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Identification and optimisation of a series of substituted 5-pyridin-2-yl-thiophene-2-hydroxamic acids as potent histone deacetylase (HDAC) inhibitors

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Abstract—Further investigation of a series of thienyl-based hydroxamic acids that included ADS100380 and ADS102550 led to the identification of the 5-pyridin-2-yl-thiophene-2-hydroxamic acid **3c**, which possessed modest HDAC inhibitory activity. Substitution at the 5- and 6-positions of the pyridyl ring of compound **3c** provided compounds **5a**–**g**, **7a**, **b**, **9**, and **13a**. Compound **5b** demonstrated improved potency, in vitro DMPK profile, and rat oral bioavailability, compared to ADS102550. Functionalisation of the pendent phenyl group of compounds **5b**, **5e** and **13a** provided analogues that possessed excellent enzyme inhibition and anti-proliferative activity.

The recent approval of SAHA (Zolinza™, Vorinostat) (Fig. 1)[1] by the FDA for once-daily oral treatment of advanced cutaneous T-cell lymphoma (CTCL) has added histone deacetylase (HDAC) inhibitors[2] to the clinician's armoury of anti-cancer therapeutics.[3,4]

Previously, we reported the discovery of ADS100380 (Fig. 1) as a sub-micromolar HDAC inhibitor, and its subsequent optimisation to provide a series of 5-(1*H*-pyrazol-3-yl)-thiophene-2-hydroxamic acids as potent HDAC inhibitors and anti-proliferative agents.[5] From this series, ADS102550 demonstrated an improved potency and PK profile compared to SAHA (Zolinza™, Vorinostat) (Fig. 1), and also provided 33% tumour growth inhibition in an HCT116 mouse tumour xenograft model. However, in vitro DMPK experiments indicated that ADS102550 exhibited significant cytochrome P450 3A4 inhibition ($IC_{50} = 0.51\ \mu M$) and lower than ideal Caco-2 permeability ($P_{app}\ 4.2 \times 10\ cm^{-6}\ s^{-1}$). The latter was consistent with the poor oral bioavailability observed.

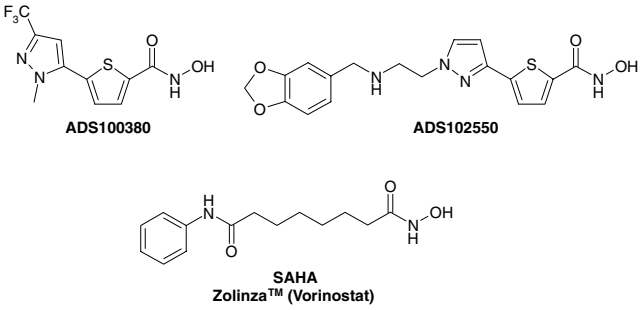

Figure 1. HDAC inhibitors.

Contemplating the competitive intellectual property position, and in order to expand and optimise further our series of thienyl-based hydroxamic acids, we elected to investigate replacement of the pyrazole ring of ADS100380 with a set of alternative aromatic rings.

We believed that this strategy would enable us to identify quickly alternative unsubstituted heterobiaryl hydroxamic acids that possessed modest HDAC inhibitor activity. Any such unsubstituted heterobiaryl hydroxamic acids could be optimised by substitution

Keywords: Histone deacetylase inhibitors; HDAC; Hydroxamic acids.
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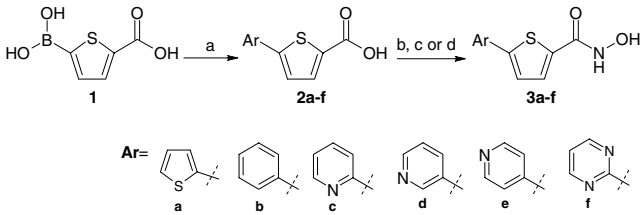
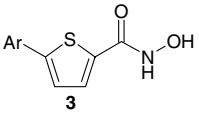
on the terminal aromatic ring system, using a similar approach to that reported previously for the identification of ADS102550.[5]

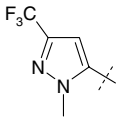
The unsubstituted biaryl hydroxamic acids **3a**–**f** were produced by coupling the corresponding carboxylic acids **2a**–**f** with *O*-THP-protected hydroxylamine, followed by acidic deprotection (Scheme 1). The carboxylic acids **2a**–**c** were commercially available, and the carboxylic acids **2d**–**f** were produced by a Suzuki coupling 'reaction between 5-(dihydroxyboryl)-2-thiophenecarboxylic acid (**1**) and the respective heteroaromatic bromides.



Scheme 1. Reagents and conditions: (a) ArBr (1 equiv), Na_2CO_3, CH_3CN, $Pd(PPh_3)_4$ (5 mol %), 80 °C, 3 h (50–85%); (b) $^{i}Pr_2Net$ (2.5 equiv), DMF, H_2NOTHP (1.1 equiv), HATU (1.1 equiv) (65–84%); (c) *p*-TSA, MeOH (58%); (d) CF_3CO_2H, CH_2Cl_2 (45–81%).

Table 1. HDAC and anti-proliferative assay results for ADS100380 and compounds **3a**–**f**

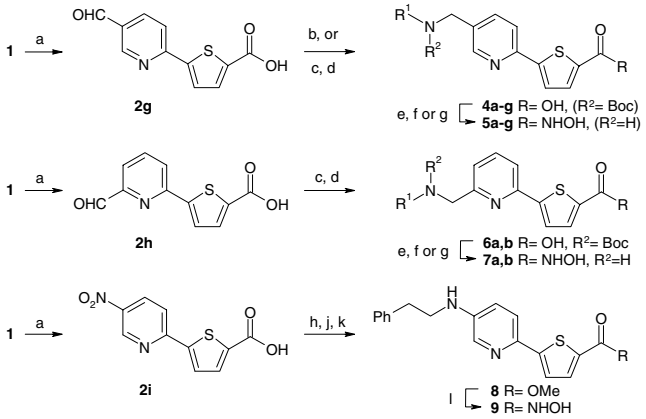


Compound	Ar	HDAC[a] (IC_{50}, μM)	Cell proliferation[a] MCF-7 (IC_{50}, μM)
ADS100380		0.750	11.4
3a		2.50	>100
3b		0.900	31.0
3c		0.243	2.25
3d		1.13	—
3e		1.26	—
3f		0.186	2.12

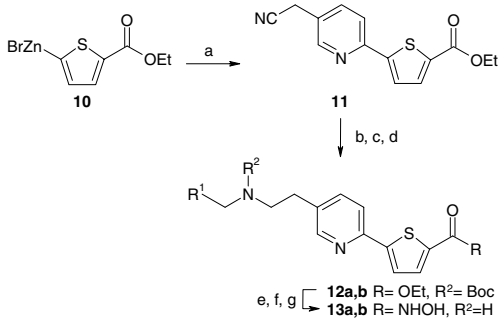
[a] Values are means of two experiments.

Replacement of the pyrazole ring of ADS100380 with a thienyl ring **3a** gave a 4-fold reduction in activity in the HDAC enzyme assay and a 10-fold reduction in the MCF-7 cell-based assay (Table 1). The phenyl analogue **3b** possessed similar HDAC potency to ADS100380. The 2-pyridyl biaryl hydroxamic acid **3c** provided the first enhancement in both HDAC and cell-based potency. However, the other two pyridyl isomers, compounds **3d** and **3e**, were both almost half an order of magnitude weaker in the HDAC assay.

Interestingly, the pyrimidine **3f** was equipotent to the 2-pyridyl analogue **3c**. These SAR data indicate a preference for a nitrogen atom adjacent to the biaryl bond for six-membered aromatic rings.



Scheme 2. Reagents and conditions: (a) ArBr (1 equiv), Na_2CO_3, CH_3CN, $Pd(PPh_3)_4$ (5 mol %), 80 °C, 3 h (73–90%); (b) i—R^1R^2NH (1.2 equiv), CH_2ClCH_2Cl, 2 h; ii—$NaBH(OAc)_3$, 16 h (63–88%); (c) i—R^1NH_2 (1.2 equiv), CH_2ClCH_2Cl, 2 h; ii—$NaBH(OAc)_3$, 16 h; (d) Boc_2O, $^{i}Pr_2NEt$, CH_2Cl_2 (53–92% two steps); (e) $^{i}Pr_2Net$ (2.5 equiv), DMF, H_2NOTHP (1.1 equiv), HATU (1.1 equiv) (45–78%); (f) 4 M HCl in dioxane, CH_2Cl_2 (25–72%); (g) CF_3CO_2H, CH_2Cl_2 (18–45%); (h) MeOH, c.HCl, reflux, 48 h (74%); (j) H_2, 5% Pd/C, CH_3CN, 1.5 h (90%); (k) i—$PhCH_2CHO$, THF, 16 h; ii—AcOH, $NaBH(OAc)_3$ (48%); (l) $NH_2OH{\cdot}HCl$, KOH, MeOH, 16 h (53%).

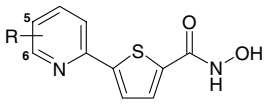


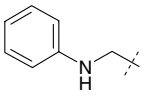
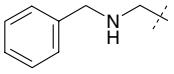
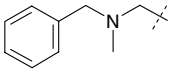
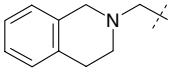
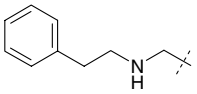
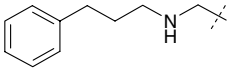
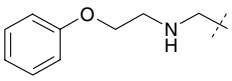
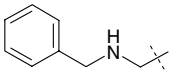
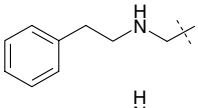
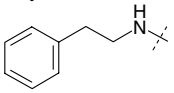
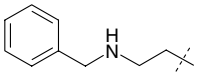
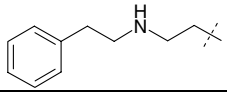
Scheme 3. Reagents and conditions: (a) ArBr (1 equiv), THF, $Pd(PPh_3)_4$ (5 mol %), 80 °C, 1 h (85%); (b) H_2, Raney Ni, EtOH/THF (2:1), 18 h (90%); (c) i—R^1CHO (1.05 equiv), MeOH, 3 h; ii—$NaBH_4$, 1 h (43–73%); (d) Boc_2O, $^{i}Pr_2NEt$, CH_2Cl_2 (>80%); (e) KOTMS, THF, 18 h (quant); (f) $^{i}Pr_2NEt$ (2.5 equiv), DMF, H_2NOTHP (1.1 equiv), HATU (1.1 equiv) (56–86%); (g) 4 M HCl in dioxane, 2 h (53–62%).

Encouraged by the results obtained for the 2-pyridyl compound **3c**, we continued to follow our strategy of incorporating additional substituents on the pyridyl ring. Functionalisation of the pyridyl ring, compared to the pyrazole ring, provided an increased number of possible attachment points for the tethered phenyl groups. Considering synthetic accessibility and the existing SAR, we elected to investigate 5- and 6-substitution patterns on the pyridine ring.

Utilising the Suzuki biaryl coupling conditions described in Scheme 1, the heterobiaryl carboxylic acid intermediates **2g**–**i** were prepared from 6-bromopyridine-3-carboxaldehyde, 6-bromopyridine-2-carboxaldehyde and 2-bromo-5-nitropyridine, respectively (Scheme 2). The aldehydes **2g** and **2h** were elaborated under reductive amination conditions using primary amines and secondary amines, followed by Boc protection where necessary, to furnish tethered heterobiaryl carboxylic acids **4a**–**g** and **6a**, **b**. Conversion of the carboxylic acids **4a**–**g** and **6a**, **b** to the respective hydroxamic acids **5a**–**g** and **7a**, **b** was achieved by coupling with *O*-THP-protected hydroxylamine, and subsequent acidic deprotection. The nitro carboxylic acid **2i** was esterified prior to reduction of the nitro group, and subsequent reductive alkylation provided the 5-phenethylamino tethered ester **8**. Conversion of ester **8** to the desired hydroxamic acid **9** was achieved using hydroxylamine under basic conditions.

Table 2. HDAC and anti-proliferative assay results for compounds **5a**–**g**, **7a**, **b**, **9** and **13a**, **b**



Compound	R	Substⁿ	HDAC[a] (IC_{50}, μM)	Cell proliferation[a] (IC_{50}, μM)	
				MCF-7	MDA-MB231
3c	H	—	0.243	2.25	11.0
5a		5	0.016	0.37	0.63
5b		5	0.016	0.08	0.22
5c		5	0.035	0.15	0.37
5d		5	0.030	0.22	0.86
5e		5	0.012	0.07	0.20
5f		5	0.018	0.12	0.25
5g		5	0.011	0.11	0.30
7a		6	0.359	1.90	—
7b		6	0.581	3.84	—
9		5	0.080	1.47	3.51
13a		5	0.009	0.08	0.21
13b		5	0.031	0.14	—

[a] Values are means of two experiments.

Compounds possessing a 5-aminoethyl substituted pyridine were produced by the route outlined in Scheme 3. The heterobiaryl ester intermediate **11** was produced via a Negishi coupling between 5-ethoxycarbonyl-2-thienylzinc bromide (**10**) and (6-bromo-pyridin-3-yl)-acetonitrile. Reduction of the nitrile **11** using Raney Nickel, followed by reductive alkylation and subsequent Boc protection, provided the tethered biaryl esters **12a** and **b**. The esters **12a** and **b** were converted to the corresponding hydroxamic acids **13a** and **b** using standard hydrolysis, coupling and deprotection conditions.

The tethered aniline **5a** provided excellent potency in the HDAC enzyme assay,[6] however, a large drop-off in activity in the MCF-7[7] assay compared to the primary assay was observed (Table 2). Homologation of aniline compound **5a** furnished the benzylamine **5b** that had identical potency in the primary assay, but significantly improved potency in both cell proliferation assays. N-methylation of the secondary amino group of compound **5b** provided the tertiary benzylamine tethered compound **5c** that displayed approximately 2-fold reduced activity both in cell proliferation and the HDAC enzyme assays. Cyclisation of the secondary amino group of compound **5b** onto the aromatic ring by an ethyl linker, compound **5d**, also led to a 2-fold reduction in activity in the HDAC and MCF-7 cell assays, and a 4-fold reduction in activity against the MDA-MB231 cell line. Homologation of the tether by replacing the benzylamine of compound **5b** with a phenethylamine group provided a marginally more active compound **5e**. Homologation of the tether of compound **5e** either by a methylene unit, compound **5f**, or by an oxygen atom, compound **5g**, had no effect on potency in either the cell-based or the HDAC enzyme assays.

The regioisomeric 6-aminopyridine **7a** of compound **5b** demonstrated a 5- to 10-fold activity loss in all assays. A similar result was also observed for the corresponding regioisomer of **5e**, compound **7b**, in which over an order of magnitude of activity was lost. These data precluded any further work being undertaken in the 6-pyridyl substituted series.

Table 3. In vitro profiling for compound **5b** compared with ADS102550 and SAHA

Criteria	Assay	**5b**	ADS102550	SAHA
In vitro potency (IC_{50}, μM)	HDAC[a]	0.016	0.029	0.125
	MCF-7[a]	0.08	0.30	1.50
	MDA-MB231[a]	0.22	0.63	12.3
	HCT116[a]	0.11	0.58	2.52
CYP450 inhibition				
at 1 μM (%)	1A2	17	9	14
	2C9	−14	0	28
	2C19	−4	71	14
	2D6	1	8	8
(IC_{50}, μM)	3A4	26	53	12
		(5.5)	(0.514)	—
Permeability ($P_{app} \times 10^{-6}$cm s^{-1})	Caco-2[b]	15.4	4.2	8.0
Microsomes remain at 30 min (%)	Hum	88	55	97
	Mou	35	5	114
Hepatocytes (μL/min/10^6cells)	Hum	7	15	3
	Rat	27	26	12

[a] Values are means of two experiments.
[b] A:B was run from pH 6.5 to 7.4.

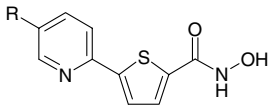
Table 4. Rat PK profile for compound **5b**, ADS102550 and SAHA after iv (1 mg/kg) and po (5 mg/kg) dosing

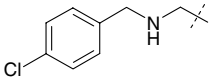
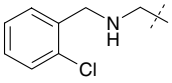
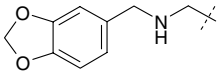
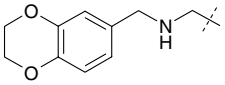
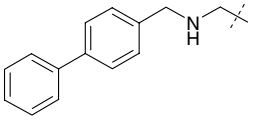
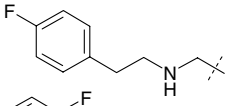
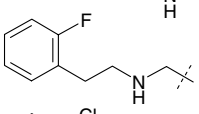
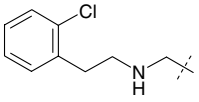
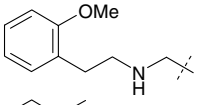
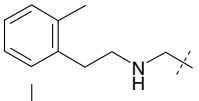
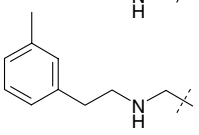
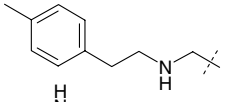
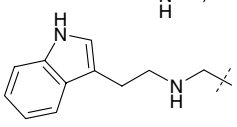
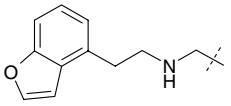
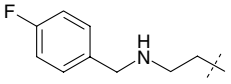
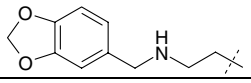
Compound	$t_{1/2}$ (h) iv	V_{dss} (L/kg) iv	CL (mL/min/kg) iv	$AUC_{0-\infty h}$ (μg h/mL) po	*F* (%) po
SAHA	0.7	1.6	67	0.4	16
ADS102550	1.6	0.9	21	0.1	2
5b	2.7	2.9	13	3.3	50

Table 5. Mouse PK profile for compound **5b**, ADS102550 and SAHA after iv (1 mg/kg), po (5 mg/kg) and ip (5 mg/kg) dosing

Compound	$t_{1/2}$ (h) iv	V_{dss} (L/kg) iv	CL (mL/min/kg) iv	$AUC_{0-\infty h}$ (μg h/mL)		*F* v(%)	
				po	ip	po	ip
SAHA	0.8	2.1	78	0.2	0.6	14	61
ADS102550	2.5	0.4	10	0.9	7.2	10	85
5b	0.17	1.1	83	0.36	1.25	36	100

Table 6. HDAC and anti-proliferative assay results for compounds **5h–u** and **13c**, **d**



Compound	R	HDAC[a] (IC_{50}, μM)	Cell proliferation[a] (IC_{50}, μM)	
			MCF-7	HCT116
5h		0.009	0.13	0.30[b]
5i		0.007	0.11	0.17[b]
5j		0.008	0.05	0.07
5k		0.011	0.05	0.09
5l		0.007	0.15	0.18
5m		0.009	0.08	0.10
5n		0.012	0.09	0.10
5o		0.006	0.08	0.08
5p		0.017	0.06	0.08
5q		0.008	0.09	0.07
5r		0.009	0.06	0.09
5s		0.014	0.06	0.06
5t		0.008	0.12	0.18
5u		0.004	0.06	0.12
13c		0.013	0.09	0.14
13d		0.009	0.07	0.11

[a] Values are means of two experiments.
[b] Values reported are for MD-MBA231 cell line.

Truncating the tether of compound **5e** by a methylene unit to provide the tethered compound **9** led to retention of HDAC inhibitory activity, but resulted in significantly reduced cell-based activity.

Moving the basic amine of compounds **5e** and **5f** further away from the pyridyl ring provided the corresponding isomeric compounds **13a** and **13b**, which possessed almost comparable activity in the HDAC and MCF-7 assays.

Table 2 includes three compounds with IC_{50} values below 20 nM in the primary assay and below 100 nM in the MCF-7 cell proliferation assay. On the basis of structural simplicity, compound **5b** was selected as a prototype compound for further profiling (Table 3). Compound **5b** was over an order of magnitude more active than SAHA in the HDAC enzyme and MCF-7, MDA-MB231, and HCT-116 cell proliferation assays. Compound **5b** was 2-fold more active than ADS102550 in the HDAC enzyme assay, and 3- to 6-fold more potent in the cell-based assays. Compound **5b** possessed a 10-fold reduction in CYP450 3A4 inhibition value compared to ADS102550, and also had a significantly enhanced permeability. Modest stability for both of the biaryl hydroxamic acids, **5b** and ADS102550, was observed in rat hepatocytes, with poor stability being demonstrated in mouse microsomes.

Compound **5b** was evaluated in rat PK experiments (iv and po, Table 4) for comparison with ADS102550 and SAHA. Compound **5b** possessed improved clearance compared to ADS102550 and SAHA. Based on the results observed in rat hepatocyte stability assays, the high clearance for SAHA was unpredicted. The good oral bioavailability observed for compound **5b** was consistent with its high Caco-2 permeability.

Encouraged by the in vivo rat PK data, we next proceeded with a series of mouse PK experiments (iv, ip, and po, Table 5). Unfortunately, compound **5b** displayed high clearance and had a very short half-life. This high clearance for compound **5b** compared to ADS102550 was not predicted from the in vitro mouse microsomal stability measurements. However, the increased oral bioavailability observed was once again consistent with the Caco-2 measurements.

Considering the promising potency, improved CYP450 3A4 profile and high permeability of compound **5b**, we elected to investigate the SAR of substituted 5-pyridin-2-yl-thiophene-2-hydroxamic acids further in an attempt to identify potent HDAC inhibitors and anti-proliferative agents possessing an improved PK profile, especially lower clearance.

A series of over fifty compounds that had substituents appended to the tethered phenyl ring of compounds **5b**, **5e**, and **13a** was prepared using the synthetic routes depicted in Schemes 2 and 3.

A selection of some of the most active compounds, **5h–u** and **13c**, **d**, that possessed IC_{50} values below 20 nM in the HDAC enzyme assay is shown in Table 6. In addition, these compounds demonstrated IC_{50} values of less than 200 nM in two cell proliferation assays. To expand the variety of cancer cell lines that the compounds were routinely tested against, the MDA-MB-231 breast cancer cell line was replaced by the HCT116 colon cancer cell line.

Generally, it was found that a very broad range of phenyl substitutions was well tolerated, with the best substituents including halogens, electron-donating groups, fused electron-rich ring systems and biaryl rings. Further profiling of selected compounds will be reported in due course.

In summary, we have expanded the initial series of thienyl-based hydroxamic acids to include substituted 5-pyridin-2-yl-thiophene-2-hydroxamic acids as potent HDAC inhibitors. One of the compounds identified, compound **5b**, has demonstrated improved potency, cytochrome P450 3A4 inhibition, Caco-2 permeability and increased oral bioavailability in rat, compared to ADS102550. Substitution of the tethered phenyl group of the 5-pyridin-2-yl-thiophene-2-hydroxamic acids **5b**, **5e**, and **13a** was shown to be well tolerated, and further expanded the SAR of substituted 5-pyridin-2-yl-thiophene-2-hydroxamic acids.

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6. The HDAC inhibitory activity of compounds was assessed using the commercially available HDAC Fluorescent Activity Assay Kit (Biomol, #AK-500) following the manufacturer instructions, but with minor modifications: the reaction was carried out at

25 °C for 30 min in the presence of 116 μM substrate. Compounds were serially diluted in DMSO and the final concentration of the solvent in the assay was 1%.

7. The anti-proliferative activity of the compounds was tested on a panel of human cancer cell lines. Cells were seeded in 96-well plates at a density of 3000 cell/well and incubated with serially diluted compounds for 72 h. The final DMSO concentration in the assay was 0.1%. The final number of cells per well was assessed using the CyQuant DNA dye (Invitrogen, #C7026) following the manufacturer instructions.