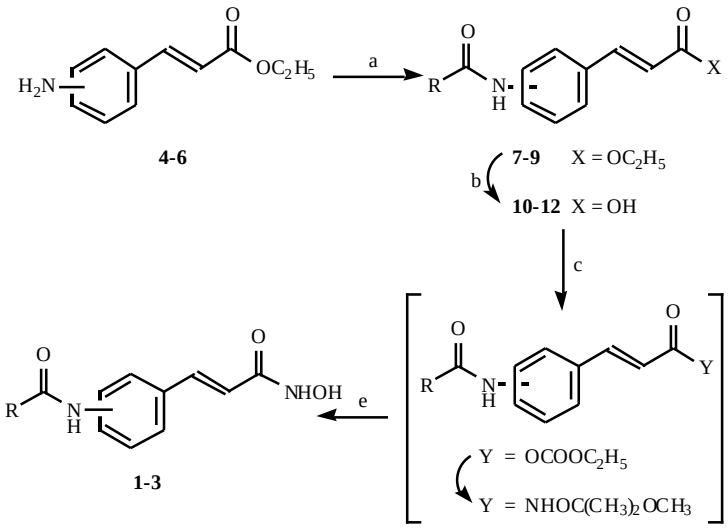


Fig. (4). 2-Acylaminocinnamyl-*N*-hydroxyamides **1**, 3-acylaminocinnamyl-*N*-hydroxyamides **2**, and 4-acylaminocinnamyl-*N*-hydroxyamides



[a](a) RCOCl, $(C_2H_5)_3N$, CH_2Cl_2, room tep. (b) LiOH, H_2O, room tem. (c) $ClCOOC_2H_5$, $(C_2H_5)_3N$, THF, 0 °C. (d) $NH_2OC(CH_3)_2OCH_3$, room temp. (e) Amberlyst 15, CH_3OH, room temp.

Scheme 1.

Table 1. Chemical and Physical Properties of Compounds 1-3

compd	R	mp, °C	recrystall. solvent	yield, %
1a	Ph	170-172	benzene	53
1b	$PhCH_2$	158-160	benzene	55
1c	$PhCH_2CH_2$	150-151	benzene	54
1d	PhCH=CH	128-130	benzene	47
1e	1-naphthyl	195-196	MeOH	65
1f	2-naphthyl	128-130	benzene	67
1g	4-biphenyl	124-125	benzene	48
2a	Ph	213-214	THF	51
2b	$PhCH_2$	183-184	THF	50
2c	$PhCH_2CH_2$	179-180	THF	56
2d	PhCH=CH	86-87	Et_2O	45
2e	1-naphthyl	191-192	MeOH	61
2f	2-naphthyl	182-183	MeOH	59
2g	4-biphenyl	213-214	MeOH	53
3a	Ph	212-213	THF	54
3b	$PhCH_2$	198-199	THF	55
3c	$PhCH_2CH_2$	204-205	THF	51
3d	PhCH=CH	218-220	THF	49
3e	1-naphthyl	210-211	MeOH	63
3f	2-naphthyl	223-224	MeOH	58

Table 2. Chemical and Physical Properties of Compounds 7-12

compd	R	X	mp, °C	recrystall. solvent	yield, %
7a	Ph	$COOC_2H_5$	137-139	toluene	73
7b	$PhCH_2$	$COOC_2H_5$	123-124	toluene	58
7c	$PhCH_2CH_2$	$COOC_2H_5$	119-120	toluene	72
7d	PhCH=CH	$COOC_2H_5$	114-116	toluene	60
7e	1-naphthyl	$COOC_2H_5$	182-183	toluene	75
7f	2-naphthyl	$COOC_2H_5$	168-169	toluene	78
7g	4-biphenyl	$COOC_2H_5$	173-174	toluene	71
8a	Ph	$COOC_2H_5$	110-111	CH_2Cl_2/*n*-hexane	75
8b	$PhCH_2$	$COOC_2H_5$	97-98	CH_2Cl_2/*n*-hexane	83
8c	$PhCH_2CH_2$	$COOC_2H_5$	90-92	CH_2Cl_2/*n*-hexane	80
8d	PhCH=CH	$COOC_2H_5$	94-95	CH_2Cl_2/*n*-hexane	68
8e	1-naphthyl	$COOC_2H_5$	155-156	toluene	81
8f	2-naphthyl	$COOC_2H_5$	115-116	toluene	83
8g	4-biphenyl	$COOC_2H_5$	174-175	toluene	69
9a	Ph	$COOC_2H_5$	144-146	CH_2Cl_2/*n*-hexane	71
9b	$PhCH_2$	$COOC_2H_5$	158-160	CH_2Cl_2/*n*-hexane	78
9c	$PhCH_2CH_2$	$COOC_2H_5$	124-126	CH_2Cl_2/*n*-hexane	77
9d	PhCH=CH	$COOC_2H_5$	153-155	CH_2Cl_2/*n*-hexane	68
9e	1-naphthyl	$COOC_2H_5$	169-170	toluene	80
9f	2-naphthyl	$COOC_2H_5$	161-162	toluene	85
9g	4-biphenyl	$COOC_2H_5$	246-248	toluene	78
10a	Ph	COOH	185-186	MeOH	75
10b	$PhCH_2$	COOH	207-208	MeOH	79
10c	$PhCH_2CH_2$	COOH	235-236	MeOH	78
10d	PhCH=CH	COOH	265-266	MeOH	65
10e	1-naphthyl	COOH	166-168	EtOH	83
10f	2-naphthyl	COOH	146-147	EtOH	79
10g	4-biphenyl	COOH	270-271	MeOH	75
11a	Ph	COOH	230-231	MeOH	74
11b	$PhCH_2$	COOH	207-209	MeOH	80
11c	$PhCH_2CH_2$	COOH	196-198	MeOH	81
11d	PhCH=CH	COOH	>280	MeOH	71
11e	1-naphthyl	COOH	>280	EtOH	87
11f	2-naphthyl	COOH	236-237	EtOH	77
11g	4-biphenyl	COOH	>280	MeOH	74

(Table 2. Contd….)

compd	R	X	mp, °C	recrystall. solvent	yield, %
12a	Ph	COOH	252-254	MeOH	78
12b	$PhCH_2$	COOH	>280	MeOH	83
12c	$PhCH_2CH_2$	COOH	>280	MeOH	76
12d	PhCH=CH	COOH	260-262	MeOH	67
12e	1-naphthyl	COOH	248-249	MeOH	91
12f	2-naphthyl	COOH	>280	MeOH	84

HD1-A are two maize enzymes homologue of mammalian class I and class II HDACs, respectively [35-38]. Class I HDACs are well-known transcriptional co-repressors, acting through the block of the expression of some tumor suppressor genes [44]. Class II HDACs have been reported to interact with one or more DNA-binding transcription factors, as well as with transcriptional co-repressors, such as MEF2 [45]. The development of new molecules able to selectively inhibit only a (sub)class of the HDAC family is a very attractive goal to pursue, because such compounds could be useful tools to distinguish the unique functions of the different HDACs, and can represent highly specific cancer therapeutic drugs with much reduced toxicity.

In the anti-HD2 assay [46-48], the percent of inhibition displayed by **1-3** at a fixed dose (ranged from 20 to 30 μM, see Table **3**) and their IC_{50} (50% inhibitory concentration) values in comparison with those of two short-chain fatty acids (sodium butyrate [49] and sodium valproate [50,51]), two hydroxamic acids (TSA [17] and SAHA [18]), and two cyclic tetrapeptides (trapoxin [52] and HC-toxin [53]) as reference drugs have been reported (Table **3**).

Table **4** shows the inhibiting effect (IC_{50} values) of **1-3** against HD1-B and HD1-A enzymes, in comparison with TSA and SAHA, and the resulting fold-selectivity values (for class I HDACs: $IC_{50\text{-}HD1\text{-}A}/IC_{50\text{-}HD1\text{-}B}$ ratio; for class II HDACs: $IC_{50\text{-}HD1\text{-}B}/IC_{50\text{-}HD1\text{-}A}$ ratio) have been assessed.

From both anti-HD2 and –HD1-B/–HD1-A assays, inhibitory data clearly showed that the 2-acylaminocinnamyl-*N*-hydroxyamides **1a-g** are endowed with poor deacetylase inhibiting activity, their IC_{50} values being in the range 8.1-52.0 μM. The most potent compound is the 2-cinnamoylamino derivative **1d**, its IC_{50} values being 8.1, 13.0, and 11.5 μM, against HD2, HD1-B, and HD1-A respectively.

Instead, the 3-acylamino- and the 4-acylamino-isomers **2** and **3** were very efficient in HDAC inhibition with inhibitory values in the nanomolar range. In particular, against HD2, the 3-acylamino-substituted compounds **2a-g** showed IC_{50} values from 85 to 130 nM. The less active derivative was the 3-benzoylamino **2a** (IC_{50} = 130 nM). By insertion of one or two carbon atom units between the benzene ring and the carbonyl group of the **2a** benzoyl portion, as well as by replacement of the benzene ring with bulkier aromatic groups (1-naphthyl, 2-naphthyl, 4-biphenyl), highly potent compounds have been obtained (**2b-g**; IC_{50} values: 85-112 nM). The activities of **2a-g** against HD1-B and HD1-A enzymes were still in the nanomolar range (IC_{50} values = 70-302 nM), but while in the anti-HD1-B assay the same structure-activity relationships (SARs) as those described in the anti-HD2 assay have been observed, against HD1-A the 3-benzoylamino derivative **2a** was the most potent (IC_{50} = 70 nM) and the most active class II-selective (class II selectivity ratio: 4.3) compound among all the synthesized cinnamyl-hydroxyamides **1-3**. Molecular modelling and docking studies with 3D-QSAR models of HD1-B and HD1-A enzymes have also been undertaken, also to provide an explanation for the **2a** activity data.

Against HD2, the 4-substituted series (compounds **3a-f)** resembled the same SAR profile as the 3-substituted counterparts, the 4-benzoylamino derivative **3a** and the 4-(3-phenylpropionylamino) derivative **3c** respectively, being, the least and the most potent compounds (**3a**: IC_{50} = 168 nM; **3c**: IC_{50} = 11 nM) of the series. In the anti-HD1-B and -HD1-A assays, again the 4-benzoylamino derivative **3a** was the less active compound, whilst the 4-(2-naphthoylamino)cinnamyl-*N*-hydroxyamide **3f** showed the highest inhibitory activity, with $IC_{50\text{-}HD1\text{-}B}$ = 36 nM and $IC_{50\text{-}HD1\text{-}A}$ = 42 nM.

CONCLUSION

A new series of 2-, 3-, and 4-acylaminocinnamyl-*N*-hydroxyamides **1-3** have been prepared, and their anti-HDAC (against maize HD2, HD1-B, and HD1-A enzymes) activities have been assessed. Cinnamyl-hydroxyamides bearing acylamino substituents at the C2 position of the benzene ring (compounds **1a-g**) showed very low HDAC inhibiting activities, with IC_{50} values in the high micromolar range. By shifting the same acylamino groups from C2 to C3 (compounds **2a-g**) as well as C4 (compounds **3a-f**) position of the benzene ring, a number of highly potent HDAC inhibitors have been obtained.

As a rule, the introduction of a benzoylamino moiety both at C3 or C4 position led to 3- and 4-benzoylamino-cinnamyl-*N*-hydroxyamides (**2a** and **3a**), with IC_{50} values in the range 130-302 nM (with the exception of the activity of **2a** against HD1-A, IC_{50} = 70 nM). The insertion of 1-2 carbon atom units between the benzene and the carbonyl group of the benzoyl portion, as well as the replacement of the benzene with the bulkier 1-naphthyl, 2-naphthyl, or 4-biphenyl moiety, increased up to 15-times the HDAC inhibitory activity of the derivatives (compare **3a**, $IC_{50\text{-}HD2}$ = 168 nM, with **3c**, $IC_{50\text{-}HD2}$ = 11 nM).

Table 3. HD2 Inhibitory Activity of Compounds 1-3[a]

compd	R	% inhbtn (fixed dose, μM)	IC_{50} ± SD (nM)
1a	Ph	43.9 (27.2)	33600 ± 1344
1b	$PhCH_2$	58 (26)	14500 ± 725
1c	$PhCH_2CH_2$	28.4 (24.8)	48200 ± 1928
1d	PhCH=CH	72.5 (25)	8100 ± 405
1e	1-naphthyl	32 (23.1)	41600 ± 2080
1f	2-naphthyl	48.6 (23.1)	24800 ± 744
1g	4-biphenyl	54.2 (21.4)	19300 ± 965
2a	Ph	94.7 (27.2)	130 ± 3.9
2b	$PhCH_2$	97.4 (25.9)	85 ± 4.2
2c	$PhCH_2CH_2$	96.8 (24.8)	90 ± 2.7
2d	PhCH=CH	95.2 (24.9)	92 ± 2.8
2e	1-naphthyl	96.2 (23.1)	112 ± 3.4
2f	2-naphthyl	96.3 (23.1)	102 ± 4.1
2g	4-biphenyl	89.2 (21.4)	96 ± 4.8
3a	Ph	93.7 (27.2)	168 ± 6.7
3b	$PhCH_2$	97.3 (26)	65 ± 3.2
3c	$PhCH_2CH_2$	98 (24.8)	11 ± 0.5
3d	PhCH=CH	94.3 (24.9)	77 ± 3.1
3e	1-naphthyl	96.4 (23.1)	69 ± 2.8
3f	2-naphthyl	96.3 (23.1)	84 ± 3.4
sodium butyrate		35 (5000)	-
sodium valproate			128000 ± 3800
TSA			7.2 ± 0.2
SAHA			50 ± 1.5
trapoxin			10 ± 0.3
HC-toxin			110 ± 4.4

[a]Data represent mean values of at least three separate experiments.

In the anti-HD2 assay, **3c** (IC_{50} = 11 nM) was the most potent compound, being >11600-, 4.5-, and 10-fold more potent than sodium valproate, SAHA, and HC-toxin, respectively, and showing the same activity as trapoxin and slightly lower (1.5-fold) activity than TSA. Moreover, in inhibiting HD2 **3c** was several times more potent than the aroyl-pyrrolyl-hydroxyamides (APHAs) previously reported by us [21-26].

HD1-B and HD1-A assays have been performed to screen the inhibitory action of **1-3** against mammalian class I (HD1-B) and class II (HD1-A) HDAC homologous enzymes. From the corresponding IC_{50} data, a selectivity ratio has been calculated. In general, compounds **1-3** showed no or little selectivity towards the class II homologue HD1-A, the most selective being **2a**, with class II selectivity ratio = 4.3. SAHA and, to a lesser extent TSA, were both class I selective (class I selectivity ratios: SAHA, 6.7; TSA, 2). About the inhibitory potency, the 4-(2-naphthoylamino) cinnamyl-*N*-hydroxyamide **3f** showed the highest inhibiting effect against the two enzymes. Even though it was 90-(HD1-B) and 52-fold (HD1-A) less potent than TSA, **3f** showed the same activity as SAHA against HD1-B, and was 4.8-fold more potent than SAHA against HD1-A.

Table 4. HD1-B and HD1-A inhibitory activity of compounds 1-3[a]

compd	R	IC_{50} ± SD (nM)		class selectivity	
		HD1-B	HD1-A	class I	class II
1a	Ph	32200 ± 1610	34100 ± 1364		
1b	$PhCH_2$	23200 ± 696	23000 ± 920		
1c	$PhCH_2CH_2$	42000 ± 1680	NI[b]		
1d	PhCH=CH	13000 ± 520	11500 ± 690		
1e	1-naphthyl	52000 ± 2080	17800 ± 1068		2.9
1f	2-naphthyl	22000 ± 1100	26100 ± 1044		
1g	4-biphenyl	23200 ± 1160	14100 ± 423		1.6
2a	Ph	302 ± 15.1	70 ± 3.5		4.3
2b	$PhCH_2$	192 ± 9.6	81 ± 3.2		2.4
2c	$PhCH_2CH_2$	114 ± 3.4	94 ± 3.8		
2d	PhCH=CH	232 ± 13.9	152 ± 6.1		
2e	1-naphthyl	216 ± 8.6	102 ± 5.1		2.1
2f	2-naphthyl	168 ± 5.0	90 ± 4.5		1.9
2g	4-biphenyl	206 ± 10.3	120 ± 3.6		1.7
3a	Ph	239 ± 7.1	130 ± 6.5		1.8
3b	$PhCH_2$	92 ± 2.8	72 ± 3.6		
3c	$PhCH_2CH_2$	102 ± 6.1	76 ± 3.0		
3d	PhCH=CH	126 ± 5.0	101 ± 5.0		
3e	1-naphthyl	115 ± 4.6	59 ± 2.9		1.9
3f	2-naphthyl	36 ± 1.4	42 ± 1.3		
TSA		0.4 ± 0.01	0.8 ± 0.03	2	
SAHA		30 ± 1.0	200 ± 9.0	6.7	

[a]Data represent mean values of at least three separate experiments. [b]NI, no inhibition.

Selected **2** and **3** compounds will be evaluated to determine their antiproliferative and cyto differentiating activities on human acute promyelocytic leukaemia HL-60 cells.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined on a Buchi 530 melting point apparatus and were incorrected. Infrared (IR) spectra (KBr) were recorded on a Perkin- Elmer Spectrum One instrument. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer; chemical shifts were reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me_4Si). All compounds were routinely checked by TLC and ^{1}H NMR. Mass spectra (MS) were obtained on a JEOL JMS-HX 110 spectrometer. TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualised by UV light. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extraction involved the use of a rotary evaporator operating at a reduced pressure of *ca*. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results were within –0.40 and +0.40% of the theoretical values. All chemicals were purchased from Aldrich Chimica, Milan (Italy) or Lancaster Synthesis GmbH, Milan (Italy) and were of the highest purity.

General Procedure for the Synthesis of Ethyl 3-(2-acylaminophenyl)-2-propenoates 7a-g, Ethyl 3-(3-acylaminophenyl)-2-propenoates 8a-g, and 4-(4-acylaminophenyl)-2-propenoates 9a-g. Example: Ethyl 3-[4-(3-phenylpropionyl)aminophenyl]-2-propenoate (9c)

3-Phenylpropionyl chloride (3.7 mmol, 0.5 mL) and triethylamine (7.7 mmol, 1.1 mL) were added to a solution of

ethyl 3-(4-aminophenyl)-2-propenoate hydrochloride **6** (3.1 mmol, 0.7 g) in dry dichloromethane (20 mL) at 0 °C under nitrogen atmosphere. After stirring at room temperature for 4 h, the reaction mixture was poured into water (50 mL), the organic layer was separated, and the aqueous one was extracted with chloroform (2 x 50 mL). The combined organic solution was washed with water (100 mL) and brine (100 mL), and was dried and evaporated to dryness. The residual solid was purified by crystallisation from dichloromethane/*n*-hexane to yield pure **9c.** ^{1}H NMR ($CDCl_3$) δ 1.31-1.34 (t, 3 H, $COOCH_2CH_3$), 2.66-2.70 (t, 2 H, $PhCH_2CH_2CO$), 3.02-3.06 (t, 2 H, $PhCH_2CH_2CO$), 4.22-4.27 (q, 2 H, $COOCH_2CH_3$), 6.32-6.36 (d, 1 H, CH=C*H*CO), 7.21-7.23 (m, 2 H, benzene H-2,6), 7.27-7.29 (m, 2 H, benzene H-3,5), 7.42-7.50 (m, 5 H, benzene H-2'-6'), 7.59-7.63 (d, 1 H, C*H*=CHCO). Low resolution MS (EI^+) *m/z* 324 (M^+).

General Procedure for the Synthesis of 3-(2-acylaminophenyl)-2-propenoic Acids 10a-g, 3-(3-acyl-aminophenyl)-2-propenoic Acids 11a-g, and 3-(4-acyl-aminophenyl)-2-propenoic Acids 12a-f. Example: 3-[2-(3-Phenyl-2-propenoylamino)phenyl]-2-propenoic Acid (10d)

A mixture of ethyl 3-[2-(3-phenyl-2-propenoylamino) phenyl]-2-propenoate (**7d**) (2.0 mmol, 0.6 g), lithium hydroxide hydrate (4.0 mmol, 0.17 g), and ethanol (15 mL) was stirred at room temperature. After 24 h, 2 N HCl was added to the mixture until the pH was 5, and the obtained solid was filtered and recrystallised from methanol to yield pure **10d**. ^{1}H NMR (DMSO-d_6) δ 6.47-6.51 (d, 1H, CH=C*H*COOH), 6.92-6.96 (d, 1H, CH=C*H*CONH), 7.23-7.27 (m, 1 H, benzene H-5), 7.40-7.46 (m, 3 H, benzene H-4,6,4'), 7.51-7.57 (m, 2 H, benzene H-3',5'), 7.61-7.64 (m, 3 H, benzene H-3,2',6'), 7.73-7.77 (d, 1H, C*H*=CHCOOH), 7.80-7.82 (d, 1 H, C*H*=CHCONH), 12.50 (bs, 1H, COOH exchangeable with D_2O). Low resolution MS (EI^+) *m/z* 294 (M^+).

General procedure for the synthesis of 3-(2-acylaminophenyl)-*N*-hydroxy-2-propenamides 1a-g, 3-(3-acylaminophenyl)-*N*-hydroxy-2-propenamides 2a-g, and 3-(4-acylaminophenyl)-*N*-hydroxy-2-propenamides 3a-f. Example: 3-[4-(2-Naphthoylamino)phenyl]-*N*-hydroxy-2-propenamide (3f)

Ethyl chloroformate (2.9 mmol, 0.3 mL) and triethylamine (3.1 mmol, 0.4 mL) were added to a cooled (0 °C) solution of 3-[4-(2-naphthoylamino)phenyl]-2-propenoic acid **12f** (1.5 mmol, 0.5 g) in dry THF (10 mL), and the mixture was stirred for 10 min. The solid was filtered off, and *O*-(2-methoxy-2-propyl)hydroxylamine (4.71 mmol, 0.35 mL) [42] was added to the filtrate. The solution was stirred for 15 min at 0 °C, then was evaporated under reduced pressure, and the residue was diluted in methanol (10 mL). Amberlyst® 15 ion-exchange resin (0.16 g) was added to the solution of the *O*-protected hydroxamate, and the mixture was stirred at room temperature for 1 h. After wards, the reaction was filtered and the filtrate was concentrated *in vacuo* to give the crude **3f** which was purified by crystallisation. ^{1}H NMR (DMSO-d_6) δ 6.39-6.43 (d, 1 H, CH=C*H*CO), 7.42-7.46 (d, 1 H, C*H*=CHCO), 7.57-7.62 (m, 5 H, benzene H-2,6 and naphthalene H-5-7), 7.75-7.76 (d, 1 H, naphthalene H-8), 7.85-7.87 (d, 2 H, benzene H-3,5), 8.00-8.02 (m, 1 H, naphthalene H-4), 8.06-8.08 (d, 1 H, naphthalene H-3), 8.16-8.18 (m, 1 H, naphthalene H-1), 9.03 (bs, 1 H, OH exchangeable with D_2O), 10.74 (s, 2 H, NH exchangeable with D_2O). Low resolution MS (EI^+) *m/z* 333 (M^+).

***In Vitro* Maize HD2, HD1-B, and HD1-A Enzyme Inhibition**

Radioactively labeled chicken core histones were used as the enzyme substrate according to established procedures [46-48]. The enzyme liberated tritiated acetic acid from the substrate, which was quantified by scintillation counting. IC_{50} values are results of triple determinations. 50 μL of maize enzyme was incubated with 5 μL of inhibitors of different concentrations for 15 min on ice, followed by incubation (30 min at 30° C) with 10 μL of total [^{3}H]acetate-prelabelled chicken reticulocyte (at 30° C) histones (1 mg/mL). Reaction was stopped by addition of 36 μL of 1 M HCl/0.4 M acetate and 800 μL of ethyl acetate. After centrifugation (10000 g, 5 min), an aliquot of 600 μL of the upper phase was counted for radioactivity in 3 mL of liquid scintillation cocktail. The compounds were tested in a starting concentration of 40 μM, and active substances were diluted further. Sodium butyrate, sodium valproate, TSA, SAHA [54], trapoxin, and HC-toxin were used as the reference compounds, and blank solvents were used as negative controls.

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