Hydroxamic Acids as Histone Deacetylase Inhibitors

Florian Thaler, Vaishali M. Patil and Satya P. Gupta

Abstract HDAC inhibition has been, for over a decade (and continues to remain), a highly competitive area. Hydroxamic acids represent the largest class of HDAC inhibitors. One product, SAHA is already approved and more than ten different chemical entities are in various clinical stages. A detailed discussion about compounds from various classes like phenyloxopropenyl, amidopropenyl analogues, spiropiperidines, biphenyl/arylamide/styrenyl, tetrahydroisoquinoline-based hydroxamic acid derivatives and N-hydroxyphenylacrylamide derivatives has been included along with the computational studies. It also covers brief details about the HDAC imaging agents. These successes as well as the enormous amount of experiences gained in preclinical and clinical studies may be useful—beyond the HDAC field—to future drug discovery programmes studying hydroxamic acid derivatives.

Abbreviations

AUC	Area under curve
B16	Murine melanoma cell line
Caco-2	Human epithelial colorectal adenocarcinoma cells
COLO205	Human colon cancer cell line
CTCL	Cutaneous T cell lymphoma

F. Thaler (✉)
Drug Discovery Unit, European Institute of Oncology, Via Adamello 16,
20139 Milan, MI, Italy
e-mail: florian.thaler@ieo.eu

V. M. Patil
School of Pharmacy, Bharat Institute of Technology, Meerut 250103, Uttar Pradesh, India

S. P. Gupta
Meerut Institute of Engineering and Technology, Meerut 250005, Uttar Pradesh, India

F	Dose-corrected area under curve (AUC) non-intravenous divided by AUC intravenous
HAT	Histone acetyltransferase
HCT116	Human colon cancer cell line
HDAC	Histone deacetylase
HDLP	Histone deacetylase-like protein
ip	Intraperitoneal
iv	Intravenous
K562	Chronic myelogenous leukaemia cell line
MM	Multiple myeloma
qd	Every day
PK	Pharmacokinetic
$t_{1/2}$	Half-life
T/C	Ratio mean relative tumour volume of the treated tumours/mean relative volume of control group
V_{ss}	Steady-state volume of distribution

Contents

1 Introduction

1.1 Chromatin

The eukaryotic genome is packaged with histone proteins to form the chromatin, which allows condensing of over a metre of DNA into the small volume of the nucleus. The fundamental repeating unit of the chromatin that occurs generally after every 157–240 base pairs is the nucleosome. The nucleosome core itself is composed of a histone octamer consisting of two copies of H2A, H2B, H3 and H4 histones around which 147 base pairs of DNA are wrapped in 1.65 turns of a flat, left-handed superhelix. The stabilisation of the nucleosome occurs through a series of protein–protein interactions within the histone octamer and by electrostatic and hydrogen bonds between the proteins and the DNA. The core histones are composed of two distinct functional domains: the "histone-fold" motif sufficient for both histone–histone and histone–DNA contacts within the nucleosome, and N and C-terminal tail domains, which remain mostly unresolved in the crystal structures (Luger et al. 1997; Davey et al. 2002). These tails extend away from nucleosomal DNA and are mainly involved in interaction with other nucleosomes or with nuclear factors (Luger and Richmond 1998).

The chromatin itself condenses to more compact structures. Short-range nucleosome–nucleosome interactions result in folded chromatin fibres ("secondary chromatin structure"). Long-range interactions between individual nucleosomes result in fibre–fibre interactions and form tertiary chromatin structures. However, these arrangements are not highly defined structural states. Rather, the current view is to consider them as a continuum of various inter-convertible states at different levels of condensation (Horn and Peterson 2002; Luger et al. 2012).

These structural states of the nucleosomes have obviously a major impact on any process requiring access to genomic DNA, such as transcription, replication and DNA repair. Not surprisingly, a plethora of studies has shown that the chromatin structure plays a crucial role in the regulation of all of these processes. These states and their variation are determined by various factors. The DNA sequence of each nucleosome is unique; and this unique DNA sequence affects the nucleosome structure through its sequence-encoded susceptibility for being distorted into the tight superhelical conformation imposed by the histone octamer. Histones are among the most highly conserved proteins in terms of sequence and structure. However, histone variants have been identified for the histone subtypes, in particular for histone H2A and H3. These variants have an influence on the structures of the nucleosomes (Malik and Henikoff 2003; Henikoff et al. 2004; Brown 2001). Furthermore, several proteins have been found interacting with the chromatin. This adds further complexity in the structural regulations. Posttranslational modifications have been one of the most intensively studied aspects as regulatory factors for structural changes of nucleosomes. Posttranslational modifications are small chemical modifications to amino acid side chains of a protein after its translation. The histone tails which account for almost 30 % of the core

histone sequences are, as already mentioned above, unstructured. Thus, they can be the subject of several posttranslational modifications, including acetylation, methylation, phosphorylation, sumoylation or ubiquitination.

1.2 The Histone Acetylase and Deacetylase Machinery

Already in 1964, Allfrey et al. first reported the isolation of acetylated and methylated histones and speculated well ahead on their time about their possible role in the regulation of RNA synthesis (Allfrey and Mirsky 1964; Allfrey et al. 1964). The authors hypothesised that these modifications of the histone structure, particularly acetylation, have an influence of RNA synthesis. Some years later, Riggs et al. (1977) found that the exposure of cultured cells to sodium n-butyrate caused a reversible accumulation of highly acetylated histones. However, the picture became clearer only during the early 1990s, when the first histone deacetylase (HDAC) was cloned in 1996 (Taunton et al. 1996). One year earlier, Kleff et al. (1995) had identified a gene encoding a yeast H4 acetyltransferase (HAT). Currently, a series of HATs and HDACs have been identified to be responsible for the tight control of the acetylation state of histones. HATs are enzymes which transfer an acetyl group from acetyl coenzyme A (acetyl CoA) onto to the ε-amino group of one or more lysine residues contained within the N-terminal tails of the histone proteins. The neutralisation of the basic charge of the tails reduces the electrostatic interactions with the DNA sequence leading to a nucleosome unwrapping (Simon et al. 2011). This relaxation of the chromatin conformation allows the transcriptional factors to access the gene promoter regions and the process of gene expression is facilitated. HATs can be classified into two different classes based on their functional localisation: the nuclear type A HATs and the cytoplasmic type B HATs. The latter ones are involved in the modification of newly synthesised histones before the assembly. Type A HATs can be further divided into five different classes based on structural and functional differences: GNAT, CBP/p300, transcriptional factors such as ATF2, nuclear hormone related (for example SRC4) and the MYST family proteins (Selvi and Kundu 2009; Grant and Berger 1999).

The counteracting histone deacetylases re-establish the positive charge in the N-terminus of the histone tails. This causes a tighter histone–DNA interaction and blocks the binding sites on promoters and thus inhibits gene transcription. The HDAC enzymes can be grouped into two families: the classical HDACs and the silent information regulator (Sir)-related protein (sirtuin) families. HDAC class III proteins (also known as sirtuins, Sirt1–Sirt7) form a structurally and mechanistically distinct class and are defined by their dependency on NAD^+ as cofactor. They catalyse the removal of the acetyl group of the acetylated lysines by transferring it to the ribose moiety of NAD^+ yielding O-acetyl-ADP-ribose and nicotinamide, which acts as a physiological inhibitor of the sirtuins by means of negative feedback (Huber and Superti-Furga 2011).

Classical HDACs are metalloenzymes harbouring a catalytic pocket with a Zn^{2+} ion. In humans, classical HDACs are grouped based on the homology to yeast enzymes in four distinct classes that vary in size and function (Grozinger et al. 1999; Gray and Ekstrom 2001; Lin et al. 2006; Ficner 2009). Class I HDACs, which comprise HDACs 1, 2, 3, and 8, share a certain degree of homology to the yeast Rpd3. These enzymes are generally nuclear proteins and are ubiquitously expressed in many human cell lines and tissues. HDAC11 is the sole member of Class IV HDACs and is found to be present in the nucleus. Class II HDACs, which are homologues to yeast Hda1, can be divided into two further sub-classes: class IIa (HDACs 4, 5, 7, 9) and class IIb HDACs (HDACs 6 and 10) (Verdin et al. 2003; Yang and Gregoire 2005). Class IIa HDACs contain a highly conserved *C*-terminal deacetylase domain with around 420 amino acids, and are homologous to yeast Hda1. However, their *N*-terminal domain, which has regulatory functions, does not show any similarity to HDACs in other classes. Class IIb HDACs have an additional deacetylase domain, although this duplication is partial in the case of HDAC10. Class II HDACs exhibit nucleocytoplasmic shuffling, suggesting their involvement in the deacetylation of non-histone substrates. For example, deacetylation of acetyl-α-tubulin is mediated by the second deacetylase domain of HDAC6 (L'Hernault and Rosenbaum 1985a, b; Hubbert et al. 2002; Haggarty et al. 2003). Recently, it has become clear that HDACs and not only the class II enzymes are involved in the deacetylation of a series of non-histone proteins (Glozak et al. 2005; Yang and Seto 2008; Singh et al. 2010; Yao and Yang 2011). Proteomic studies allowed to identify a remarkable amount of acetylation sites: Kim et al. (2006) found 388 acetylation sites on 195 proteins in HeLa cells and mouse liver mitochondria by immunoaffinity purification using an anti-acetyl lysine antibody; high-resolution mass spectrometry experiments permitted to identify even more impressive number of 3,600 lysine acetylation sites on 1,750 proteins (Choudhary et al. 2009). These results propose that acetylation/ deacetylation is a regulatory modification that rivals phosphorylation in number of substrates (Kouzarides 2000; Choudhary et al. 2009). Furthermore, these acetylation sites were found on proteins involved in diverse cellular processes, such as chromatin remodelling, cell cycle, splicing, nuclear transport, signal transduction and apoptosis.

Several of these proteins were found to be relevant for tumorigenesis and cancer cell proliferation (Buchwald et al. 2009; Yao and Yang 2011). On the other hand, acetylation of histones results in changes of chromatin structures and this has an impact on any process requiring access to genomic DNA. Thus, it is not surprising that abnormal activities of HDACs and HATs have been found involved in the development of several diseases. Indeed, HDACs expression and their activity showed to be altered in many cancers. For example, HDACs are associated with the function of oncogenic-translocation products, such as PML-RARα in acute promyelocytic leukaemia. PML-RAR associate with a corepressor complex containing HDAC activity. This complex is able to inhibit the transcription of genes involved in haematopoietic differentiation, and thus contributing to the differentiation block found in this form of leukaemia (Grignani et al. 1998; Lin

Table 1 Classification of hydroxamic acid-based HDAC inhibitors

Cap structure	Hydroxamic acid class
Absent	Short-chain fatty hydroxamic acids
Relatively small hydrophobic group	TSA
	Hybrid polar compounds: SAHA, Pyroxamide
	Benzamide-based HA
	Arylketones
	Amino acid-contains, benzamide-based HA
	Indole amide-based HA
	Aroyl-heterocyclic-based HA
	Aroyl-pentadienoic HA
	Aroyl-urea-based HA
	Sulfonamide-based HA: Oxamflatin, PXD101
	(Bi-)aryl-(heterocyclic)-based HA
	SK-7041, NVP-LAQ824
	A-161906
	Aroyl-pyrrole HA
Cyclic peptides	Cyclic hydroxamic acid-containing peptides
	Cyclic hexapeptides
Macro- or monocyclic succinimide	Succinimide-based HA
N- and C-substituted cysteine	Cysteine-based HA
1,3-dioxane	1,3-dioxanes: Tubacin

et al. 1998; Richon and O'Brien 2002). Subsequently, it has been shown that inhibition of HDACs resulted in a cell-cycle arrest and differentiation through an increased expression of p21WAF1/CIP1. Inhibition also affected tumour survival by blocking angiogenesis through the increased acetylation of HIF-1α and protein degradation through the acetylation of Hsp90. These findings have made HDACs as attractive targets for cancer therapy (Federico and Bagella 2011; Mercurio et al. 2010). The diverse mechanisms through which HDAC inhibitors exert their cytotoxic activity include induction of apoptosis by both intrinsic and extrinsic pathways, selective expression of repressed genes, cell-cycle arrest, DNA damage and repair, inhibition of angiogenesis, cell death due to accumulation of reactive oxygen species (ROS), autophagy and immunomodulatory effects (Bolden et al. 2006).

During the last 20 years, a variety of HDAC inhibitors have been developed based on the structures of the naturally occurring inhibitors, or discovered randomly in HDAC screening assays. The currently available HDAC inhibitors can be classified according to the nature of the metal binding group into different categories, i.e. hydroxamic acid-based, carboxylic acid-based, disulfide-based, epoxide-based, and anilide-based inhibitors. The hydroxamic acid-based HDAC inhibitors can be sub-classified according to the nature of cap structure (Table 1) (Elaut et al. 2007) or broadly classified as small-capped and large-capped hydroxamic acid-based inhibitors.

2 Histone Deacetylase Inhibitors: From *n*-Butyrate to SAHA

The first example of HDAC inhibition was reported in 1977. Riggs et al. (1977) found an accumulation of acetylated histones in the presence of sodium *n*-butyrate (Fig. 1). It was then questioned, if the accumulation of highly acetylated histones caused by this carboxylic acid was really linked to the observed induced differentiation in tumoral HeLa and Friend erythroleukemia cells. It took some years until Yoshida et al. (1987) found that Trichostatin A (TSA) (Fig. 1), a hydroxamic acid derivative originally found as fungistatic antibiotic (Tsuji et al. 1976), caused induction of Friend leukaemia cell differentiation at low nanomolar concentration. Three years later, the same authors disclosed that the (*R*) isomer of TSA was able to cause an accumulation of highly acetylated histones in vivo and to inhibit the activity of the partially purified histone deacetylase in vitro. The (*S*) isomer as well as the trichostatic acid did not show inhibitory activity and had no effect on the induction of Friend cell differentiation and the inhibition of the cell-cycle progression (Yoshida et al. 1990a). Furthermore, (*R*)-TSA was found to exhibit a significant lower inhibitory activity in a tumour cell line containing mutated histone deacetylases. These important observations provided the first evidence that the effect of TSA on cell proliferation and differentiation was directly related to the inhibition of histone deacetylases (Yoshida et al. 1987, 1990b). After 16 years later, suberoylanilide hydroxamic acid (SAHA) (Fig. 1) gained approval by FDA as the first HDAC inhibitor to be used for the treatment of cutaneous T cell lymphoma (CTCL) (Marks 2007; Marks and Breslow 2007). SAHA, disclosed in 1993 (Breslow et al. 1993), was the result of two decades of research activities carried out at the Columbia University and the Memorial-Sloan Kettering Cancer Center. In the early 1970s, Friend et al. (1971) found that DMSO acted as differentiating agent—murine erythroleukemia cells (MELC) after being placed in
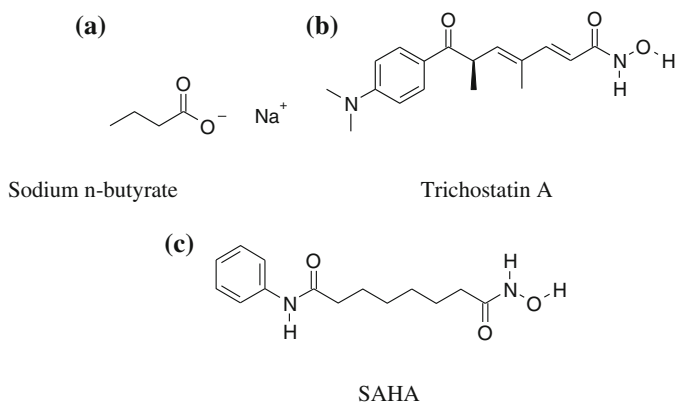

(a) Sodium n-butyrate

(b) Trichostatin A

(c) SAHA

Fig. 1 Chemical structures of Sodium *n*-butyrate (**a**), Trichostatin A (**b**), and SAHA (**c**)

culture with dimethylsulfoxide turned red, suggesting the presence of haemoglobin. The search for new derivatives able to induce cytodifferentiation and growth arrest resulted in the discovery of polar solvent species, such as *N*-methylformamide or acetamide (Tanaka et al. 1975). While these compounds showed only a slight increase of activity compared to DMSO, dimeric amides proved to be much more potent. They were able to induce differentiation at low millimolar concentrations (Reuben et al. 1976, 1978). One example of this series, hexamethylene bisacetamide (HMBA), was selected for clinical studies. The trials showed that the compound was able to induce minor and partial remission in myelodysplastic syndrome and acute myelogenous leukaemia. However, the remission resulted to be transient and the doses required were not tolerated by cancer patients (Andreeff et al. 1992). Even though the biological target of these compounds was not identified, it was hypothesised that the dimeric amide structures may possibly act as chelating agents of metal ions. Exploration of new chemical entities led to the discovery of hydroxamic acids as well as bishydroxamates (Breslow et al. 1991; Richon et al. 1996). One example, SAHA, (but also other hydroxamic acid derivatives), was approximately 2,000-fold more potent than HMBA and was able to induce differentiation in MELC cells at low micromolar concentrations. Richon et al. (1998) confirmed that SAHA, like TSA, showed an HDAC inhibitory activity and caused accumulation of hyperacetylated histone H4 in cultured cells. One year later, the X-ray crystallographic structures of SAHA and TSA bound to the *Aquifex aeolicus* HDAC homologue histone deacetylase-like protein (HDLP) revealed that the hydroxamic acid moiety of both compounds is doubly coordinated to a zinc atom at the bottom of a cavity (Finnin et al. 1999). In 2000, SAHA entered clinical studies and, after intravenous and the oral administration, showed a good safety profile and antitumor activity in different malignancies, in particular haematological malignancies (Kelly et al. 2003, 2005). Finally, in October 2006, FDA approved the compound as first HDAC inhibitor for the treatment of CTCL, after that a significant response rate in prior therapy-resistant CTCL patients was observed (Mann et al. 2007).

3 Histone Deacetylase Inhibitors: Orally Available Hydroxamic Acid Derivatives

While SAHA showed a considerable efficacy in different haematological malignancies in clinical trials, patient response in other cancers remained much more uncertain and often rather limited (Graham et al. 2009; Mercurio et al. 2010). SAHA had demonstrated some activity in patients with advanced solid tumours in phase I trials. However, phase II studies in patients with breast, colorectal or non-small cell lung cancer showed a limited drug exposure, which did not allow a reliable efficacy analysis (Vansteenkiste et al. 2008). SAHA was well tolerated, but exhibited only medium potency (Marks and Breslow 2007) and was cleared

rapidly (Kelly et al. 2003). Short half-lives in vitro as well as in vivo are quite common to several first generation hydroxamic acid inhibitors (Elaut et al. 2007), and thus hydroxamates are often considered as poor drugs. They are frequently down prioritised in drug discovery programmes because of their poor physico-chemical and ADME properties, despite their good in vitro potency (Elaut et al. 2007; Flipo et al. 2009).

However, the overall impressive in vitro and in vivo data of the first drugs made HDACs and their inhibition an attractive target for several drug discovery pro-grammes. Research efforts were directed to highly potent compounds with a prolonged in vivo exposure and generally a better pharmacokinetic (PK) profile. In fact, we can find a significant number of research groups, both from industry and academia, involved in the exploration of novel chemical entities with HDAC inhibitory properties. Almost 400 PCT patent applications claiming new HDAC inhibiting agents is just one indicator of the impressive amount of efforts done in this field during the last decade. It is noticeable that more than 50 % of the applications are related to hydroxamic acid derivatives (Thaler 2012). Another sign is that the significant number of molecules have entered clinical studies. Around 20 chemical entities, either alone or in combination, have entered clinical studies for the treatment of several diseases, mainly tumours. Among them there is a considerable amount of hydroxamic acid derivatives e.g. LBH589 or panobi-nostat (Novartis) (Atadja 2009; Neri et al. 2012), and PXD101 or belinostat (Topotarget) (Steele et al. 2008) are currently in phase III studies, ITF-2357 or givinostat (Italfarmaco) (Rambaldi et al. 2012), PCI-24781 or abexinostat (Servier, Pharmacyclics) (Buggy et al. 2006), SB939 or pracinostat (S*BIO Pte Ltd) (Novotny-Diermayr et al. 2010; Wang et al. 2011), JNJ-26481585 or quisinostat (Johnson & Johnson) (Arts et al. 2009; Tong et al. 2010) and 4SC-201 or res-minostat (4SC) (Mandl-Weber et al. 2010; Brunetto et al. 2009) have reached clinical phase II (Fig. 2). Examples in phase I can be given as AR-42 or OSU-HDAC-42 (Arno Therapeutics) (Kulp et al. 2006; Lu et al. 2005), CG-200745 (CrystalGenomics Inc) (Hwang et al. 2012), CHR-3996 (Chroma Therapeutics) (Donald et al. 2010; Banerji et al. 2010), the HDAC6 selective ACY-1215 (Acetylon Pharmaceuticals Inc) (Santo et al. 2012) and the dual kinase and HDAC inhibitor CUDC-101 (Curis and Ligand Pharmaceuticals) (Cai et al. 2010; Lai et al. 2010; Shimizu et al. 2010) (Fig. 3).

In the following sections, the research efforts directed towards the development of hydroxamic acid derivatives with improved PK properties are discussed.

3.1 From SB639 to Pracinostat

Scientists from the Singaporean biotech company S*Bio Pte explored different fused heterocyclic rings such as benzimidazoles as new linkers for low molecular weight HDAC inhibiting agents. This research led to the discovery of the first lead compound, SB639 (Fig. 4) (Wang et al. 2009). The compound showed good
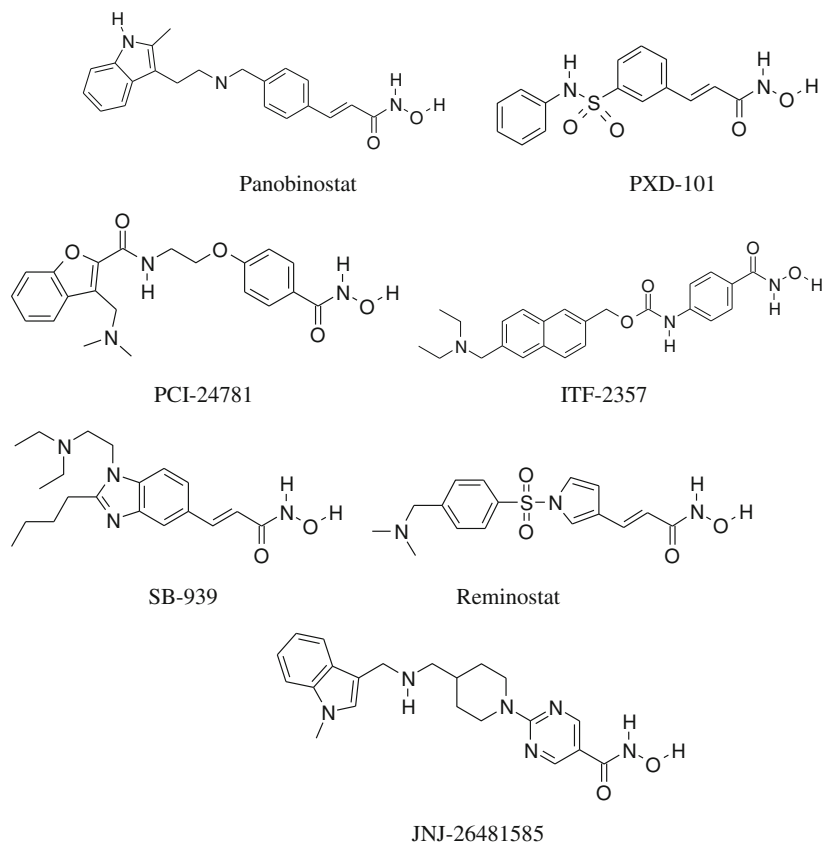
Fig. 2 HDAC inhibitors in phase II and Phase III clinical trials

in vitro potency with an IC$_{50}$ value of 0.035 ± 0.016 μM against HDAC1 and antiproliferative activity against the human colon cancer cell line COLO205 in the submicromolar range (IC$_{50}$ = 0.14 ± 0.05 μM). The compound was stable in human and dog liver microsomes with a half-life of around 1 h. However, the half-life dropped in rat and mouse microsomes to 6 and 3 min, respectively. The compound showed high clearance rates in mice (15.8 L/h/kg) and in rats (3.84 L/h/kg). The oral bioavailability was 13 % (mice) and 10.5 % (rats), respectively (Venkatesh et al. 2007). Nevertheless, the plasma concentration of the compound reached levels above the HDAC IC$_{50}$ value and the compound showed antitumor activity in HCT116 tumour-bearing nude mice after oral administration (Wang et al. 2009).

Metabolic profiling experiments in rat liver hepatocytes showed that the major metabolic paths of SB639 were the oxidation of the pyrrolidinylethyl group and the reduction of the hydroxamic acid to amide (Wang et al. 2011). Further development of this series was carried out considering particularly the metabolic stability of the compounds. This expansion resulted in several compounds. Among

ACY-1215

CUDC-101

(S)-Ar-42

CG200745

CHR-3996
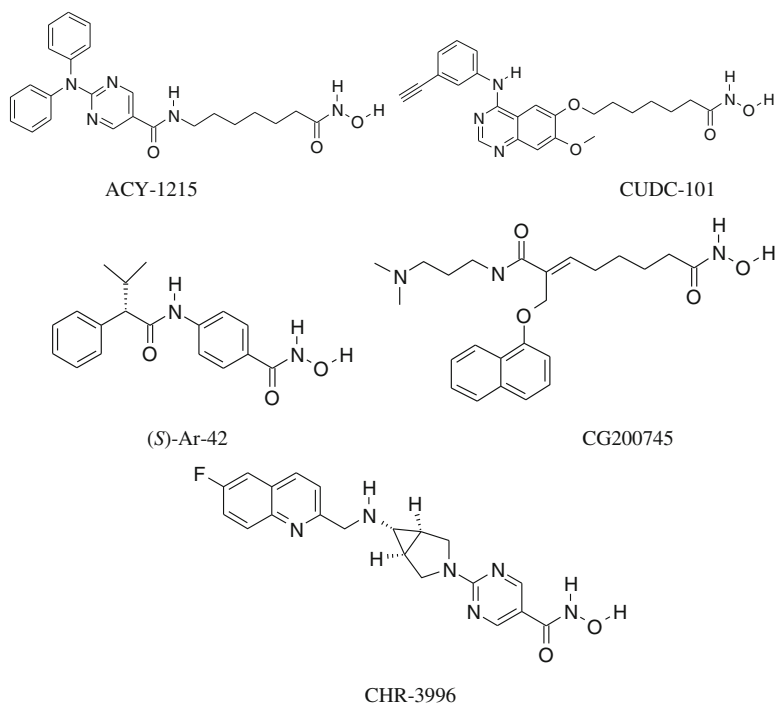
Fig. 3 HDAC inhibitors currently in phase I clinical trials

them, compound SB939 (Fig. 4) was found to be potent against HDAC1 with an IC_{50} value of 0.077 ± 0.014 µM and showed antiproliferative activity against a series of cancer cell lines in the submicromolar range. For example, the compound with an IC_{50} of 0.48 ± 0.27 µM was around six times more active than SAHA ($IC_{50} = 2.85 \pm 0.27$ µM) against HCT116 cells. An interesting observation was that the microsomal liability in human and mouse microsomes generally increased with higher lipophilicity of the molecules. SB939 as well as several other derivatives were relatively stable in human and dog microsomes. On the other hand, a major variability was found in rodents. Consistent with the in vitro microsomal data, SB939 exhibited an oral bioavailability of 65, 34 and 3.1 % in beagle dogs,

(a)

(b)
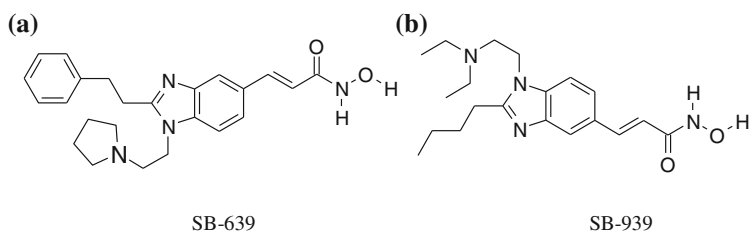
SB-639

SB-939

Fig. 4 Structures of **a** SB-639 and **b** SB-939

nude mice and Wistar rats, respectively. Furthermore, the compound had high systemic clearance (relative to liver blood flow) of 1.5, 9.2 and 4.5 L/h/kg and high volume of distribution at steady state of 4.2, 3.5 and 1.7 l/kg in dog, mouse and rat, respectively (Jayaraman et al. 2011). The superior in vitro activities and PK properties compared to SAHA translated into a higher antitumor efficacy in a HCT116 tumour xenograft model. SB939 was approximately twice as efficacious at the maximum tolerated dose as SAHA: the tumour growth inhibition for SAHA was 48 % at 200 mg/kg compared to 94 % for SB939 at 100 mg/kg. In addition, an accumulation of the compound was observed in tumour tissues (Novotny-Diermayr et al. 2010). This was not found after administering the reference of SAHA. In addition, SB939 has good aqueous solubility and high permeability in human Caco-2 cells with low efflux. These characteristics are indicative of a high-intestinal absorption in vivo (Jayaraman et al. 2011). In fact, phase I studies conducted in patients with advanced solid malignancies showed that the pharmacologically active concentrations were achieved already at the lowest dose of 10 mg (Jayaraman et al. 2011). The drug was rapidly absorbed (t_{max} = 1–3 h). The mean elimination half-life was around 7 h and oral clearance was 53 ± 8.5 L/h. C_{max} and AUC were dose proportional between 10 and 60 mg doses. Furthermore, no substantial accumulation of SB939 on day 15 following repeated dosing was found. At the 60 mg dose high acetylation levels was found in all patients indicating sustained target inhibition. Two patients experienced prolonged disease stabilisation (Yong et al. 2009). An interesting aspect is that the human PK was successfully predicted based on the in vitro ADME data using an ADME simulator and allometric scaling (Jayaraman et al. 2011).

3.2 CHR-3996

The HDAC project at the British biotech company, Chroma Therapeutics, was focused on the synthesis of pyrimidine hydroxamate derivatives bound to bicyclic hexahydropyrrolo[3,4-c]pyrrole and azabicyclo[3,1,0]hexane linkers (Moffat et al. 2010). The inhibitory activities of around 25 described azabicyclo[3,1,0]hexanes varied only slightly and were in the low nanomolar range against HDACs from nuclear HeLa extracts. This narrow range in activity despite substantial variations of the linker structures is not uncommon; for example, as already described above, a similar behaviour was experienced in house for several examples within the amidopropenyl and the spiropiperidine series (cf. Sects. 3.4 and 3.5). The

Fig. 5 Structure of CHR-3996
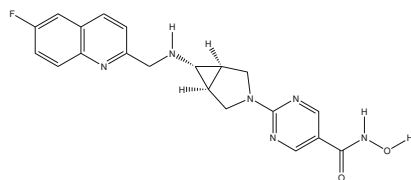
compounds showed good antiproliferative activity in tumoral HCT116 cells with GI_{50} values in the micro- or submicromolar range. In particular, it was found that diminishing the polar surface area was beneficial for the cell growth inhibition. PK studies using oral cassette dosing allowed identifying CHR-3996 as one lead compound (Fig. 5). The compound was potent against HDACs 1, 2 and 3 with IC_{50} values of 3, 4 and 7 nM, respectively. On the other hand, it was significantly less active against the class II members HDACs 5 and 6. CHR-3996 showed good oral bioavailability in rat and dog of 27 and 40 %, respectively. Furthermore, the compound exhibited high systemic clearance (relative to liver blood flow) of 4.2 (dog) and 8.0 L/h/kg (rat), respectively. In vivo HCT116 xenograft experiments showed that the compound had a significant antitumor activity: administration of 50 mg/kg once daily orally resulted in almost complete inhibition of the tumour. CHR-3996 advanced into clinical studies. Phase I clinical studies showed that the compound was rapidly absorbed, had a mean elimination plasma half-life of 3 h and mean AUC values exceeding the levels effective in xenograft studies at doses $\geq$40 mg. Five patients demonstrated stable disease for at least two cycles (Banerji et al. 2010, 2012).

3.3 Phenylhydroxamic Acid Derivatives (AR-42)

The phenylhydroxamic acid derivative AR-42 (known also as OSU-HDAC-42 or (*S*)-HDAC-42) (Fig. 6) was originated at the Ohio State University. The compound resulted from an exploration of new HDAC inhibitors composed of short-chain fatty acids linked to a hydroxamic acid group as Zn^{2+}-chelating motif (Lu et al. 2005). The compound showed good HDAC inhibitory potency with an IC_{50} value of 16 nM and was around five times more potent than its corresponding *R*-isomer. Antiproliferative activities in three myeloma cell lines, IM-9, RPMI-8226 and U266, were in the submicromolar range. The compound was around four to seven times more active than SAHA (Bai et al. 2011). This good potency, but even more its pharamacokinetic behaviour in rodents, has made the compound an attractive agent for further studies. Indeed, Ar-42 displayed a good oral bioavailability in rats (Cheng et al. 2006a) and in mice (Cheng et al. 2006b): the oral bioavailability was ∼ 100 % in rats and 27.4 % in mice. Total body clearance was 1.40 L/h/kg (rats) and 1.47 L/h/kg (mice). The good in vitro potency as well as the PK profile translated in good antitumor activity in different in vivo efficacy models. For example, the compound administered 25 mg/kg daily and 50 mg/kg

Fig. 6 Structure of (*S*)-Ar-42
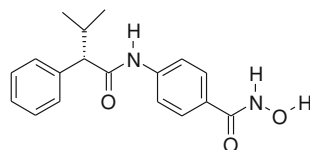
every other day significantly inhibited tumour growth in mouse PC-3 tumour xenografts by 52 and 67 %, respectively (Kulp et al. 2006). The compound was licensed to Arno Therapeutics, which is currently conducting phase I/IIa trials in adult patients with relapsed or recurrent haematological malignancies and solid tumours (http://clinicaltrials.gov/ct2/show/NCT01129193?term=AR-42&rank=1).

3.4 Phenyloxopropenyl and Amidopropenyl Analogues

The HDAC inhibitory programme in DAC was initiated in collaboration with Antonello Mai from the University of Rome, who had intensively studied aroyl-pyrrolyl hydroxyamides (APHAs) as histone deacetylase inhibitors (Massa et al. 2001; Mai et al. 2005a, b, 2006) and identified several class II selective compounds. It is noticeable that these research efforts have origins in programmes carried out by the same group in the late 1980s, when pyrrole-containing analogues of trichostatin were explored for their antifungal, antibacterial and antiviral activities (Massa et al. 1990) but ended with HDAC inhibitory (Massa et al. 2001) activity.

A small library of aryl- and heteroaryl-hydroxamic acid fragments was designed and subjected for biological characterisation (Thaler et al. 2010a). The HDAC inhibitory activity was performed using HeLa nuclear extract as enzyme source and the percentage of inhibition of the enzymes was assessed at 0.1, 1.0 and 10 μM concentration of the inhibitors. As shown in Table 2, the phenyl hydroxamate (1) was almost inactive under the experimental conditions, whereas 2

Table 2 HDAC inhibitory activity of a fragment library
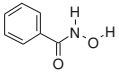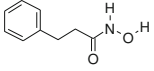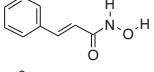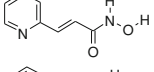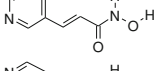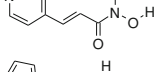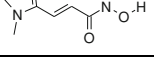
Compounds	Structure	Percentage of inhibition at		
		0.1 (μM)	1 (μM)	10 (μM)
1		0	1	19
2		0	36	89
3		18	55	93
4		34	82	100
5		13	75	96
6		0	34	87
7		9	13	53

Table 3 HDAC enzyme, antiproliferative activity, and microsomal stability data of the phenyloxopropenyl series
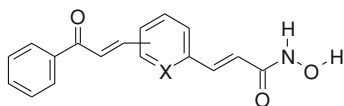
Compound	X	Position	Enzyme (μM)	K562 (μM)	Microsomes	
					Mouse (%)	Human (%)
8	CH	5	1.48	2.28	2	5
9	N	4	0.323	1.06	14	16
10	N	5	0.022	0.69	6	14
11	CH	6	0.085	3.21	6	9
12	N	6	0.020	0.51	11	38

was moderately active with 36 % enzyme inhibition at 1 μM. Introduction of an acrylic group (**3**) led to a further increase in activity. Within the pyridine-acryl-hydroxamic acid series, the pyridine-2-yl derivative (**4**) demonstrated to be the most potent compound and the activity decreased from the pyridine-2-yl to the pyridine-4-yl analogue (**6**). The electron-rich pyrrolyl derivative (**7**), present in the APHA series, was less potent than the other acryl hydroxamates.

The heteroaryl-hydroxamate **4** and the phenylacryl-hydroxamate **3** were selected for further expansion. In the first step, a scaffold with a phenyloxopropenyl moiety (Mai et al. 2005a) positioned in *meta* or *para* position with respect to the acrylhydroxamate substituent was prepared. The *meta* phenyl analogue **11** (Table 3), with an IC_{50} value of 0.085 μM, was almost 20 times more potent than the corresponding *para* derivative **8** (IC_{50} = 1.48 μM). A slightly different behaviour was found in the pyridinyl series: the 2,5 di-substituted derivative **10** and the 2,6 di-substituted analogue **12** exhibited similar IC_{50} value ($\sim$0.02 μM) and were around 15 times more active than the 2,4-substituted derivative **9**. Further, the compounds and in particular the pyridinyl derivatives showed good antiproliferative effects in the chronic myelogenous leukaemia cell line K562. In specific, the cellular IC_{50} values for **10**, **12** and **9** ranged between 0.5 and 1 μM. The phenylacrylates **8** and **11** exhibited IC_{50} values of 2.3–3.2 μM, respectively.

The good biochemical and cellular inhibitory activity of this series, however, was compromised with an overall low metabolic stability in microsomes. It was observed that the compounds were almost completely degraded in mouse microsomes after 30 min incubation: the percentage of the recovery ranged from just 2 % (**8**) to 14 % (**9**) (Table 3). Similar data were obtained in human microsome preparations with the exception of compound **12**, which was more stable than the other examples. Metabolic profiling experiments of **8** in mouse microsomes showed that the major sites of metabolism were the hydroxamic acid moiety and the unsaturated ketone (Thaler et al. 2009). While the major metabolite of the hydroxamic acid moiety was the acrylic acid (**8a**), α,β-unsaturated ketone was either reduced to the corresponding alcohol (**8b**) or to the saturated ketone derivative (**8c**) (Fig. 7).
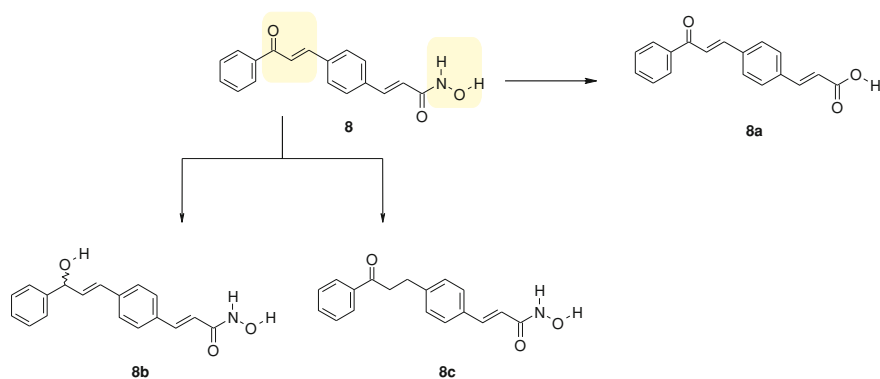
Fig. 7 Metabolic profiling of compound **8** in mouse microsomes

A similar outcome was found for the pyridine derivative **9**. Since the hydroxamic acid was essential for the biological activity, research efforts were directed towards new chemical entities devoid of the metabolically unstable α,β-unsaturated ketone. One approach was the replacement of the phenyl by 4-phenylpiperazine group (Thaler et al. 2010b) (Fig. 8).

Similar to the phenyloxopropenyl series, 4-phenylpiperazinyl analogues of the following four linkers were prepared: phenylacrylate, *meta* and *para* substituted, and pyridin-2-ylacrylate, substituted in positions 5 or 6. As summarised in Table 4, replacement of the phenyl led to a minor decrease in the HDAC inhibitory activity. However, the trends within both series were quite comparable: the *meta* substituted phenylacrylate derivative **15** exhibited an IC_{50} value of 0.354 µM, and was
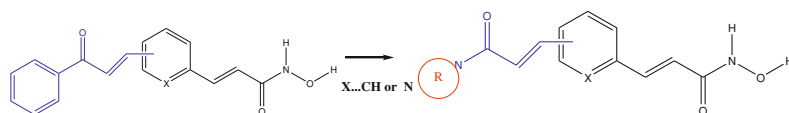
Fig. 8 From phenyloxopropenyl to amidopropenyl series

Table 4 SAR of 4-methyl- and 4-phenyl-piperazinyl analogues **13–16**

Compound	X		R	Enzyme (µM)	Cells (µM)		Microsomes (%)	
					K562	HCT116	Mouse	Human
13	CH	5	Ph	5.60	3.37	5.77	27	51
14	N	5	PPh	0.299	1.41	1.33	91	82
15	CH	6	PPh	0.354	0.980	0.780	72	60
16	N	6	PPh	0.069	1.13	1.07	74	77

about 15 times more potent than the corresponding *para* substituted (**13**). In the pyridinyl series, the differences between 2,5 and 2,6 di-substituted pyridin-2-yl-acrylates were smaller: compound **16** exhibited an IC_{50} value of 0.069 μM and was the most potent example in this series, around four times more than **14**. Comparison of the compounds containing a central phenyl ring with those with the pyridinyl ring showed the inhibitors with the pyridinyl ring were generally more potent. Compounds **14**, **15** and **16** exhibited comparable antiproliferative potency in leukemic K562 and human colon cancer HCT116 cells with IC_{50} values of around 1 μM. Hydroxamate **13**, in line with the lower enzymatic inhibition, had IC_{50} values in K562 and HCT116 cells of 3.77 and 5.77 μM, respectively.

The major difference between the phenyloxopropenyl and the 4-phenylpiper-azinyl series was their microsomal stability: all four amidopropenyl compounds were metabolised <50 % in the tested conditions and were remarkably more stable than the ketone analogues. The only exception was **13** with a recovery of just 27 % in mouse microsomal preparation. These results confirmed that the α,β-unsaturated ketone moiety was a major site of metabolic liability and replacement with an amide group resulted in stable compounds in microsomal preparations.

These encouraging data prompted to explore this series in a greater depth and the hydroxamic acid **16** was selected as starting point for this expansion. The synthetic efforts were concentrated on a series of cyclic amine derivatives substituted by phenyl. This selection was based on the fact that hydrophobic aromatic groups represent a common surface recognition domain in several HDAC inhibitors (Miller et al. 2003). As shown in Table 5, IC_{50} values ranged between

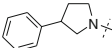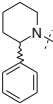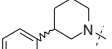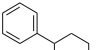**Table 5** SAR of phenyl-cycloamine analogues **17–21**
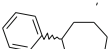
Compound	R	Enzyme (μM)	Cells (μM)		Microsomes (%)	
			K562	HCT116	Mouse	Human
17		0.294	1.220	1.300	66	66
18		0.269	0.616	0.432	52	90
19		0.025	0.445	0.294	74	86
20		0.146	1.730	1.470	75	98
21		0.286	0.568	0.581	29	46

Table 6 Pharmacokinetic profile for **15**, **16** and **19** (administered 5 mg/kg, i.v. and 15 mg/kg, orally)

Compound	15	16	19
AUC$iv_{0-\infty}$ (ngh/mL)	440	590	1191
$t_{1/2}$ (h)	1.22	0.27	0.67
Cl (L/h/kg)	11.37	8.47	4.19
V_{ss} (L/kg)	5.2	0.8	0.55
F (%)	10	2.9	3.1

0.025 μM (**19**) and 0.294 μM (**17**). These differences in activities are quite small compared to the substantial structural variations. These findings can be ascribed to the fact that the surface region of the HDAC enzyme is highly flexible and able to accommodate inhibitors with different capping groups (Somoza et al. 2004; Wang et al. 2005). The compounds exhibited comparable antiproliferative activities in K562 and HCT116 cells; compound **19** (IC$_{50}$ = 0.445 μM) was around four-fold more active than the least potent analogue **20** (IC$_{50}$ = 1.730 μM) in K562 cells. Furthermore, hydroxamic acid **19** was around five times more active than **20** (IC$_{50}$ = 1.470 μM) in HCT116 cells.

All compounds exhibited a good stability in mouse and human microsomes with <50 % of the inhibitors metabolised. The only outlier was compound **21**, with 29 and 46 % remaining in mouse and human microsomes, respectively.

However, the remarkable stability of the compounds in microsomes was not confirmed by in vivo PK experiments in mice. Three selected derivatives, the aryl-hydroxamate **15** and the two heteroaryl-hydroxamates **16** and **19**, displayed a low oral bioavailability with $F \leq 10$ % (Table 6). The compounds also showed very high clearance rates. In particular, inhibitors **15** and **16** showed clearance rates, which exceed the hepatic blood flow of 5.4 L/h/kg in mice (Davies and Morris 1993).

Further investigations revealed that the three representative compounds had high clearance rates in rat and human hepatocytes. These observations indicated a metabolic degradation of these hydroxamic acid derivatives catalysed preferentially by non-microsomal enzymes. Reduction of the hydroxamic acid group to its corresponding amide was identified for the three compounds as one relevant biotransformation pathway. The same outcome was found for SAHA, which was studied as reference compound. Similar results had been previously reported for TSA (Elaut et al. 2002). These observations suggest that this metabolic behaviour is likely to be common to further hydroxamic acids.

These results are in agreement with the observed in vivo PK behaviour in mice. They show that the metabolic stability of these hydroxamates in microsomes was neither found in hepatocytes nor in vivo. ADME experiments employing microsomes are widely used and they are often very suitable indicators for the in vivo PK behaviour. However, our experiences as well as other cases have shown that microsomes may not be sufficient for predicting in vivo clearances of structures containing hydroxamic acid moieties. Hepatocyte preparations, even though more expensive, demonstrated to be a more valid alternative for predicting the in vivo clearance behaviour of the studied hydroxamates.

3.5 Spiropiperidines

The phenyloxopropenyl and the amidopropenyl series resulted in the discovery of several compounds with good in vitro biochemical and antiproliferative activity. However, none of them exhibited an oral bioavailability superior to 20 % in mice (Thaler et al. 2010a, b). Therefore, various other scaffolds were evaluated. Major focus was particularly on privileged structures, which already had provided derivatives with good oral bioavailability. Privileged structures with their inherent affinity for diverse biological targets represent an ideal source of core scaffolds for the design of molecules able to target various receptors (DeSimone et al. 2004; Costantino and Barlocco 2006). One of them is the conformationally constraint 4-oxospiro[chroman-2,4′-piperidine] ring system, which is, for example, present in antiarrhythmic agents (Elliott et al. 1992).

Some 4-oxospirochromanes linked to an acrylhydroxamic acid group as zinc binding motif were prepared based on the results obtained during the exploration of different hydroxamic acid fragments (Fig. 9) (Thaler et al. 2010a; Varasi et al. 2011). In the first run, unsubstituted and the *N*-methyl, *N*-acetyl and *N*-benzyl substituted piperidine analogues (22–25, Table 7) were synthesised. As shown in Table 7, all compounds inhibited the HDAC activity in the submicromolar range. No major differences in potencies were found: the unsubstituted spiropiperidine (22) with an IC_{50} value of 0.082 μM was around eight-fold more active than the least potent one within this series, the acetyl analogue (24). Compound 24 was also the least potent compound within this group against leukemic K562 and colon cancer HCT116 cells, while the other analogues showed good antiproliferative activity in the micromolar or submicromolar range. Next, synthetic efforts were focused on modifications of the central spirochromane core structure. Shift of the acrylamide moiety to position 7 and reduction of the chromane ring size to spirobenzofuran furnished compounds, which were generally less active (Varasi et al. 2011). Then, the importance of the ketone group was assessed. The hydroxy derivative 25a (Fig. 10) and the chromene 25b exhibited IC_{50} values of 1.21 and 1.19 μM, respectively, and were around ten-fold less active than the 4-oxo-chromane 25. Also the chromane 25c ($IC_{50} = 9.0$ μM) and the *N*-acetyl derivatives 25d ($IC_{50} = 5.15$ μM) were significantly less active. In addition, these four compounds showed a lower antiproliferative activity in K562 and HCT116 cells
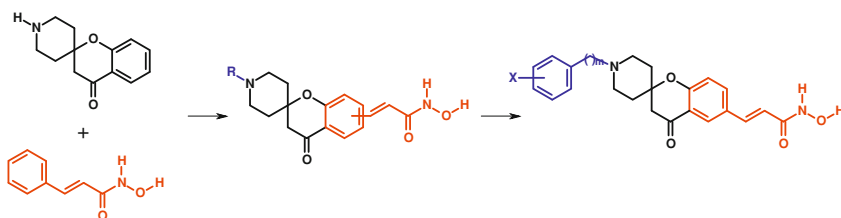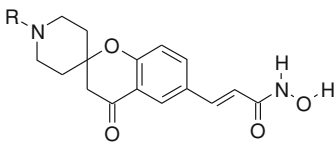

Fig. 9 Spiropiperidines

Table 7 Spiro [chromane-2,4′-piperidine]-N-hydroxy-acrylamides and their activities

Compound	R	Enzyme	Cells (μM)		Microsomes (%)	
		(μM)	K562	HCT116	Mouse	Human
22	H	0.082	5.77	1.59	nd.	nd.
23	CH$_3$	0.288	0.806	0.266	nd.	nd.
24	Acetyl	0.641	9.37	5.66	nd.	nd.
25	Benzyl	0.121	0.399	0.477	32	35
26	Ph	0.410	0.913	1.261	23	4
27	Ph-CH$_2$-CH$_2$-	0.113	0.180	0.201	57	66
28	Ph-(CH$_2$)$_3$-	0.171	0.449	0.445	29	46
29	2-F-Bn	0.118	0.742	0.920	23	36
30	3-F-Bn	0.251	0.585	1.026	32	54
31	4-F-Bn	0.108	0.681	0.777	47	63
32	2-MeO-Bn	0.197	0.150	0.151	40	47
33	3-MeO-Bn	0.199	0.385	0.473	38	36
34	4-MeO-Bn	0.175	0.184	0.205	43	35
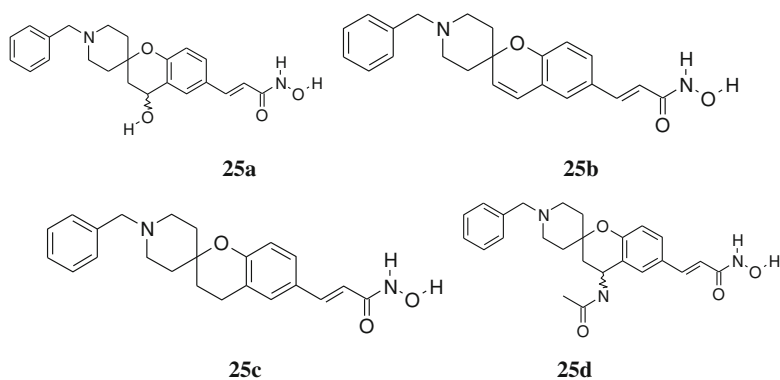
Fig. 10 Analogues of the 4-Oxospirochromane **25** (Table 1)

confirming the importance of the carbonyl group for the biochemical and cellular potency (Varasi et al. 2011).

These explorations consolidated the 4-oxospiro[chromane-2,4′-piperidine]-6-yl series as the most promising scaffold for a further expansion. For this purpose, different N-piperidine substituents and a number of compounds were made around the benzyl derivative **25** (Thaler et al. 2012). The few representatives **22–25**, prepared in the first run, showed no major differences in potencies. This indicated that variations on this site were likely well tolerated. But at that stage, one of the

major objectives was to obtain inhibitors with a good ADME profile with an acceptable activity.

Not surprisingly, no major differences in HDAC inhibitory activities were found. As shown in Table 7, the phenyl spiropiperidine (26) exhibited an IC_{50} value of 0.41 μM, whereas all other tested compounds had IC_{50} values in the range of 0.10–0.28 μM. All compounds showed good antiproliferative activities in K562 and HCT116 cells with IC_{50} values in the submicromolar to low micromolar range. The 2-phenylethyl analogue (27) was the most potent and the spirocycle (26) the least potent compound among the unsubstituted derivatives. Further elongation of the alkyl chain did not result in an increase of activity. Compound 32 with IC_{50} value of 0.15 μM was the most potent compound against K562 and HCT116 cells among the substituted benzyl analogues.

But as already mentioned earlier, the main selection criteria were set by the stability experiments. In fact, the tested compounds already showed different metabolic behaviours in microsomal preparations. In specific, the phenyl analogue (26) was rapidly metabolised both in mouse and human microsomes, with only 23 and 4 % of the product recovered unmodified after 30 min of incubation. The benzyl spirocycle (25) demonstrated a better stability with 32 % remaining in the mouse and 55 % in the human preparation. The 2-phenylethyl analogue (27) was even more stable with 57 % (mouse) and 66 % (human) remaining, respectively. However, this trend did not continue, when the alkyl chain was further increased: the 3-phenylpropyl spirocycle (28) had a microsomal stability similar or even lower than the benzyl derivative (25). The 4-fluorobenzyl derivative (31) emerged as the most stable derivative among the substituted benzyl analogues with 47 and 63 % of the compound recovered in mouse and human microsomes, respectively. Within the fluoro series, the 2-fluorobenzyl derivative (29) had the lowest stability compared to the 3- and 4-fluoro analogues (30) and (31) with 23 and 36 % remaining in mouse and human microsomes, respectively. On the other hand, no major differences were observed among the *ortho*, *meta* and *para* methoxybenzyl inhibitors (32–34).

Three representative examples 25, 27 and 31 displayed good oral bioavailability in mouse PK experiments with an oral bioavailability F of 31.5, 51.1 and 27.6 %, respectively (Table 8). Spirocycle 25 showed a high systemic plasma clearance with 5.88 mL/h/kg, similar to the hepatic flood flow of 5.4 mL/h/kg in mice (Davies and Morris 1993). The other two examples, 27 and 31, exhibited lower clearance rates with 4.79 and 3.35 mL/h/kg, respectively. This outcome is in line

Table 8 Pharmacokinetic properties of 25, 27 and 31 in mice

| Compound	25	27	31
$AUC_{iv0-\infty}$ (ngh/mL)	847	1042	1494
$t_{1/2}$ (h)	8.4	9.1	13
Cl (L/h/kg)	5.88	4.79	3.35
V_{ss} (L/kg)	8.9	8.6	5.9
F (%)	31.5	51.1	27.6

with their superior stability in human and mouse microsomes compared to **25**. Estimated elimination half-lives for the three examples ranged between 8 and 13 h. All three examples had very high estimated steady-state volume of distribution (V_{ss}), suggesting substantial distribution into tissues. The V_{ss} of **27** with 8.6 L/kg was in the same range of the compound **25** (8.9 L/kg). The 4-fluorobenzyl derivative (**31**) had a V_{ss} of 5.9 L/kg, lower than the other two spirocycles, **25** and **27**, but still ∼8 times the total body water in mice (Table 8) (Davies and Morris 1993).

The three spirocycles also showed good in vivo antitumor activity in an HCT116 xenograft model after oral administration. Consistent with the PK experiments, the 2-phenylethyl and the *para* fluorobenzyl analogues (**27** and **31**) were slightly more potent than **25**. In specific, the calculated T/C were 0.2 at 75 mg/kg for **31** and 0.2 and 0.34 at 150 mg/kg for **27** and **25,** respectively (Varasi et al. 2011; Thaler et al. 2012).

3.6 Biphenyl/Arylamide/Styrenyl Hydroxamic Acid Analogues

In mid 1999, the Abbott Medicinal Chemistry started to work on HDAC programme with the preparation and evaluation of several series of hydroxamic acids. Two HDAC inhibitors, the macrocyclic hydroxamate (**35**) and the α-ketoamide (**36**), were evaluated for their selectivity profile in comparison to SAHA and MS-275, but no selectivity was observed for a particular class of HDAC.
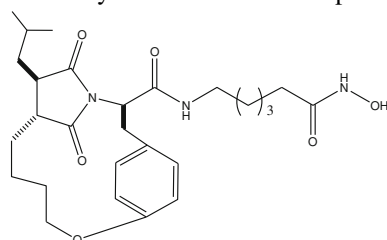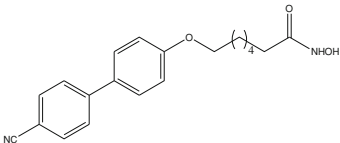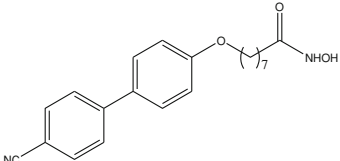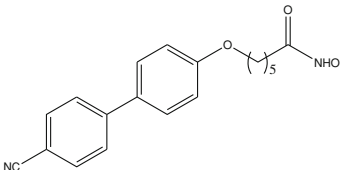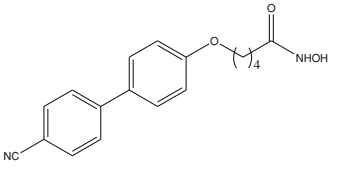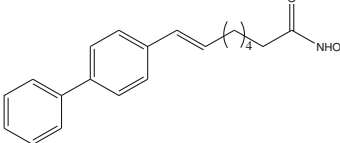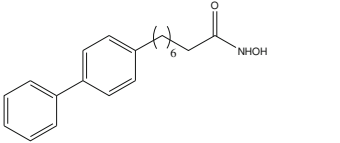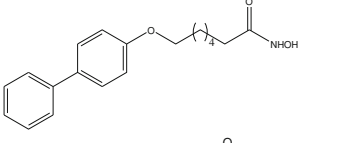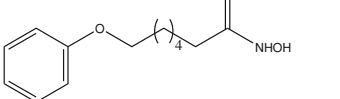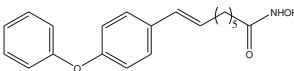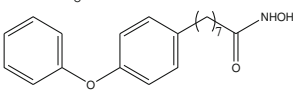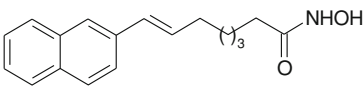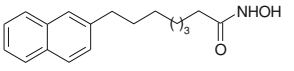

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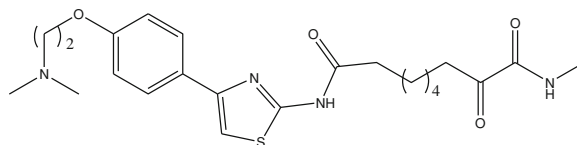
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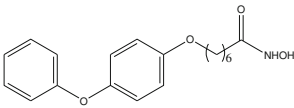
The biphenyl ether series was among the first examined families leading to the identification of **37a** (Table 9) as a screening hit and having complimentary fit to the proposed general structure for HDAC inhibitors (Jung et al. 1999; Jung 2001). The SAR studies concluded that the 6-methylene linker was optimal and a longer/shorter tether showed loss of potency (**37a**, **37b–d**) (Table 9). Reduction of double

Table 9 A series of biphenyl hydroxamic acids

Compound	Structure	HDAC IC$_{50}$ (nM, K562 nuclear extract))
37a		9
37b		31
37c		76
37d		181
37e		5
37f		550
37g		17
37h		178

Table 9 (continued)

Compound	Structure	HDAC IC$_{50}$ (nM, K562 nuclear extract))
37i		31
37j		6
37k		552
38a		6
38b		25

bond to saturated alkyl ether (**37f**) showed loss of inhibitory activity. Similar results were observed for compounds **38a–b** (Woo et al. 2002).

The nitrile group on the biphenyl was not important for activity (**37f** and **37g**), but replacement of the biphenyl moiety with phenyl resulted in a significant loss of potency (**37h**). Replacement of the tether ether of **37i** with alkenyl (**37j**) and alkyl (**37k**) showed similar trends as with compounds **37g**, **37e** and **37h**. All compounds had modest cellular potency (>1 μM) against HT1080 and MDA435 cell lines and were not considered for further studies.

The poor cellular activity profile of SAHA and the 'reverse-amide' series stimulated the evaluation of some biphenyl analogues (**39a–i,** Table 10). The 'SAHA-like' amides, the *para*-biphenyl and *meta*-biphenyl isomers (**39a** and **39b**) were found equipotent and had shown submicromolar cellular activity against both cell lines (Remiszewski et al. 2002). The series has shown beneficial effect by introducing a 4-phenylthiazol-2-yl moiety (**39c**) as compared to **39a** or **39b**. The *meta*-biphenyl analogue (**39e**) was more potent than the *para*-biphenyl isomer (**39d**); the reverse-amide 4-phenyl thiazole (**39f**) was equipotent with the *meta*-biphenyl analogue (**39e**), but exhibited a better cellular activity. Some of the favourable replacements include the 5-phenyl oxazol-2-yl (**39g**), 5-phenyl fur-2-yl (**39h**) and 5-phenylthiophen-2-yl (**39i**) (Curtin et al. 2002).
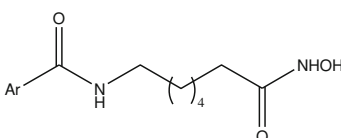
Some heteroaromatic phenyl replacements on the 'reverse-amide' template (**40a**) are shown in Table 11. The 2-pyridyl and 2-pyrrole substituents (**40b** and **40c**) had given equipotent inhibitors and the polycyclic heterocycles (**40d** and **40e**) had shown improved potency. *N*-substitution on the indole moiety of **40d** with phenyl (**40h**) or substituents on the indole phenyl ring (**40i**) led to an increase of potency, while the *N*-methyl analogue (**40g**) was less active (Wada et al. 2003).
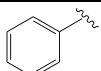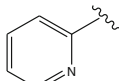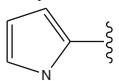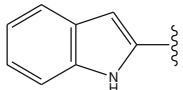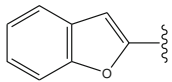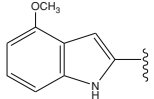
Examples of expected improvements of inhibitory activity by using a highly rigid, lipophilic tether like analogues are shown in Table 12. Submicromolar

Table 10 A series of arylamide hydroxamic acids along with cellular and enzymatic potency
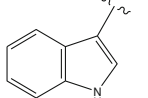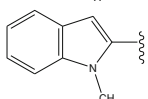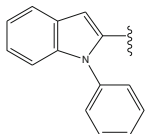
Compound	Structure	HDAC IC$_{50}$ (nM)	HT1080 proliferation IC$_{50}$ (µM)	MDA435 proliferation IC$_{50}$ (µM)
SAHA	–	120	2.4	1.9
39a		6	0.47	0.38
39b		14	0.67	0.50
39c		0.7	0.13	0.04
39d		23	1.5	0.52
39e		3	2.3	9.1
39f		4	0.52	0.47
39g		22	0.34	0.31
39h		5	1.5	0.38
39i		4	0.40	0.20

Table 11 Arylamide hydroxamic acids and their enzymatic and cellular potency

Compoundd	Ar	HDAC IC$_{50}$ (nM)	HT1080 proliferation IC$_{50}$ (μM)	MDA435 proliferation IC$_{50}$ (μM)
40a		1000	–	–
40b		1680	–	–
40c		524	–	–
40d		15	0.14	0.15
40e		30	0.76	0.49
40f		38	1.0	0.72
40g		56	0.78	0.39
40h		4	2.5	0.76
40i		3	0.12	0.13

activity was observed with the styrenyl tethers (**41a**, **41b**) and the potency was diminished with saturation of double bond (**41c**). The para substituted phenyl derivative **41a** resulted to be slightly more active than the corresponding 1,3-phenyl analogue (**41d**). The 2-anilino-2-oxo-ethyl analogue of **41a** resulted to be almost three times more active in the HDAC inhibitory assay and showed an

Table 12 Series of styrenyl hydroxamic acids and their enzymatic and cellular potency

Compound	Structure	HDAC IC$_{50}$ (nM)	HT1080 proliferation IC$_{50}$ (μM)	MDA435 proliferation IC$_{50}$ (μM)
41a		260	–	–
41b		640	–	–
41c		3200	–	–
41d		450	–	–
41e		97	0.58	0.37
41f		>5000	–	–
41g		15	0.88	0.63
41h		6	0.10	0.07

antiproliferative activity with IC$_{50}$ values in the submicromolar range. Further modifications of **41e** as in *meta*-biphenyl analogue (**41g**) and 4-phenylthiazol-2-yl-hydroxamate (**41h**) had shown excellent inhibitory and cellular activity (Curtin et al. 2002).

In a further study, the linking amide functionality in the arylamide **39c** was removed giving hydroxamates **42a** and **42b**. Both compounds showed a lower

HDAC inhibitory activity with IC_{50} values of 19 nM (**42a**) and 9 nM (**42b**), respectively, as well as a reduced cellular activity (Dai et al. 2003).

42a

42b
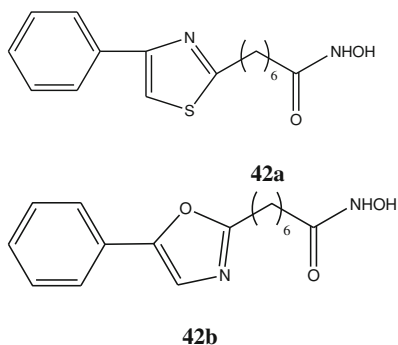
As illustrated in the previous examples, variations of the cap group are in general well tolerated. Even significant structural variation, e.g. by introducing a macrocyclic cap group resulted in derivatives with potent HDAC inhibitory activity (Sternson et al. 2001). For example, the hydroxamic acid (**43**) having a cyclic tetrapeptide terminal group has shown reversible HDAC inhibition (HDAC1 (IC_{50} = 1.9 nM) with a slight selectivity over HDAC6 (IC_{50} = 19 nM).

43
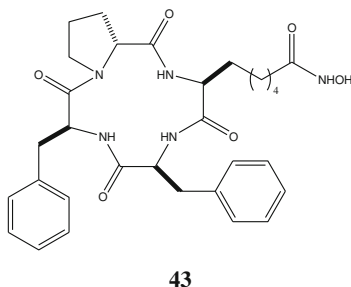
Modifications of the peptidic analogue **43** by replacing the cyclic tetrapeptide with the peptidomimetic core of Abbott's macrocyclic matrix metalloproteinase inhibitor (Steinman et al. 1998) resulted in derivatives, which had reduced HDAC inhibitory activity. For example, the macrocyclic hydroxamic acid inhibitors **44** and **45a** (Fig. 11) exhibited HDAC IC_{50} values of 2.1 μM and 38 nM, respectively (Curtin et al. 2002).

The studies concluded that the parent macrocycle succinimide **45a** had significant antiproliferative activity, but any further modifications did not make any significant improvement in the HDAC and cellular growth inhibitory activity (Table 13).

The nature of the amino acidic side chain was important for activity. Compound **45g**, devoid of benzyl moiety, resulted to be a weak HDAC inhibitor with no measureable antiproliferative potency at the tested conditions. Compound **45h**,
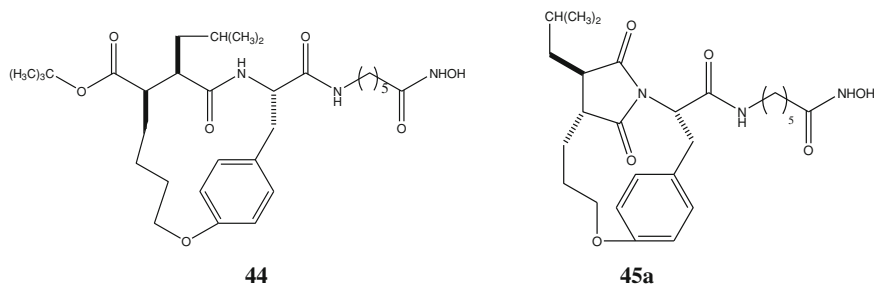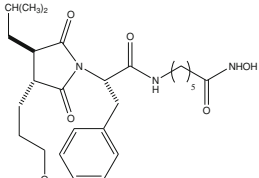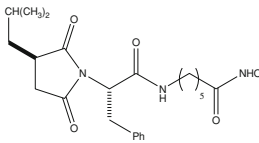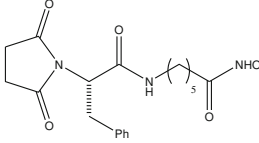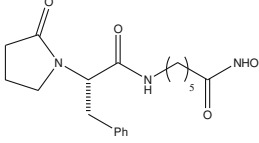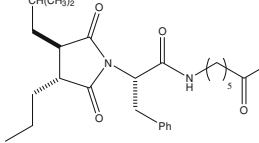
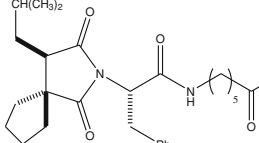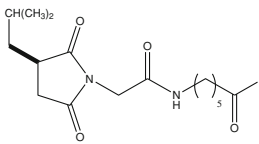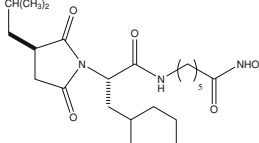**Fig. 11** Macrocyclic hydroxamic acid analogues

which comprises an L-cyclohexylalanine residue, regained the potency and the *para* methoxy-Ph analogue **45i** exhibited potencies similar to the macrocyclic inhibitor **45a**. The penta- and hexa-methylene tethered analogues of the disubstituted succinimide (**45e** and **45j**) had similar activity, while the tetra-methylene analogue (**45k**) was much less potent (Curtin et al. 2002; Curtin and Glaser 2003).

3.7 Tetrahydroisoquinoline-Based Hydroxamic Acid Derivatives

A novel class of tetrahydroisoquinoline-based hydroxamic acid analogues was evaluated in vitro and in vivo as potential anticancer and HDAC inhibitor agents at the Shandong University (Zhang et al. 2010, 2011a, b). The designing was based on the common pharmacophore having three parts, the Zn^{2+} binding group and a linker and the surface recognition domain (Miller et al. 2003). The 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid having distinct geometrical conformation and biological activity (Klutchko et al. 1986) was used to design novel HDAC inhibitors (**46**, **47**, **48**, Table 14) (Zhang et al. 2010). Most of the target compounds showed an inhibitory activity against HDAC8 comparable to SAHA, some of them were more potent than the reference compound. Modifications of the R_1, both in the BOC series **46** and well as for the free amine analogues **47**, were well tolerated with IC_{50} values ranging from 1.06 (**47l**) to 8.21 μM (**47a**). Compounds with aromatic R_1 groups (**46a–m**, **47a–m**) were slightly more potent than the compounds bearing aliphatic R_1 groups (**46n–p**, **47n–p**).

A further expansion led to compound **48a**, which resulted to be more potent than the derivatives in the **46** or **47** series, such as **46d**. On the other hand, the rigid compounds **48b** and **48c** were found almost inactive. These results indicated that variations of the substituents on the secondary amine influence the inhibitory activity more than the R_1 groups in the **46** or **47** series. Some of the compounds showed also better antiproliferative activity against HCT116, SKOV3 and HL60 cell lines.

Table 13 Structures of succinimide acids and their enzymatic and cellular potency

Compound	Structure	HDAC IC$_{50}$ (nM)	HT1080 proliferation IC$_{50}$ (µM)	MDA435 proliferation IC$_{50}$ (µM)
45a		38	0.25	0.15
45b		99	2.3	0.67
45c		660	14	10
45d		>5000	–	–
45e		51	0.40	0.57
45f		66	1.5	0.7
45g		5000	–	–
45h		640	–	–

(continued)

Table 13 (continued)
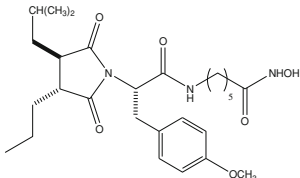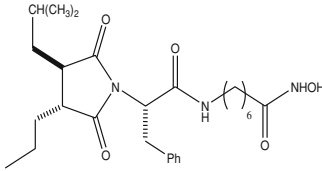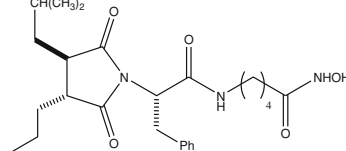
Compound	Structure	HDAC IC$_{50}$ (nM)	HT1080 proliferation IC$_{50}$ (µM)	MDA435 proliferation IC$_{50}$ (µM)
45i		38	0.53	0.18
45j		230	2.9	1.6
45k		1600	–	–

Table 14 Structures and HDAC8 inhibitory activities of compounds **46a–p** and **47a–p**

46a-p   **47a-p**
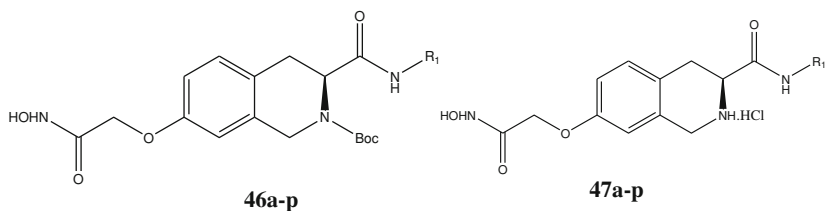
Compound	R$_1$	IC$_{50}$ (µM, HDAC8)
46a	-Ph	1.29
47a		8.21
46b	-CH$_2$-Ph	3.41
47b		5.10
46c	-(CH$_2$)$_2$-Ph	2.67
47c		4.07
46d	-(p-OCH$_3$)Ph	1.00
47d		5.57
46e	-(p-CH$_3$)Ph	1.65
47e		3.82

Table 14 (continued)
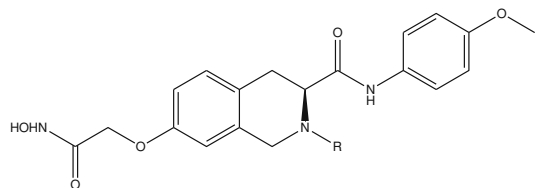
Compound	R_1	IC$_{50}$ (μM, HDAC8)
46f	-(o-CH$_3$)Ph	4.00
47f		4.13
46g	-(m-CH$_3$)Ph	1.77
47g		3.62
46h	-(p-F)Ph	2.56
47h		3.23
46i	-(m-Cl)Ph	1.17
47i		3.20
46j	-(o,p-diCH$_3$)Ph	3.78
47j		3.39
46k	-(m-Cl,p-F)Ph	1.55
47k		3.34
46l	-1-naphthyl	4.25
47l		1.06
46m	-(p-Ph)Ph	1.98
47m		2.21
46n	-(CH$_2$)$_4$CH$_3$	3.02
47n		3.54
46o	-(CH$_2$)$_5$CH$_3$	4.42
47o		5.77
46p	-C(CH$_3$)$_3$	4.58
47p		12.17

48a

48b

48c
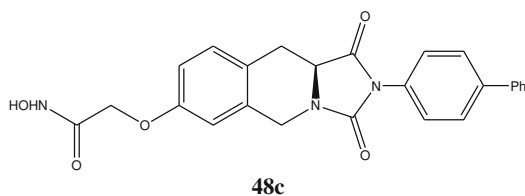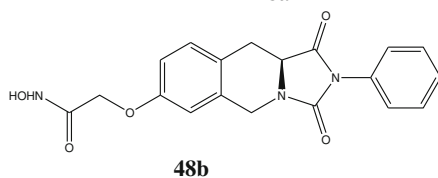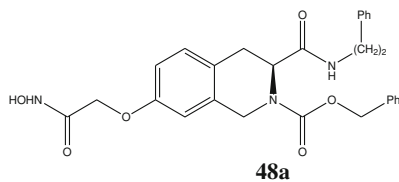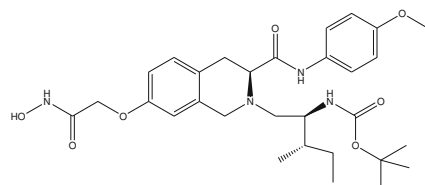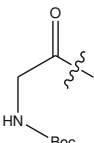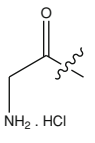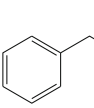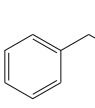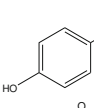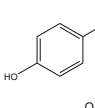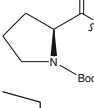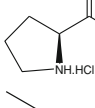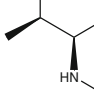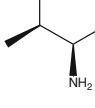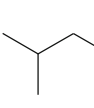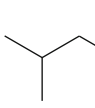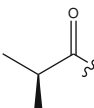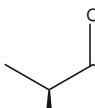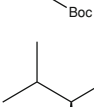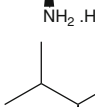
49a
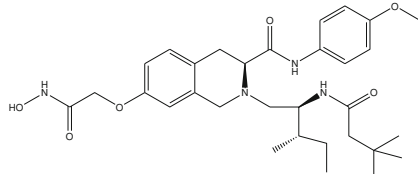
49b
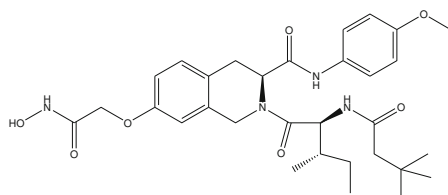
49c

Based on the lead structure **46d**, further extensive structure–activity relationship studies were performed in order to optimise anticancer activities of this series (Zhang et al. 2011b). For this purpose, the R_1 moiety (4-OCH$_3$–Ph) was kept fixed and the Boc group was replaced with other functional groups as shown in Table 15; the less polar BOC protected compounds (**50a–h**) were more potent than the corresponding deprotected analogues (**51a–h**). The reduction of the amide group to a tertiary amine group was found detrimental (**50l–n**). The antiproliferative activities of potent compounds were found similar to that of SAHA in A549 (lung cancer) and MDA-MB-231 (breast cancer) cell lines (Zhang et al. 2011b). Furthermore, compound **50e** showed in vivo antitumor activity comparable to SAHA.
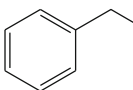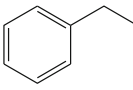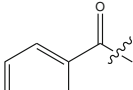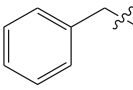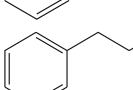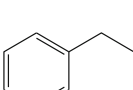
As further exploration, the synthetic efforts were directed towards compounds (examples **52a–c**) having the tertiary butyloxycarbonyl group and a better water solubility (Fig. 12), but the in vitro results were inferior to those of **50e**. A further exploration culminated in compounds **53a**, **b**, (Fig. 13) which exhibited mid-nMIC$_{50}$ values against HDAC8 and potent growth inhibition in multiple tumour cell lines. Compounds **50e** and **52a–c** were selected for in vivo activity experiments and showed good anticancer potencies comparable to SAHA in a human breast carcinoma (MDA-MB-231) xenograft model after ip administration.

Table 15 Structures and HDAC8 inhibitory activities of compounds **50a–n** and **51a–h**

Compound	R	IC$_{50}$(µM) HDAC8	Compound	R	IC$_{50}$ (µM) HDAC8
50a		0.51	**51a**		2.14
50b		0.103	**51b**		0.368
50c		0.175	**51c**		0.634
50d		0.212	**51d**		0.481
50e		0.139	**51e**		1.04
50f		0.163	**51f**		0.675
50g		0.182	**51g**		1.28
50h		0.104	**51h**		1.02

(continued)

Table 15 (continued)

Compound	R	IC$_{50}$(μM) HDAC8	Compound	R	IC$_{50}$ (μM) HDAC8
50i		0.141	**50m**		1.02
50j		0.164	**50n**		1.92
50k		0.114	**50l**		1.72

52a-c

52a (ZYJ-34a), R =

52b (ZYJ-34b), R =

52c (ZYJ-34c), R =
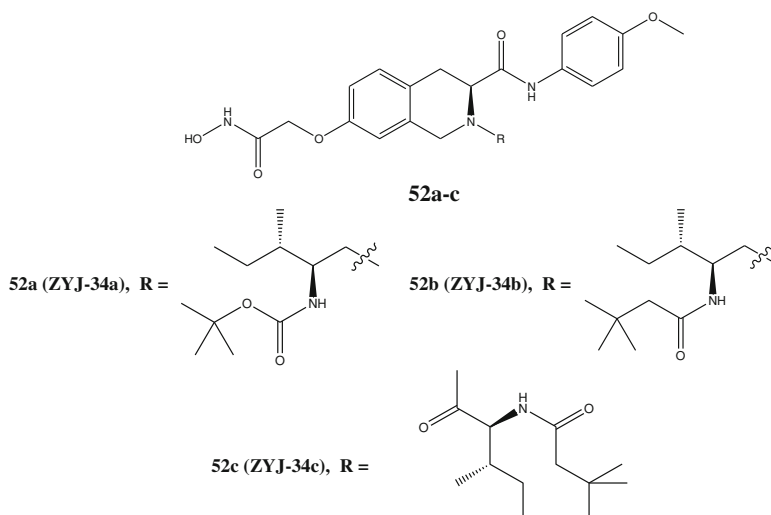
Fig. 12 Structures of hydroxamic acid based HDAC inhibitors having tertiary butyloxycarbonyl group

3.8 N-*Hydroxy Phenylacrylamide Derivatives*

Research activities at the Novartis Institutes for Biomedical Research resulted in a series of potent HDAC inhibitors, among them the clinical candidates LAQ824 and LBH589 in the early 2000 (Remiszewski et al. 2003). The development of second-generation HDAC inhibitors was aimed towards improving in vitro potency and in vivo efficacy of hydroxamate-based HDAC inhibitors and simultaneous elimination of interactions with cardiac ion channels linked to QT prolongation (Shultz et al. 2011). The N-hydroxy phenylacrymide pharmacophore

Fig. 13 Structures of compounds **53a** and **53b**

53a-b

53a, R=   **53b, R=**
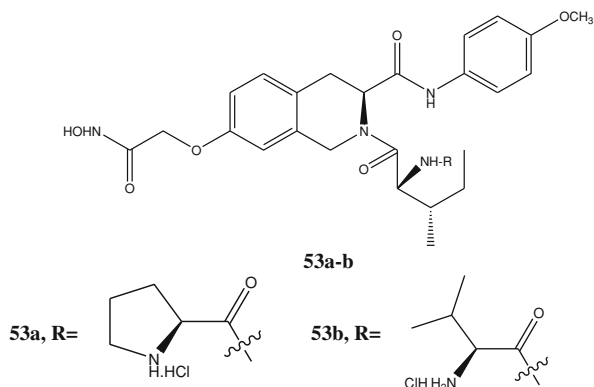
explored also in their first-generation HDAC inhibitors was modified using structure-based drug design, physicochemical property modulation and a matched molecular pair approaches and a series of data were prepared (Shultz et al. 2011, see Table 16). The cardiac safety profile was measured using the *i*CSI (in vitro cardiac safety index) parameter. Compounds **54a**, **54b** and **54c** were found to be highly potent, efficacious, and having greater in vitro cardiac safety as compared to several HDAC inhibitors in clinical trials. Steric effect and amphiphilicity were identified as the contributing parameters for the activity (Table 16). Compounds **54a** and **54c** showed significant in vivo antitumor activity in an HCT116 xenograft model. The tumour growth regression in mice treated with 10 mg/kg of **54a**, administrated iv, qd for eight total doses, or with **54c**, administered 50 mg/kg iv, qd for 13 total doses, resulted to be 22 or 9 %, respectively.

Table 16 Azaindole analogues and their inhibitory activity and *i*CSI against HDAC1, HCT116 and hERG

Compound	R_1	R_2	IC_{50} (nM)			iCSI
			HDAC-1	HCT116	hERG (%)	
54a		-CH(CH$_3$)$_2$	4	4	36	>7500
54b		-H	5	122	20	>256
54c		-H	3	4.5	31	>6667

4 Isoform Selective HDAC Inhibitors

Sodium *n*-butyrate, trichostatin and SAHA have in common that their biological targets were unknown at the time when their antitumor activity in different cell lines was assessed. Their activity against HDACs has been only ascertained afterwards. Most of HDAC inhibitors are natural products or derivatives, such as the cyclic tetrapeptide trapoxin, which contains an epoxide group capable of binding irreversibly with histone deacetylase enzymes (Itazaki et al. 1990; Kijima et al. 1993), and the thiol FK228. FK228 was the second HDAC inhibitor reaching the market after gaining approval by the FDA in November 2009 (Ueda et al. 1994; Nakajima et al. 1998; Bertino and Otterson 2011; Grant et al. 2010; Jain and Zain 2011). After the discovery of the above-mentioned inhibitors, the knowledge on HDACs and their biological functions has grown rapidly. New assays have been developed and crystal structures of several HDACs have been disclosed after the structure of *Aquifex aeolicus* HDAC homologue HDLP (Finnin et al. 1999). Thus, with the advances of the biological knowledge on HDACs, there is no surprise that the selectivity profile for TSA and SAHA was found to be typical for "pan-inhibitors". The compounds were able to inhibit all enzymes with similar potency, though this belief has been recently challenged (Bradner et al. 2010).

Currently, the more advanced compounds can be classified either as pan-HDAC inhibitors, such as the majority of the hydroxamic acids, or as class I selective inhibitors, such as the pyrimidinhydroxamates CHR-3996 or JNJ-26481585 (Arts et al. 2009) as well as some non-hydroxamic acid derivatives like aminoanilide MGCD0103 (Garcia-Manero et al. 2008; Younes et al. 2011). In addition, there are compounds, like the thiol FK228, which can be described as a potent class I inhibitor and a moderate class II inhibitor. The clinical studies of these HDAC inhibitors have shown a similar toxicity profile, which includes gastrointestinal disturbances, fatigue and in several cases cardiotoxicity (Marsoni et al. 2008; Hymes 2010; Lynch et al. 2012). Toxicities were similar in pan-HDAC inhibitors, class I selective inhibitors and FK228. Thus, the clinical outcome has left open the question, whether selective inhibitors of HDACs would be less toxic and overall more advantageous compared to the more advanced HDAC inhibitors as anti-cancer agents.

As mentioned above, the growing understanding of HDACs and their biological functions provided new tools or knowledge, which is crucial for the design of new selective HDAC inhibitors. Recombinant HDAC proteins as enzymes for the assays have become available. Furthermore, class IIa and IV specific substrates have been identified, which allow a precise profiling of compounds against bio-chemically active HDAC1-11 (Hauser and Jung 2009; Lahm et al. 2007; Bradner et al. 2010; Madsen and Olsen 2012). Interestingly, it has been recently found that HDAC3 showed in vitro decrotonylase activity (Madsen and Olsen 2012). Thus, the knowledge on HDACs continues to grow and it won't be surprising to find that these enzymes may be possibly involved in further activities in cellular environments. In this context, it should be remembered that HDACs are components of

multiprotein complexes (frequently having multiple enzymatic activities); and testing isolated recombinant HDACs in biochemical assays may not necessarily represent the best mode to reveal their cellular enzymatic functions. The crystal structures of human histone deacetylases HDAC2 (Cronin et al. 2009), HDAC3 (Watson et al. 2012), HDAC4 (Bottomley et al. 2008), HDAC7 (Schuetz et al. 2008) and HDAC8 (Somoza et al. 2004; Vannini et al. 2004) revealed the differences in the active enzymatic sites. These discoveries have provided new tools for the design of isoform selective compounds or compounds with a different selectivity profile than the older ones. These compounds may clarify if the toxicities seen in the more advanced HDAC inhibitors could be associated to one or more HDAC isoform(s).

At present, there are some examples, which exhibit isoform selectivity different from that of the above described HDAC inhibitors (Fig. 14). One example is the HDAC8 selective inhibitor PCI-34051 (Pharmacyclics) (Balasubramanian et al. 2009). PCI-34051 was found to be over 200-fold selective for HDAC8 compared to HDAC1-3, HDAC6 and HDAC10. The compound selectively induced apoptosis in cell lines derived from T cell lymphomas and leukaemias, but not in other haematopoietic or solid tumour cells. A further example is compound **55**, presented as A8B4 (Tang et al. 2011). The compound showed IC_{50} values of 0.023 μM against HDAC8, 3.6 μM against HDAC2, and 15 μM against HDAC3/nCoR2. No further data are yet available. Other examples comprise some *ortho* substituted *N*-hydroxycinnamates, e.g., **56** (Huang et al. 2012). Compound **56** exhibited IC_{50} values of 27.2 ± 3.1 nM against HDAC8, 3 μM against HDACs 1 and 3 and ≥20 μM against HDACs 2, 4, 6, 10 and 11.

More examples can be found in the field of HDAC6 selective compounds (Fig. 15). The first example, tubacin, was already discovered in 2003, but its development was hampered by its poor drug properties (Haggarty et al. 2003). A
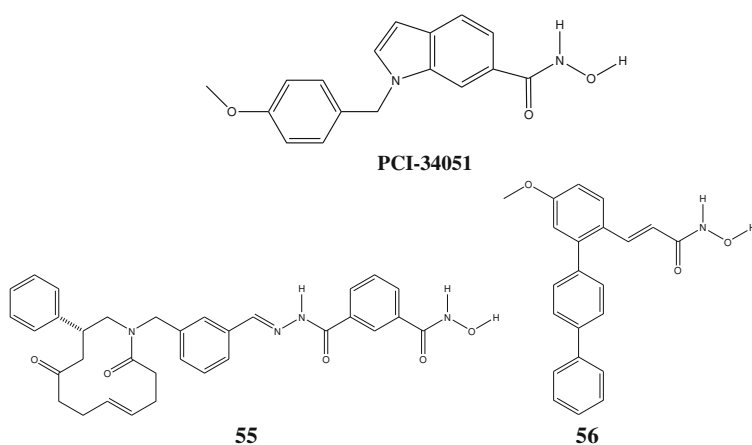
Fig. 14 HDAC8 selective compounds

Tubacin

Tubastatin A

Nexturastat A

57

(S)-9

ACY-1215
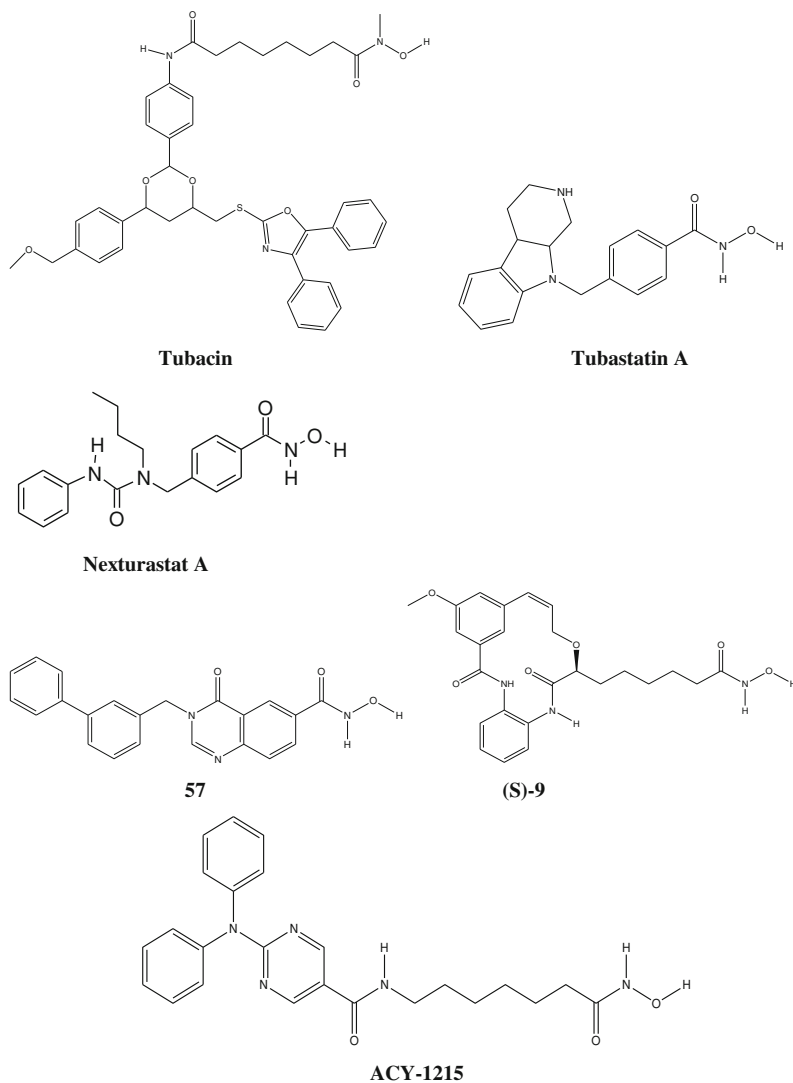
Fig. 15 HDAC6 selective compounds

homology model of HDAC6 revealed that the dimensions of the outer rim of the catalytic channel differ greatly between HDAC6 and HDAC1 (17.5 Å vs. 12.5 Å) (Butler et al. 2010). This structural diversity allowed designing compounds containing a cap moiety large and rigid enough to accommodate the rim region of HDAC6 but not of HDAC1. The resulting lead compound Tubastatin A was more than 1,000-fold selective versus all isoforms excluding HDAC8, where it had approximately 57-fold selectivity. The same laboratory disclosed recently the urea derivative Nexturastat A, which—compared to Tubastatin A—exhibited an

improved inhibition of HDAC6, while maintaining the selectivity of approximately 600-fold relative to the inhibition of HDAC1 (Bergmann et al. 2012). The compound showed also a superior antiproliferative activity against B16 melanoma cells (GI_{50} = 14.3 ± 1.15 μM) than Tubastatin (40.5 ± 1.21 μM), but lower than LBH589 (0.150 ± 0.001 μM).

Also the HDAC6 selectivity has been found by exploring different hydroxamic acids linked to a macrocyclic structure (Auzzas et al. 2010). Representative compound (**S**)-**9** inhibited HDAC6 with an IC_{50} value of 0.84 nM. The inhibitor was around 30 times more selective over HDAC1 and HDAC7 and even more over the other isoforms. HDAC6 selective inhibitors have been also described by scientists from Millennium Pharmaceuticals. For example, representative compound **57** (exemplified as I-126 in the in WO2012/054332) blocked HDAC6 activity by 95.6 % at 0.37 μM (England et al. 2012).

Currently, ACY-1215 has attracted great interest. The HDAC6 selective inhibitor initiated phase I/II clinical studies in July 2011 (www.clinicaltrials.gov/ct2/show/NCT01323751). The compound exhibited an IC_{50} value of 5 nM against HDAC6 and was around ten times less active against HDACs 1, 2 and 3. The activities against the class II enzymes HDAC 4, 5, 7 9 and against HDAC11 were in the micromolar range (Santo et al. 2012). Antiproliferative activities in Multiple Myeloma (MM) cell lines resulted with IC_{50} values ranging from 2 to 8 μM. A synergistic effect was found, when the compound was dosed in combination with bortezomib. These synergistic effects were seen both in vitro and in vivo in a human MM xenograft mouse model.

Inhibitors selective for other enzymes are even less explored. A series of diphenylmethylene hydroxamic acids as class IIa selective HDAC inhibitors have been described by scientists from Methylgene (Fig. 16) (Tessier et al. 2009). One of the described compounds, *N*-hydroxy-9*H*-xanthene-9-carboxamide (**58**), was somewhat more selective for HDAC7 with an IC_{50} of 0.05 μM. Other class II selective compounds have been disclosed in WO2012/103008 (CHDI Foundation Inc. 2012). Representative compound **59** had IC_{50} values of 0.02 μM against HDAC4 and 0.22 μM in human T cell lymphocyte Jurkat E6.1 cells. These structures are quite different from the hydroxamic acids described so far. In fact,

Fig. 16 Class IIa selective compounds

58   **59**
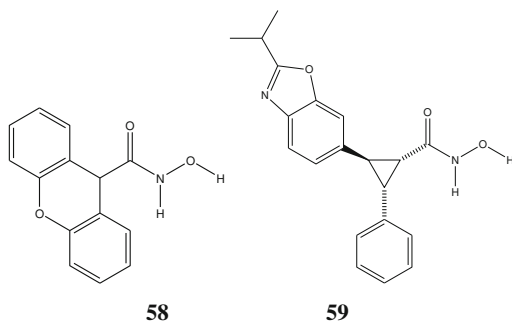
the X-ray structure of HDAC7 (Schuetz et al. 2008) revealed that the catalytic domain of class IIa HDACs is significantly different from that of the previously reported class I and class IIb-like HDACs. The presence of a hydrophobic pocket in the enlarged active site of HDAC7 is absent in class I and IIb and allows to accommodate compounds with bulky moieties adjacent to the hydroxamic acid as Zn^{2+}-chelating motif.

5 Computational Studies

In 1999, the histone deacetylase-like protein (HDLP) in complex with TSA and SAHA has been elucidated (Finnin et al. 1999). Later, various structure-based and ligand-based computational studies were carried out towards development of HDAC inhibitors (Wang 2009) and the results of various studies are as summarised below:

- Very few isoforms selective HDAC inhibitors have been studied and rarely any compound having high selectivity has been found.
- The zinc-binding groups are present in the majority of HDAC inhibitors. The ionisation states of the HDAC enzymes and the bound ligands need further study.
- Future consideration is required towards the flexibility of the HDAC8's pocket and surface mobility.
- For rapid virtual screening of libraries, a more effective scoring function with predictive power is required.

Very few QSAR studies on HDAC inhibitors have been reported until now. The first QSAR model was developed using TSA- and SAHA-like hydroxamic acids (Lan-Hargest et al. 2002) suggesting the importance of shape and area of the molecules for biological activity (Wang et al. 2004). Further, on a data set of 124 compounds, a QSAR study has been reported. The model has shown role of van der Waals surface area and hydrophobicity towards biological activity (Xie et al. 2004). A series of substituted biaryl hydroxamates and mercaptoacetamides as HDAC inhibitors against pancreatic cancer cell growth with nanomolar potency have been designed and synthesised (Kozikowski et al. 2008) and QSAR derived equations have shown significant correlations between different HDAC isoforms (Wang 2009).

The binding mode of indole amide analogues in human HDAC1 catalytic core has been explored by Guo et al. (2005). Further, 3D-QSAR model using CoMFA and CoMSIA has been established for 29 substituted hydroxamic-based HDAC inhibitors with an indole amide residue at the terminus. A comparison between both studies concluded good correlation between the two analyses. On the same set of compounds, a multiple regression analysis by Bajpai et al. (2013) revealed that the inhibition of the histone deacetylase by this series of compounds might involve the dispersion interaction with the receptor where charge transfer between pairs of atoms might greatly help to polarise the molecule. The results obtained by this

multiple regression analysis were in good agreement with those obtained by CoMFA and CoMSIA.

Juvale et al. (2006) performed CoMFA and CoMSIA studies on a data set of 40 hydroxamate analogues reported by Jung et al. (1999), Remiszewski et al. (2002) and Woo et al. (2002). The derived models have shown significance of steric and electronic fields along with lipophilicity as contributing parameters. A 3D-QSAR pharmacophore model has been developed using 30 known HDAC inhibitors (Chen et al. 2008). It concluded the essential ligand features, i.e. hydrogen-bond acceptor and hydrogen-bond donor features corresponding to the metal-binding function and coordination to the Zn(II) ion. Along with this, the hydrophobic/π-π stacking interactions between ligand and enzyme play a critical role for the inhibitory activity.

6 Development of HDAC Imaging Agents

As outlined above, a significant number of HDAC inhibitors have entered various clinical phases. In vivo imaging of HDACs and their inhibition is important and hence development of appropriate imaging agents has become a major challenge.

The conventional anatomic imaging modalities used to monitor clinical response can only help to identify tumour size or rudimentary physiologic changes and both of them occur relatively late after treatment onset. The ex vivo techniques can be utilised to analyse readily accessible tissue specimens. Thus, imaging probes are needed to investigate the efficiency of existing novel HDAC inhibitors with the hope that they may provide basic scientific insights which could lead to novel clinical applications.

Very few compounds for in vivo HDAC imaging have been reported. Some examples include 6-([^{18}F]fluoroacetamide)-1-hexanoicanilide (FAHA) (**60**) (Reid et al. 2009) and [^{11}C]MS-275, a carbon-11-labeled version of the benzamide class HDAC inhibitor MS-275 (entinostat) (**61**) for cerebral imaging (Hooker et al. 2010). These efforts were terminated because the short half-life of carbon-11- ($\sim$20 min) compared to fluorine-18 ($\sim$110 min) presents technical challenges and limits potential applications. Ronen and co-workers studied Boc-lysine trifluoro- acetic acid 6 (BLT) as a substrate for HDACs using ^{19}F magnetic resonance spectroscopy (Sankaranarayanapillai et al. 2008). Hendricks et al. reported devel- opment of ^{18}F-suberoylanilide hydroxamic acid (^{18}F-SAHA) (**62**), a close analogue of the clinically relevant SAHA. This compound results to be one of the first ^{18}F- position emission tomography (PET) HDAC imaging agent (Hendricks et al. 2011).
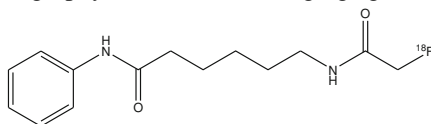
60
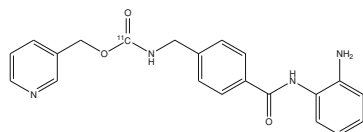
61
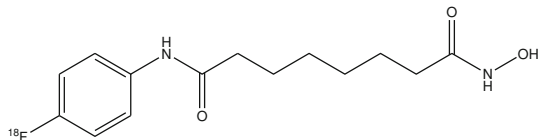
62

7 Conclusion

Hydroxamic acids demonstrated to be very potent inhibitors of several enzymes, such as matrix metalloproteinases, carbonic anhydrases, lipoxygenases, ureases and many others (Muri et al. 2002; Gupta and Sharma 2013). However, hydroxamic acid derivatives are often considered as poor drugs and are down prioritised in drug discovery programmes despite their good in vitro potency, the reasons being their poor physicochemical and ADME properties. Drug discovery programmes related to hydroxamic acids as HDAC inhibitors faced the same issues; nevertheless, the research efforts in this field have been appreciable and several successes achieved such as discovery of acetohydroxamic acid (Marwick 1983), adrafinil (Siwak et al. 2003) and SAHA (Grant et al. 2007). SAHA was considerably efficacious in different haematological malignancies, even though having just medium potency and being cleared rapidly (Kelly et al. 2003; Marks and Breslow 2007), while the clinical outcome in other cancers remained much more uncertain and often rather limited (Graham et al. 2009; Mercurio et al. 2010). However, the notable in vitro and in vivo data of these first inhibitors made HDACs an attractive target for drug discovery programmes. Intensive research efforts resulted in a remarkable number of clinical candidates—over ten hydroxamic acid derivatives—for tumours and some other diseases. This chapter presents a case history of the drug discovery for the treatment of cancers based on HDAC inhibition by hydroxamic acids with a particular attention for the PK properties of the drugs discovered. Extensive in vitro ADME assays, for example by studying the stability of the inhibitors in hepatocytes, or PK cassette experiments as well as computational tools for simulating the ADME properties have been proved fundamental for overcoming the poor drug properties.

The toxicity profile of SAHA and of other more "mature" HDAC inhibitors are not strictly related to the hydroxamic acid groups, but can also be related to 2-aminoanilides, such as MGCD0103, or to thiols such as FK228. To date, a major topic in the HDAC inhibitory field is the development of selective HDAC inhibitors

with a different selectivity profile than those already in advanced clinical studies. These compounds may clarify if the toxicities observed in the more advanced HDAC inhibitors might be associated with one or several HDACs. For future drug discovery programmes based on hydroxamic acid derivatives, the experiences gained from these preclinical and clinical studies may be of great value.

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