Bioorganic & Medicinal Chemistry Letters 20 (2010) 3314–3321

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Acylurea connected straight chain hydroxamates as novel histone deacetylase inhibitors: Synthesis, SAR, and in vivo antitumor activity

Haishan Wang [a,*], Ze-Yi Lim [a], Yan Zhou [a], Melvin Ng [a], Ting Lu [a], Ken Lee [a], Kanda Sangthongpitag [b], Kee Chuan Goh [b], Xukun Wang [b], Xiaofeng Wu [b], Hwee Hoon Khng [b], Siok Kun Goh [b], Wai Chung Ong [b], Zahid Bonday [b], Eric T. Sun [a]

[a] Department of Chemistry Discovery, S*BIO Pte Ltd, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore
[b] Department of Biology Discovery, S*BIO Pte Ltd, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore

ARTICLE INFO

Article history:
Received 9 February 2010
Revised 1 April 2010
Accepted 10 April 2010
Available online 14 April 2010

Keywords:
HDAC inhibitor
Acylurea
Hydroxamic acid
SAR
Anticancer
Efficacy

ABSTRACT

Thirty-six novel acylurea connected straight chain hydroxamates were designed and synthesized. Structure–activity relationships (SAR) were established for the length of linear chain linker and substitutions on the benzoylurea group. Compounds **5g**, **5i**, **5n**, and **19** showed 10–20-fold enhanced HDAC1 potency compared to SAHA. In general, the cellular potency pIC_{50} (COLO205) correlates with enzymatic potency pIC_{50} (HDAC1). Compound **5b** (SB207), a structurally simple and close analogue to SAHA, is more potent against HDAC1 and HDAC6 compared to the latter. As a representative example of this series, good in vitro enzymatic and cellular potency plus an excellent pharmacokinetic profile has translated into better efficacy than SAHA in both prostate cancer (PC3) and colon cancer (HCT116) xenograft models.

Histone deacetylase (HDAC) enzymes are divided into four different classes.[1] Class I and II isozymes, specifically HDACs 1, 2, 3, 6 and 8, have been associated with uncontrolled tumor growth. For example, selective knockdown studies on HDACs suggest that the class I HDACs, particularly HDACs 1 and 3, are essential to the proliferation and survival of mammalian carcinoma cells.[2] HDAC inhibitors have been studied for their therapeutic effects on cancer cells.[3] Suberoylanilide hydroxamic acid (SAHA, vorinostat), the first HDAC inhibitor approved by the FDA in 2006 for the treatment of cutaneous T-cell lymphoma, validates HDAC inhibition as a strategy for cancer therapy.[4]

There are a number of HDAC inhibitors which are currently undergoing clinical trials either as a single therapy or in combination for treatment of solid and hematologic malignancies (Fig. 1). The morphology of the HDAC inhibitor pharmacophore, as exemplified by SAHA (Fig. 1), is characterized by three portions: a metal- or zinc-binding group (ZBG), a hydrophobic group (CAP) for protein surface recognition or interaction, and a linker to connect both ZBG and CAP. The common linkers are aliphatic chain (e.g., six carbon chain in SAHA), aromatic ring (e.g., phenylene in MS-275)[5] and vinyl-aromatic (e.g., styryl in PXD-101).[6] The most common ZBGs are hydroxamic acid and benzamide (e.g., MS-275). Hydroxamic acid remains among the most potent and popular ZBGs reported for inhibition of Class I HDACs. There is also a connection unit (CU) or connector between the CAP and the linker. Examples of CUs used for a variety of types of HDAC inhibitors are shown below (Fig. 2).[7]

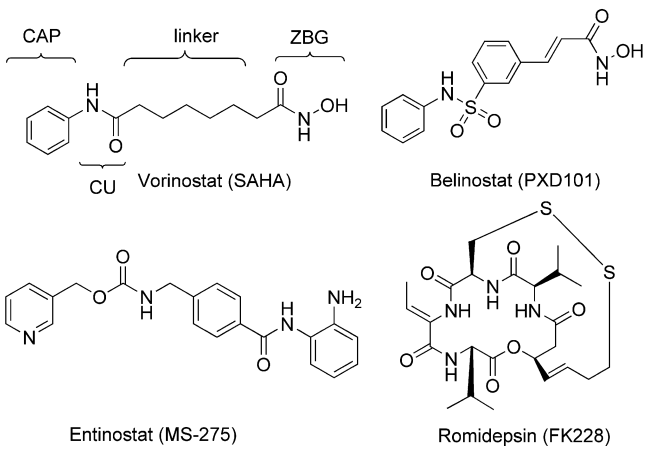

Figure 1. Examples of clinically tested HDAC inhibitors.

* Corresponding author. Tel.: +65 68275019; fax: +65 68275005.
E-mail address: haishan_wang@sbio.com (H. Wang).

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doi:10.1016/j.bmcl.2010.04.041

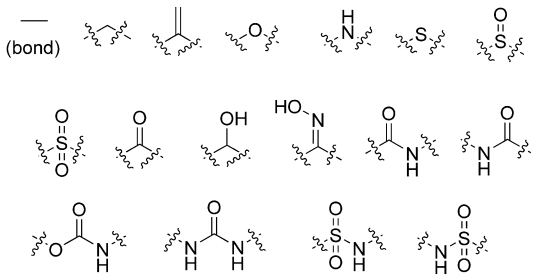
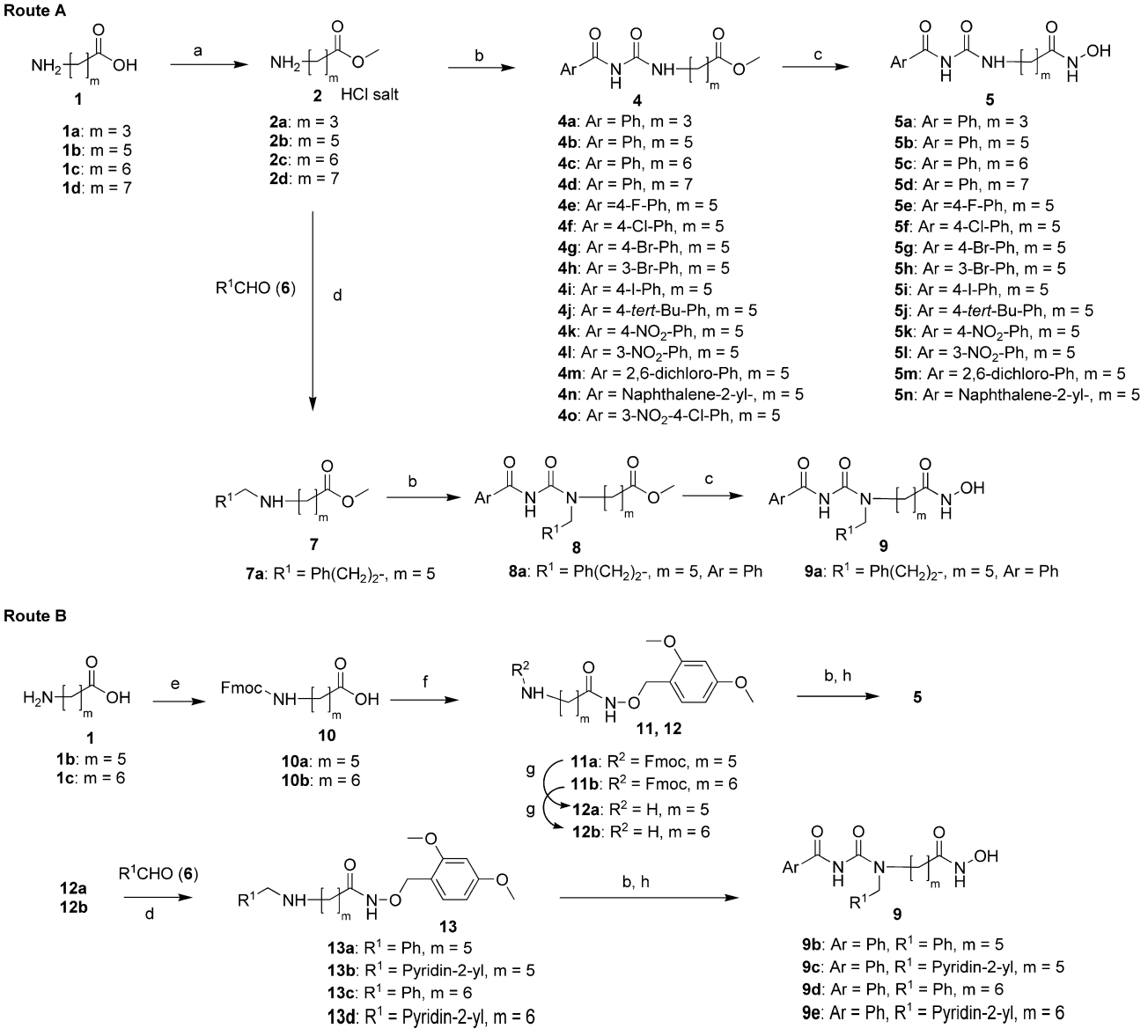


Figure 2. Examples of connection units (CUs) used for HDAC inhibitors.

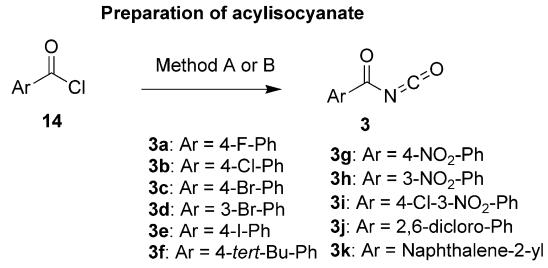
As part of our ongoing efforts to discover novel anticancer agents, we have examined acylurea and sulfonylurea connected hydroxamates as low molecular weight novel HDAC inhibitors.[8] To the best of our knowledge, acylurea (–CONHCONH–), which can be considered as a combination of amide and urea, is a new CU for HDAC inhibitor. This CU can be used not only for generating novel HDAC inhibitor from a patenting perspective, but also for investigating interactions with the region between the binding channel and the entrance area of the HDAC catalytic pocket.[9,10] The optimal location for such an interaction is tunable by adjusting the length of the linker. The acylurea moiety has more hydrogen bond acceptors and donors available compared to either amide or urea, hence the conformation or orientation of the linker and/or the CAP may change, thus influencing potency and/or isoform-selectivity. This unit will likely change the overall drug-like properties as well.

In this report, we describe the synthesis and evaluation of acylurea connected straight chain hydroxamates as novel HDAC inhibitors, some of them have shown promising pharmacological and pharmacokinetic properties.

Scheme 1, Route A, illustrates the general procedure used for preparing acylurea connected hydroxamic acids **5** and **9**. Amino acid ester **2**, if not commercially available, was prepared from ami-



Scheme 1. Reagents and conditions: (a) $SOCl_2$ (1.6 equiv), MeOH, −78 °C to rt; (b) ArCON=C=O (**3**), Et_3N, CH_2Cl_2, −78 °C to rt; (c) $NH_2OH{\cdot}HCl$ (10 equiv), NaOMe (12 equiv), MeOH, 0 °C to rt; (d) $NaBH(OAc)_3$, CH_2Cl_2; (e) Fmoc-Cl, Na_2CO_3, H_2O/dioxane; (f) *O*-(2,4-dimethoxy-benzyl)-hydroxylamine, DCC, CH_2Cl_2, rt; (g) piperidine, MeOH, rt; (h) 5% TFA in CH_2Cl_2, rt.

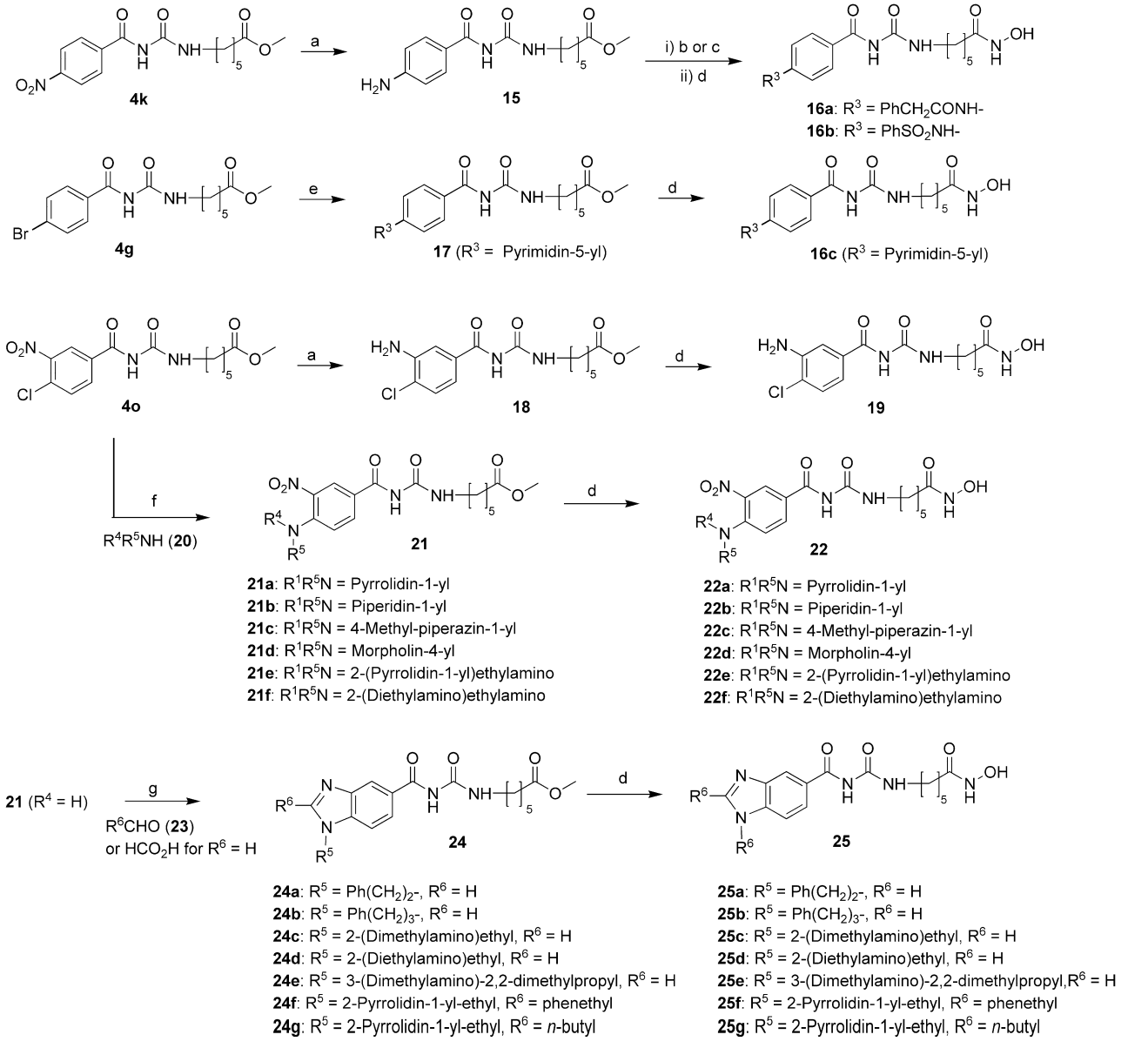


Scheme 2. Reagents and conditions: Method (A) AgOCN (1.1 equiv), CH_2Cl_2, rt; Method (B) NaOCN (1.3 equiv), $SnCl_4$ (0.06 equiv), 1,2-dichlorobenzene, 180 °C.

no acid **1**, and then reacted with acylisocyanate **3** to give acylurea connected methyl ester **4**, which was treated with excessive hydroxylamine and afforded hydroxamate (**5**) in good yield. N-alkylated hydroxamate **9** was made by using similar procedures but starting from secondary amine **7** which was prepared by reductive alkylation of **2** with aldehyde **6**. For compounds containing a functional group labile to strong basic conditions (NaOMe/MeOH) or for easy workup during parallel synthesis, *O*-2,4-dimethoxy-benzyl[11] protected hydroxamate precursor **11** was used to prepare hydroxamates **5** and **9** (Scheme 1, Route B).

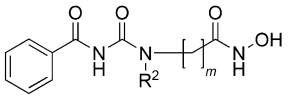
Benzoylisocyanate (**3**, Ar = phenyl) is commercially available, more complex acylisocyanates **3** were prepared according to Scheme 2. Method A is a traditional way of making acylisocyanates by reaction of silver cyanate with acid chloride **14**.[12] Alternatively, much cheaper sodium cyanate was used to react with acid chloride **14** under elevated temperature to give crude **3**,[13] which was used without further purification. Acylisocyanates **3** with electron-withdrawing groups, such as **3a**–**c** and **3g**–**j**, were prepared by method B. **3d**–**f** and **3k** were made by method A.

To further diversify the CAP group (the Ar moiety) of **5** (Scheme 1), a series of synthetic routes were developed to install Ar as a mono- or di-substituted phenyl, or a (substituted) fused ring starting from methyl ester **4** (Scheme 1) as depicted in Scheme 3. Nitro compounds **4k** was reduced to aniline **15**, and then subsequently acylated or



Scheme 3. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, CH_2Cl_2–MeOH; (b) $PhCH_2COCl$, CH_2Cl_2, Et_3N; (c) $PhSO_2Cl$, CH_2Cl_2, Et_3N; (d) $NH_2OH{\cdot}HCl$ (10 equiv), NaOMe (12–20 equiv), MeOH, 0 °C to rt; (e) $R^3B(OH)_2$, $Pd(PPh_3)_4$, PhMe–MeOH (9:1), $NaHCO_3$ (sat), 80 °C; (f) Et_3N, 1,4-dioxane, 80 °C; (g) $SnCl_2{\cdot}2H_2O$ (5 equiv), HOAC–MeOH (1:9), 40 °C.

Table 1
SAR of linker length (m) for **5a–d** and R^2 for **9a–e**



5a-d: R^2 = H, m = 3, 5, 6, 7
9a-e: R^2 = R^1CH_2-, m = 5, 6

Compound	m	R^2	HDAC1 IC_{50}[a] (nM)	HDAC8 IC_{50}[a] (nM)	COLO205 IC_{50}[b] (μM)
5a	3	H	>10,000	>10,000	NT[c]
5b	5	H	53 ± 17	608 ± 113	2.21 ± 0.41
5c	6	H	145 ± 35	270 ± 14	2.68 ± 0.28
5d	7	H	97 ± 4	405 ± 106	8.28 ± 2.85
9a	5	$Ph(CH_2)_3$–	392[d]	328[d]	NT[c]
9b	5	$PhCH_2$–	355 ± 49	NT[c]	3.19 ± 0.95
9c	5	Pyridin-2-yl-CH_2–	245 ± 49	NT[c]	14.7 ± 6.1
9d	6	$PhCH_2$–	1600 ± 749	NT[c]	NT[c]
9e	6	Pyridin-2-yl-CH_2–	555 ± 191	NT[c]	9.06 ± 0.38
SAHA	—	—	121 ± 37	272 ± 127	2.20 ± 0.14

[a] Values are expressed as mean ± standard deviation of at least two independent duplicate experiments.
[b] Values are expressed as mean ± standard deviation of at least two independent triplicate experiments.
[c] NT: not tested.
[d] Values obtained from only one duplicate or triplicate experiment.

sulfonylated to afford hydroxamates **16a** or **16b** after reacting with excessive hydroxylamine. Halogenated **4g** was attached with an aryl or heteroaryl group via Suzuki reaction, then biaryl **17** was converted to hydroxamate **16c**. Compound **19**, a 3-amino analogue of **5f**, was made by reducing the nitro group of **4o** to aniline **18** first before converting it to hydroxamic acid. The chloro of **4o** was displaced by an amine R^4R^5NH (**20**) to afford **21**, which was further converted to hydroxamate **22**. Compound **21** (when R^4 = H) was also transformed to benzimidazole **24** by reacting it with aldehyde R^6CHO (**23**) or formic acid in the presence of a nitro reducing reagent (e.g., $SnCl_2$).[14] Reacting the methyl ester **24** with hydroxylamine afforded 3-(1*H*-benzimidazole-5-carbonyl)-ureido containing hydroxamate **25**.

The CAP or Ar group for initially synthesized hydroxamates (Table 1) was fixed as phenyl, for comparison with SAHA, derived from readily available benzoyl isocyanate. Historically, we had both HDAC8 and HDAC1 as respective target for our two in-house HDAC programs, and human colon cancer cell line COLO205 was used for routine anti-proliferation assay.[15] Thus these compounds were profiled against both HDAC1 and HDAC8 enzymes and COLO205 cells (Table 1). Clearly m = 5 is optimal for both HDAC1 and COLO205 potency when comparing compounds (**5a–d**) with an increasing number of methylenes in the linker. This SAR is inline with some similar studies on straight chain hydroxamates; with the shorter amide group as the CU, a six-methylene linker is optimal for HDAC enzymatic acitivity.[16] As for urea as the CU, a five-methylene is optimal for HDAC enzymatic activity.[7a] When acylurea is CU, it is longer than both amide and urea, thus it is not a surprise that the optimal length is five-methylene.

Compounds with N-alkylated side chains showed much reduced HDAC potency (**9a–c** vs **5b**; **9d–e** vs **5c**). It seems that these branched groups are not well tolerated probably due to unfavorable interactions at the binding pocket rim region. Remiszewski et al. reported that potency of urea connected straight chain hydroxamates also reduced about threefold after N-methylation of the urea moiety.[16a]

These compounds in Table 1 are much weaker inhibitors of HDAC8 enzyme activity compared to HDAC1. As HDAC1 plays an important role in cell proliferation,[2] the HDAC1 assay was selected as the only HDAC isoform for SAR studies.

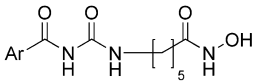
Compound **5b** with m = 5 is more potent than SAHA in the HDAC1 assay and was selected for further modification. The mono-substituted benzoylureas (**5e–l, 16a–c**, Table 2) were made according to Scheme 3. A preference for 4-substituted phenyls emerges (**5g** vs **5h**; **5k** vs **5l**), the potency is related to the size and lipophilicity of the substituent, in the order of I > Br > Cl > H, NO_2 > F. For example, the enzymatic potency was enhanced 4- and 7-fold respectively by simple 4-bromo or 4-iodo substitution. Neither amide **16a** nor sulfonamide **16j** is improved over **5b** in terms of HDAC1 potency. Their cellular activities are even worse, probably due to their poor physicochemical properties. Lipophilic *tert*-butyl group (**5j**) is well tolerated at position 4. Rigid pyrimidine (**16c**) can maintain the enzymatic potency, but has weaker cellular activity.

Disubstituted phenyls (**5m**, **22a–f**, Table 3) were also investigated. Substitutions at both 2 and 6 positions (**5m**) are not good for enzymatic potency. 4-Amino substituted compounds (**22a–f**) can maintain or slightly enhance enzymatic potency, but less bulky (**22a**) or flexible basic chains (**22e–f**) are optimal for both enzymatic and cellular activities. Compound **19** is the most potent disubstituted analogue of **5b** in Table 3. Compared to **5f**, the 3-amino group of **19** slightly improves both the enzymatic and cellular potency. Compounds **25a–g** are cyclic analogs of **22e–f**, but there are no significant changes in their HDAC1 potencies. The benzimidazole ring is basic, and not particularly lipophilic (as in an all-carbon ring like **5n**) hence solubility is good. Despite lower solubility, compound **5n** is the most potent fused ring analog of **5b**. This confirms that lipophilicity is important for the CAP group.

The above studies have demonstrated that the potency of these acylurea connected straight chain hydroxamates can be improved by modifying the CAP region of **5b**, compounds **5g**, **5i**, **5n**, and **19** are representative examples with low nanomolar HDAC1 IC_{50} values. We also assessed the relationship between enzymatic and cellular potency. In general, the cellular potency pIC_{50} (COLO205) correlates significantly (p = 0.017, n = 32) with the enzymatic potency pIC_{50} (HDAC1) although linear regression is not excellent (Fig. S1, Supplementary data). This is not surprising due to the pivotal role HDAC1 plays in cell proliferation and survival.

HDAC isoform profiling[15] work confirmed that compounds **5b** and **5g** are pan-HDAC inhibitors (Table 3). Compound **5b** shares a similar profile as SAHA,[17] but it is more potent against HDAC1 and HDAC6[18] than the latter. Compound **5g** is much more potent against all the isoforms except HDAC8. Compounds **5b** and **5g** are relatively poor HDAC8 inhibitors, because they share the similar straight alkyl chain linker as SAHA. The linker region is more involved in direct interactions with the hydrophobic pocket of HDAC8, trichostatin A has a bulkier linker and exhibits better

Table 2
Biological activities of compounds with Ar being mono-substituted phenyl (**5e–l**, **16a–c**), disubstituted phenyl (**5m**, **19**, **22a–f**), or fused aromatic ring (**5n**, **25a–g**)



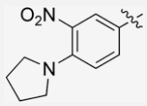
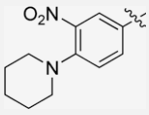
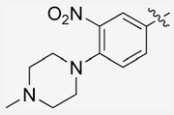
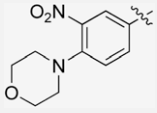
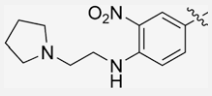
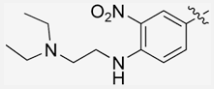
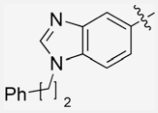
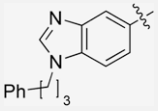
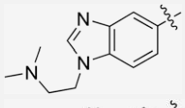
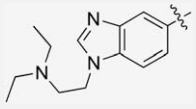
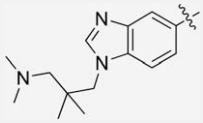
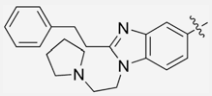
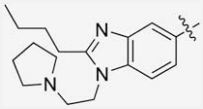
Compound	**Ar**	HDAC1 IC_{50}[a] (nM)	COLO205 IC_{50}[b] (μM)
5b	Ph	53 ± 17	2.21 ± 0.41
5e	4-F-Ph	115 ± 7	2.55 ± 0.21
5f	4-Cl-Ph	26 ± 1	1.60 ± 0.14
5g	4-Br-Ph	13 ± 5	0.87 ± 0.49
5h	3-Br-Ph	60 ± 4	3.15 ± 0.21
5i	4-I-Ph	8 ± 1	0.87 ± 0.19
5j	4-*tert*-Bu-Ph	24 ± 1	2.35 ± 0.07
5k	4-NO_2-Ph	45 ± 2	2.31 ± 0.09
5l	3-NO_2-Ph	92 ± 1	2.80 ± 0.00
5m	2,6-Dichloro-Ph	185 ± 21	2.95[c]
5n	Naphthalene-2-yl	6 ± 1	2.15 ± 0.21
16a	4-($PhCH_2CONH$)-Ph	44 ± 6	13.2 ± 5.3
16b	4-($PhSO_2NH$)-Ph	97 ± 5	>100
16c	4-(Pyrimidin-5-yl)-Ph	26 ± 2	10.0 ± 2.9
19	3-NH_2–4-Cl-Ph	11 ± 1	1.25 ± 0.21
22a		20 ± 1	3.45 ± 1.48
22b		46 ± 6	3.25 ± 0.64
22c		43 ± 10	11.5 ± 2.2
22d		55 ± 2	12.0 ± 1.4
22e		22 ± 3	2.42 ± 0.43
22f		20 ± 1	1.70 ± 0.28
25a		25 ± 3	9.70 ± 0.14
25b		20 ± 2	3.75 ± 1.77
25c		49 ± 2	19.5 ± 0.7
25d		21 ± 1	5.10 ± 0.71
25e		20 ± 1	3.70 ± 0.71

Table 2 (*continued*)

Compound	Ar	HDAC1 IC_{50}[a] (nM)	COLO205 IC_{50}[b] (μM)
25f		34 ± 1	5.38 ± 0.10
25g		22 ± 0	2.91 ± 1.26

[a,b,c] See footnote of Table 1.

Table 3
Compounds **5b**, **5g**, and SAHA are pan-HDAC inhibitors

HDAC[a]	K_i[b] (nM)		
	5b	**5g**	SAHA
1	16 ± 2	8 ± 2	63 ± 13
2	44 ± 2	12 ± 1	40 ± 4
3	23 ± 5	8 ± 3	29 ± 9
4	20 ± 4	6 ± 1	16 ± 1
5	46 ± 14	13 ± 2	33 ± 14
6	7 ± 2	8 ± 0	18 ± 5
7	NT[c]	31 ± 5	126 ± 60
8	252 ± 25	315 ± 48	205 ± 35
9	55 ± 9	6 ± 2	65 ± 26
10	40 ± 9	9 ± 2	50 ± 7
11	39 ± 6	9 ± 2	36 ± 6

[a] HDAC isoenzymes.
[b] Dissociation constant.[15]
[c] NT: not tested.

Table 5
Profiling of selected compounds

Compound	Solubility[a] (μM)	Solubility[b] (μg/mL)	Log D[c]	c log P[d]	PSA[e]
5b	177	526	0.75	1.07	108
5g	51	15	1.72	1.86	108
16a	28	NT	NT	2.11	137
16c	17	NT	NT	0.65	133
22e	>205	NT	NT	1.09	169
25f	>250	NT	NT	3.45	129
25g	>211	NT	NT	2.97	129
SAHA	>250	166	1.04	1.84	78

[a] Kinetic solubility (pH 7).
[b] Thermodynamic solubility (pH 7.4).
[c] Determined in *n*-octanol/sodium phosphate buffer (pH 7.4).
[d] c log P (octanol/water) and
[e] PSA (polar surface area) were calculated using MOE 2008.10, Chemical Computing Group.

inhibitory effect on HDAC8 compared to SAHA.[19] Compounds **16a–c**, **19**, **22a–f**, and **25a–g** have structurally diverse CAP groups, hence their HDAC isoform inhibitory profiles may be expected to be different from those of **5b** and **5g**.

Compounds with representative Ar groups were selected for further evaluation in cellular assays. Broad activity against human tumor cell lines, such as HCT116 (colon cancer), A2780 (ovarian cancer) and PC3 (prostate cancer) was encouraging (Table 4). Compounds **5b** was also further profiled against more human tumor cell lines, for example, NCI-H522 (non-small cell lung cancer, IC_{50} = 0.50 ± 0.31 μM), Ramos (human Burkitt's lymphoma, IC_{50} = 0.46 μM). As comparison, SAHA showed IC_{50} = 1.28 ± 0.35 μM and 0.65 ± 0.36 μM for these two cell lines, respectively.

A hallmark of HDAC inhibition is the increase in the acetylation level of histones.[3,20] Histone acetylation, including H3, H4 and H2A can be detected by Western immuno-blotting. The mechanism of action of compounds **5b–c**, **5g**, **5k**, **9b**, **16a**, **16c**, **22e**, and **25f–g** was confirmed in vitro at 10 μM as evidenced by hyperacetylation of histone H3 in COLO205 cells, other compounds were not tested. Compound **5b** also increased the level of both acylated histone 3[3,20] and α-tubulin,[18] and up-regulated cyclin-dependant kinase inhibitor p21[21] protein expression in HCT116 cells in a dose-dependent manner (Fig. S2, Supplementary data). In one of our preliminary studies on histone acetylation pharmacodynamics, compound **5b** and SAHA were dosed to COLO205 tumor-bearing mice at 160 mg/kg via ip injection, and both compounds demonstrated hyperacetylation of histone H3 and H4 in tumor tissues at 1.5 h time point, but compound **5b** seemed more efficient (Fig. S3, Supplementary data).

Kinetic solubility of selected compounds (Table 5) was assessed. Compounds **5b** and compounds with protonable basic centers at physiological pH (**22e**, **25f–g**) showed relatively high solubility. Compound **5b** was further assessed on its thermodynamic solubility in pH 7.4 buffer, the value (526 μg/mL or 1.8 mM) is higher than that of SAHA (166 μg/mL, 0.63 mM). SAHA has a reported value of 0.1 mg/mL.[4] Compound **5b** has a measured log D = 0.75, which is more polar than SAHA (log D = 1.04), thus more soluble.

In vitro metabolic stability ($t_{1/2}$) of selected compounds assessed in different species liver microsomes is presented in Table 6. In general, these compounds showed good stability ($t_{1/2}$ >30 min) in human liver microsomal assay except compound **25f** which has increased liability due to its lipophilicity (c log P). The metabolic stability of **5b** was comparable with that of SAHA in human, rat and mouse liver microsomes.

Table 4
Cellular IC_{50} (μM) of selected compounds

Compound	HCT116	A2780	PC3
5b	1.58[a]	0.70 ± 0.06	0.74 ± 0.21
5g	0.60[a]	0.45 ± 0.05	0.66 ± 0.07
22e	1.33[a]	0.99 ± 0.16	0.92 ± 0.20
25g	3.52[a]	1.35 ± 0.07	1.25 ± 0.12
SAHA	1.43 ± 0.29	1.62 ± 0.47	1.21 ± 0.85

[a] Values obtained from only one triplicate experiment.

Table 6
Liver microsomal stability ($t_{1/2}$, min) of selected compounds

Compound	Human	Rat	Mouse
5b	>60	>60	>30
5g	>60	53 ± 10	31 ± 3
16c	>30	NT	NT
22e	>30	NT	NT
25f	21 ± 2	NT	NT
25g	>30	NT	NT
SAHA	>60	>60	>30

NT: not tested.

The permeability of **5b** was also assessed in Caco-2 cells. The rate of transport (PappAtoB) of **5b** was 3.12×10^{-6} cm/s, which was higher than that reported for SAHA (1.70×10^{-6} cm/s).[4]

The results of PK studies on compound **5b**, SAHA, LBH-589 and PXD-101 in nude mice have been published.[22] After a single iv dose of 10 mg/kg injection, the systemic plasma clearance of **5b** (5.03 L/h/kg) was lower than that of SAHA (6.73 L/h/kg) as well as the hepatic blood flow of 5.4 L/h/kg in mice. The estimated steady-state volume of distribution (Vss) of **5b** (2.16 L/kg) was greater than that of SAHA (0.81 L/kg). The estimated elimination half-life for **5b** was 1.54 h, which was longer than that of SAHA (0.38 h). After an oral single dose of 50 mg/kg, the AUC and C_{max} of **5b** were 9 and 12 times greater than those of SAHA, respectively. Compound **5b** has an oral bioavailability in mouse of 62%, which is much higher than that of SAHA (8.3%).

Compound **5b** was selected for evaluation in vivo to demonstrate whether the acylurea connected hydroxamate is efficacious and has any advantages over SAHA. As SAHA had demonstrated efficacy in CWR22 human prostate xenograft in nude mice,[3] our in vivo xenograft studies were also initiated in a human prostate cancer PC3 tumor-bearing nude mice.[23] Compound **5b** and SAHA were given daily via intraperitoneal (ip) injection for 21 days at doses of 160, 200, and 250 mg/kg (**5b** only) (Fig. S4, Supplementary data). Compound **5b** demonstrated significant antitumor activity compared to vehicle control. Tumor growth inhibition (TGI) on day 22 was 73% (200 mg/kg, maximum tolerated dose (MTD), p <0.01) and 57% (160 mg/kg, p <0.05), but 250 mg/kg dose was toxic with 2/9 treatment-related deaths on day 4. SAHA was used as a positive control and showed moderate activity at 160 mg/kg (MTD) with TGI = 35% ($p > 0.05$), but the 200 mg/kg dose was toxic in this experiment, with 2/9 treatment-related deaths on days 16 and 22, respectively.

Compound **5b** was also given orally once per day to HCT116 tumor-bearing mice (Fig. 3), with TGI on day 21 of 80% (100 mg/kg, $p < 0.01$), but it was not tolerated at 200 mg/kg, with 2/10 treatment-related deaths on days 6 and 10, respectively. SAHA showed moderate activity at 200 mg/kg with TGI = 50% ($p < 0.01$) in this experiment. Compared to SAHA, compound **5b** can achieve better efficacies at the same or lower dose level.

According to the published pharmacokinetic study results of **5b**,[22] when dosed orally at 50 mg/kg, the C_{max} (22.4 μM) was well above the IC_{50} levels for HDAC1 (0.053 μM), HCT116 (1.58 μM), and PC3 (0.74 μM). Plasma concentration was maintained above the HDAC1 IC_{50} for about 22 h, above HCT116 and PC3 IC_{50}s for 2.2 and 4.3 h, respectively. Given that the doses in the PC3 and HCT116 xenograft experiments were much higher than this PK study, it is not surprising that **5b** can achieve such good efficacy as compared to SAHA in both xenograft studies.

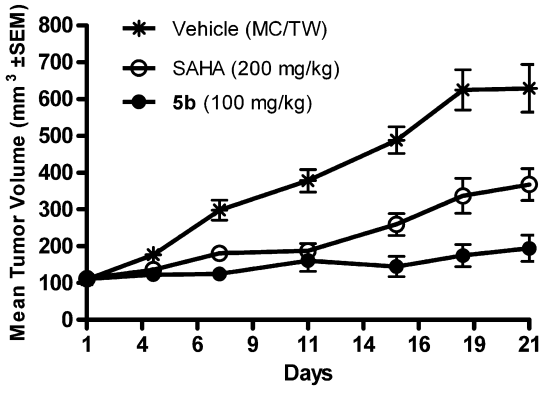


Figure 3. Antitumor activity of **5b** and SAHA in HCT116 xenograft model. $n = 10$ female BALB/c nude mice (Animal Resources Centre, Australia) per dose group, po, qd × 21 days.

In summary, we have designed and synthesized a series of acylurea connected straight chain hydroxamates, established structure–activity relationships for the length of linear chain linker and substitutions on the benzoylurea group. Compounds **5g**, **5i**, **5n**, and **19** showed 10–20-fold enhanced HDAC1 potency compared to SAHA. A structurally simple and representative compound, **5b** (SB207), has shown very good drug-like properties, and has demonstrated good antitumor efficacy in both PC3 and HCT116 xenograft models. These results encourage us to further evaluate and develop this series as well as to better understand the HDAC isoform inhibitory profiles of those with complex CAP groups such as substituted benzimidazole, naphthalene, indole, and other polycyclic ring systems.

Acknowledgments

We thank the S*BIO sample management team for preparing sample solutions for assays, the PKDM department for DMPK studies on **5b**, Dr Ignacio Segarra for early animal work on **5b**, and Dr. Brian W. Dymock for critical review of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.041.

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