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Design and campaign synthesis of pyridine-based histone deacetylase inhibitors

David M. Andrews,* Keith M. Gibson, Mark A. Graham, Zbigniew S. Matusiak, Craig A. Roberts, Elaine S. E. Stokes, Madeleine C. Brady and Christine M. Chresta

Cancer and Infection Research, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

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Abstract—A lead benzamide, bearing a cyanopyridyl moiety (**3**), was identified as a potent and low molecular weight histone deacetylase (HDAC) inhibitor. Various replacements of the cyano group were explored at the C3-position, along with the exploration of solubility-enhancing groups at the C5-position. It was determined that cyano substitution at the C3-position of the pyridyl core, along with a methylazetidinyl substituent at the C5-position yielded optimal HDAC1 inhibition and anti-proliferative activity in HCT-116 cells.

In the eukaryotic cell, DNA is routinely compacted to prevent transcription factor accessibility. When the cell is activated this compacted DNA is made available to DNA-binding proteins, thereby allowing the induction of gene transcription.[1] Nuclear DNA associates with histones to form chromatin and the N-terminal tails of the core histones contain lysine residues that are sites for post-transcriptional acetylation.[2,3] This reversible process is important in transcriptional regulation and cell-cycle progression.[4] Histone deacetylases (HDACs) are zinc-containing enzymes which catalyze the removal of acetyl groups from the ε-amino termini of lysine residues clustered near the amino terminus of nucleosomal histones and inhibition of this process is intimately linked to the induction of gene transcription.[5] In addition, HDAC disregulation has been associated with several cancers and HDAC inhibitors such as the commercially launched Zolinza (**1**)[2] and MS-275 (**2**)[6] are undergoing study for the potential treatment of cutaneous T-cell lymphoma and various haematological malignancies (Fig. 1).[7,8]

As part of an ongoing effort to identify novel HDAC inhibitors, compound **3** and its corresponding des-cyano analogue were identified as potent, low molecular weight, but moderately soluble leads, capable of being

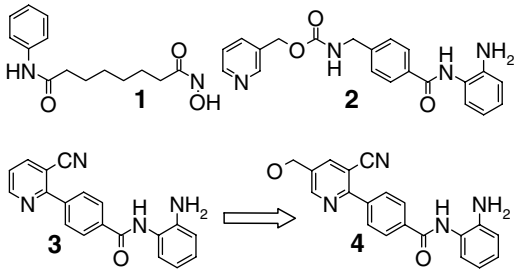

Figure 1. Zolinza (**1**), MS-275 (**2**) and cyanopyridine lead **3**.

elaborated into drug-like compounds.[9] The cyano compound **3** was shown to be more potent than the des-cyano analogue by approximately 5- to 10-fold in an HDAC isolated enzyme assay[10] and therefore became the focus of our optimization efforts (data not shown). We rationalized that the introduction of a hydroxymethyl moiety at the 5-position would provide **4** which could be used in suitably activated form to provide a large range of base-containing and therefore more soluble analogues. Further expansion of the lead structure, substituting methyl, chloro and fluoro for cyano at the 3-position provided valuable SAR, which is described later.

Our synthetic strategy relied upon the ability to access the key boronate ester **6** in good yield and to exploit it in a series of Suzuki coupling reactions with the known or

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* Corresponding author. Tel.: +44 1625 517126; e-mail: david.andrews@astrazeneca.com

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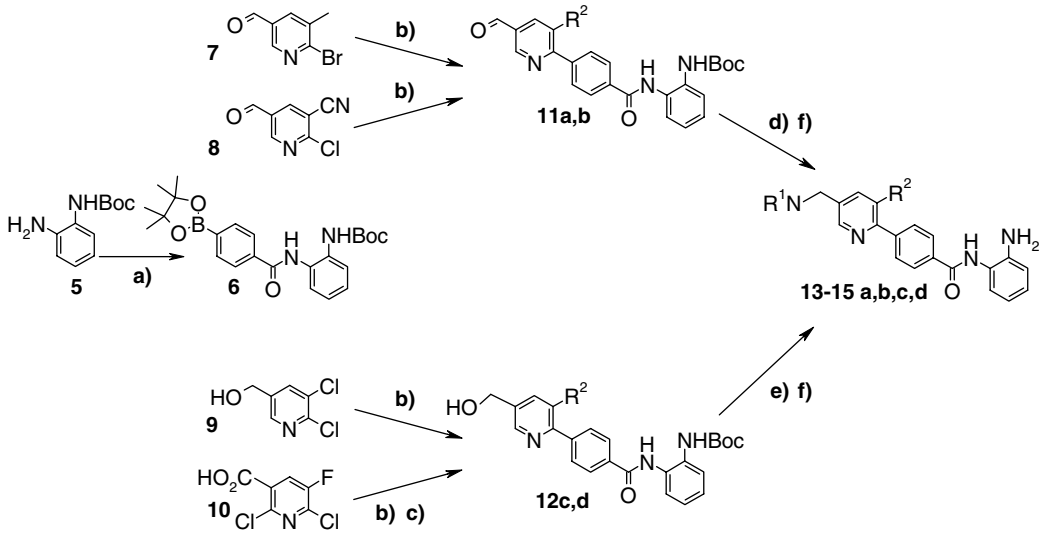


Figure 2. Synthesis of aminomethylpyridine derivatives (a) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, DMTMM, acetonitrile, rt, 90%; (b) **7** or **8** or **9** or **10**, **6**, $PdCl_2$(dppf), $NaHCO_3$, DME, H_2O, 60 °C, 5–9 h, 50–76%; (c) $LiAlH_4$/THF, rt 15 min, H_2O, 0 °C, NaOH, 2 h; 10% Pd/C, EtOH, Et_3N, H_2, rt 18 h 21%; d) R^1NH, $NaBH(OAc)_3$, DCM, rt, 3 h, H_2O 50–80%; (e) MsCl, DCM, Et_3N, **12c** or **12d**, rt, 2 h, R^1NH, rt, 18 h 50–80%; (f) DCM, TFA, rt 30 min, SCX2 cartridge elution—DCM, MeOH, 2 M NH_3/MeOH.

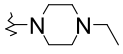
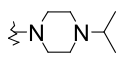
commercially available haloheterocycles **7**–**10**. (Fig. 2) **6** was obtained in 90% yield on a >500 g scale by coupling amine **5** with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid using *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-*N*-methylmorpholinium chloride (DMTMM) in acetonitrile at room temperature. The haloheterocycles **7**–**10** were coupled by heating with **6** in water/DME/$NaHCO_3$ at 60 °C and in the presence of tetrakis(triphenylphosphoranyl) palladium as the catalyst.

The appropriate pyridine precursors for the methyl,[11] cyano[12] and chloro analogues were either commercially available, or prepared according to the literature precedent. The precursor to the fluoroanalogue was derived by coupling commercially available **10** with the boronate ester **6**, as above, followed by reduction using lithium aluminium hydride to adjust the oxidation level. The intermediate 6-chloro compound was dechlorinated using hydrogen gas over 10% palladium on charcoal in ethanol/triethylamine overnight at room temperature. Although the yield was only modest (21%), this was more than compensated by the directness of the approach.

Aldehydes **11a** and **11b** were then subject to multiple parallel synthesis, using sodium triacetoxyborohydride in dichloromethane and a range of secondary amines, the yields typically being in the range 50–80%. Alcohols **12c** and **12d** were activated using methanesulfonyl chloride and displaced with the same set of secondary amines, again with overall yield typically in the range 50–80%. The final test compounds were liberated in 70–95% yields by Boc deprotection using TFA/DCM and were characterized by LC–MS and proton NMR.

The biological data for an illustrative subset of the tertiary amines generated by this approach is summarized in Table 1. In all, exploitation of the four different 3-substituted pyridine aldehydes and alcohols led to the synthesis of approximately 300 secondary and tertiary amine test compounds.

Table 1. Efficacy and non-efficacy properties of aminomethylpyridine-based HDAC inhibitors

Compound	R1	R2	M_{Wt}	HDAC1 enzyme mean pIC_{50}[10]	HCT116 proliferation mean pIC_{50}[13]	ACD log *D* (7.4)	hERG mean pIC_{50}	pH 7.4 mean solubility (μM)	pH 7.4 measured log *D*
3	—	CN	314	7.91	6.54	2.05	<4.5	35	1.58
13a		CH_3	372	7.67	6.51	0.87	<4.6	2240	1.18
13b		CN	383	8.01	6.86	0.38	<4.6	746	1.22
13c		Cl	393	7.73	6.52	1.5	4.65	1340	NV
13d		F	376	7.30	6.43	1.3	<4.6	841	1.69
14a		CH_3	430	7.73	6.64	1.98	<4.6	>2350	1.20
14b		CN	441	8.01	6.86	1.09	<4.6	>1940	1.14
14c		Cl	450	7.79	6.47	2.27	5.08	NT	1.81
14d		F	434	7.42	6.36	2.04	<4.5	>2420	NV
15a		CH_3	444	7.72	6.62	2.31	<4.6	>2620	1.44
15b		CN	455	7.98	6.85	1.42	4.72	1385	1.28
15c		Cl	464	7.88	6.51	2.6	5.15	>1490	1.68
15d		F	448	7.48	6.19	2.37	<4.5	>2160	1.60

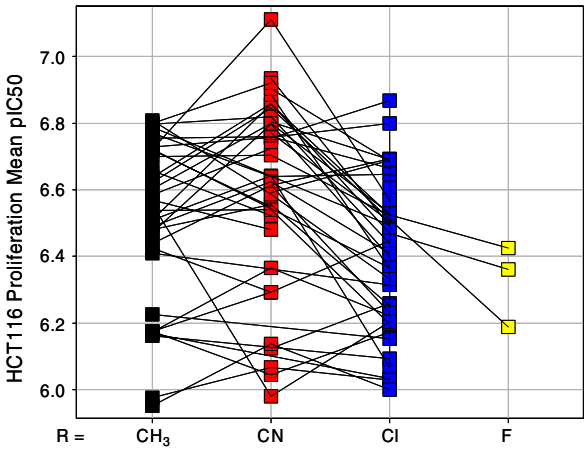


Figure 3. Pyridine core vs cell potency; line connectors show matched pairs.

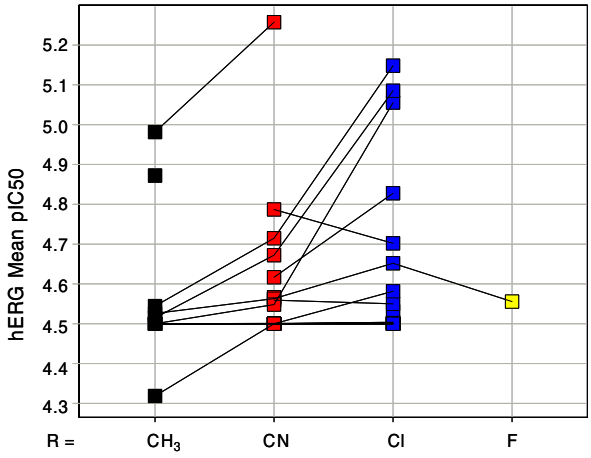


Figure 5. Pyridine core vs IonWorks hERG pIC_{50}; line connectors show matched pairs.

The successful parallel synthesis allowed rapid systematic testing and visualization of the results by matched pair analysis. When the data for a small number of compounds are studied (as in Table 1), the SAR for cell potency appears to be fairly flat, but when the whole dataset is analyzed, modest but clear trends in potency for the 3-substituent can be observed. Figure 3 shows that when matched pairs are compared, the chloro and fluoro-substituted pyridines are consistently less potent than their methyl and cyano counterparts. Interestingly, the enhanced potency of the cyano series does not appear to arise as a consequence of increased lipophilicity as the cyano compounds generally display log *D* 0.4 lower than their halo matched pairs (Fig. 4). Extensive use of the calculated log *D* was used pre-synthesis to select compounds with moderate predicted log *D*, there being a reasonable correlation between the calculated and measured values. In each series, compounds were selected from those enumerated with log *D* generally below 2.5.

No correlation was observed within the dataset as a whole for the relationship between hERG IC_{50} and lipophilicity or potency,[14] but once again, matched pair analysis suggested that the lowest hERG liabilities resided within the methyl and cyano subseries, as illustrated in Figure 5.

Having established that the cyano and methyl pyridines offered the best balance of efficacy and non-efficacy

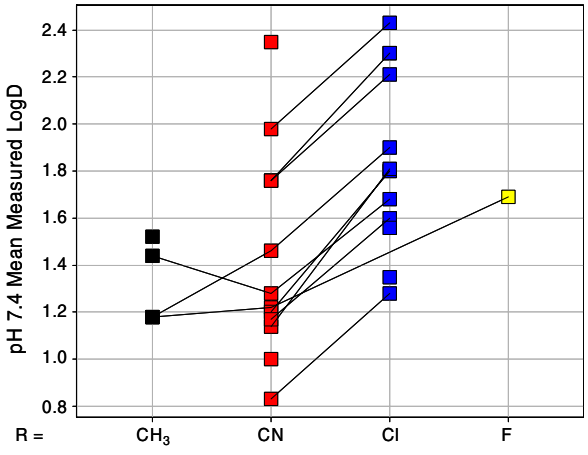


Figure 4. Pyridine core vs measured log *D* (pH 7.4); line connectors show matched pairs.

Table 2. Rat iv DMPK properties of aminomethylpyridine-based HDAC inhibitors

Compound	Dose iv (μmol/kg)	CL (mL/min/kg)	$t_{1/2}$ iv (h)	V_{ss} (l/kg)	$AUC_{(0-t)}$ iv norm (h kg/L)
3	5.0	56.1	NV	1.0	0.30
13a	5.0	30.2	3.0	6.9	0.55
13b	5.0	19.6	3.8	5.4	0.84
13c					
13d	5.0	34.0	3.6	6.3	0.49
14a					
14b	2.5	47.5	1.1	3.6	0.35
14c					
14d					
15a					
15b	2.5	44.0	2.0	5.1	0.37
15c					
15d					

Table 3. Rat oral DMPK properties of aminomethylpyridine-based HDAC inhibitors

Compound	Dose oral (μmol/kg)	$t_{1/2}$ oral (h)	$AUC_{(0-t)}$ oral norm (h kg/L)
3	10.0	NV	0.19
13a	67.1	5.5	0.68
13b	65.2	5.5	0.74
13c	63.6	5.2	0.51
13d	10.0	5.4	0.25
14a	4.9	NV[a]	0.02
14b	56.7	3.2	0.32
14c	55.6	4.4	0.16
14d	4.6	NV[a]	0.26
15a	56.4	3.4	0.18
15b	55.0	4.3	0.33
15c	3.9	NV[a]	0.08
15d	4.5	NV[a]	0.21

[a] Compound administered as part of an oral cassette.

Table 4. DMPK properties of the HDAC inhibitor **13b** in a range of pre-clinical species

Species/route	Dose (μmol/kg)	CL (mL/min/kg)	V_{ss} (l/kg)	$t_{1/2}$ (h)	$AUC_{(0-t)}$ norm (μM h kg/μmol)	Oral %*F*
Nude mouse/oral	65.2	—	—	3.0	0.51	—
Beagle dog/iv	0.8	30.0	2.32	1.9	0.55	—
Beagle dog/oral	3.0	—	—	2.1	0.07	13%

properties, we sought to more broadly elucidate the structure–property relationships by measuring the DMPK properties of the four subseries. The oral exposure of the lead compound **3** had been limited by high clearance in the rat and we were also concerned that its modest solubility would limit its utility in the higher doses needed for a xenograft efficacy study. In general, the normalized oral exposures of the halopyridines were in a similar range to that observed for **3**; those with exposure exceeding the reference generally showed greater hERG inhibition (Tables 2 and 3).

The methyl pyridines **14a** and **15a** showed lower oral exposure than their cyano analogues **14b** and **15b**, precluding further study. In the case of the cyclobutyl analogues **13a** and **13b**,[15] the oral AUCs were comparable, however, clearance of the methylpyridine **13a** was higher than that of cyanopyridine **13b** (approximately 30 vs 20 mL/min/kg plasma clearance).

The solubility of **13b**, coupled with its low plasma protein binding (mouse, 54%; rat, 53%; dog, 48% free), made it an ideal candidate for further evaluation. Compound **13b** showed excellent oral exposure in the rat and moderate dog oral bioavailability as shown in Table 4.

The development of compounds in the HDAC inhibitor class has previously been reported as an iterative exercise,[16] building upon the structure–activity relationships derived for the hydroxamate and benzamide families. In an effort to better understand the utility of the pyridine-based benzamide inhibitors, we have adopted an optimization campaign style of approach. Pre-synthesis calculation of properties was used to define a large compound set capable of being prepared from a small number of advanced, flexible intermediates. Extensive use of R-group stripping and matched pair analysis was used to select compounds for further synthesis and testing, the focus being upon generating structure–property as well as structure–activity relationships. Further profiling of **13b** will be reported in due course, along with other novel HDAC inhibitors.

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