Bioorganic & Medicinal Chemistry Letters 19 (2009) 1866–1870

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of lysine derived sulfamides as histone deacetylase inhibitors

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ARTICLE INFO

Article history:
Received 29 January 2009
Revised 16 February 2009
Accepted 19 February 2009
Available online 23 February 2009

Keywords:
Sulfamides
HDAC inhibitors
HDAC1
HDAC6

ABSTRACT

We have recently reported on a novel class of histone deacetylase (HDAC) inhibitors bearing a sulfamide group as the zinc-binding unit. Herein, we report on the synthesis of sulfamide based inhibitors designed around a lysine scaffold and their structure–activity relationships against HDAC1 and HDAC6 isotypes as well as 293T cells. Our efforts led us to an improvement of the originally disclosed lysine-based sulfamide, **2a** to compound **12h** which has equal potency in enzyme and cell-based assays as well as enhanced metabolic stability and PK profile.

The acetylation status of lysine residues on the N-terminus of core H2A/B, H3 and H4 is known to play a crucial role in chromatin structure and hence the regulation of gene expression.[1] Hyperacetylation of histones is catalyzed by a family of histone acetyl-transferases (HAT) and leads to the relaxation of chromatin and, thus, gene activation. Conversely, hypoacetylation, which is controlled by the histone deacetylases (HDAC), causes the condensation of chromatin resulting in transcriptional repression. The HDAC family is classified into two categories: zinc-dependent amidohydrolases (classes I, II and IV) and the NAD^+ dependent enzymes (class III) also known as sirtuins. Class I of the zinc-dependent HDAC family is composed of isotypes 1, 2, 3 and 8. Class II is further subdivided into two subclasses: class IIa (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 10). HDAC11 is the only known member of class IV.[1a–c]

HDAC inhibitors have shown attractive anti-cancer properties by inducing transcriptional events involved in growth arrest, cell proliferation, and apoptosis.[1,2] The HDAC inhibitor vorinostat (Zolinza®, formerly known as SAHA)[3] was approved in 2006 for the treatment of cutaneous T-cell lymphoma (CTCL),[4a] and a number of other inhibitors are in different stages of clinical development.[4] Various structural classes of HDAC inhibitors have been developed, yet most feature a common pharmacophore comprised of a zinc-binding group, a linker (scaffold), and a surface recognition domain (cap) which together impart potency and isotype selectivity.[2] Examples of inhibitors are depicted in Figure 1 and range from hydroxamic acids such as vorinostat to short-chained fatty acids such as valproic acid.[5] Vorinostat typically exhibits non-selective inhibition against most of the zinc-dependent HDAC's, while MGCD0103,[6] an aminobenzamide currently in Phase I/II clinical trials, is more selective for class I HDAC's over class II. Apicidin[7],

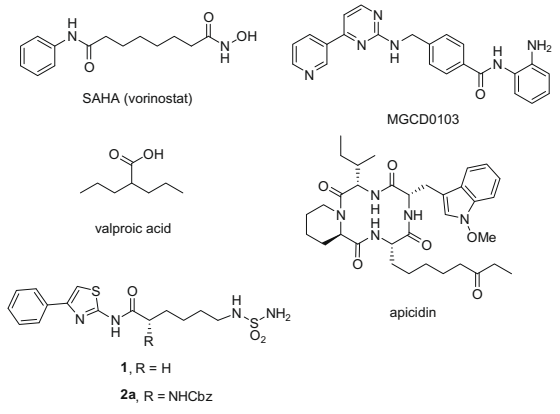

Figure 1. Examples of HDAC inhibitors in preclinical or various stages of clinical development.

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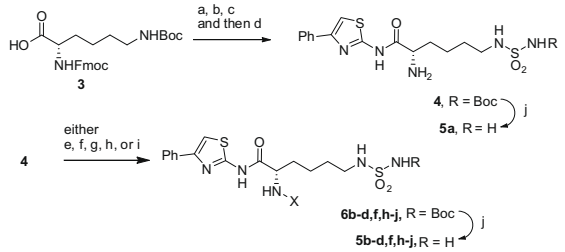
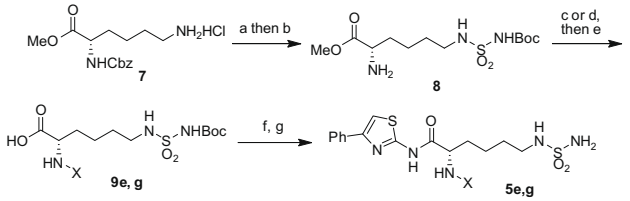
doi:10.1016/j.bmcl.2009.02.075



Scheme 1. Reagents and conditions: (a) $POCl_3$, pyr, 4-phenylthiazol-2-amine; (b) 4 N HCl, dioxane/DCM; (c) $ClSO_2NCO$, *t*BuOH, Et_3N, DCM; (d) piperidine, DCM; (e) BOP, Et_3N, DCM and 2-phenylacetic acid; (f) *N,N′*-disuccinimidyl carbonate, 2,6-lutidine DCM/MeCN and alcohol; (g) Et_3N, THF and 4-fluorobenzylisocyanate; (h) $PhCH_2SO_2Cl$, pyr/DCM; (i) 2-(5-methoxy-2-methyl-1*H*-indol-3-yl)acetic acid, $POCl_3$, pyr; (j) TFA, DCM.



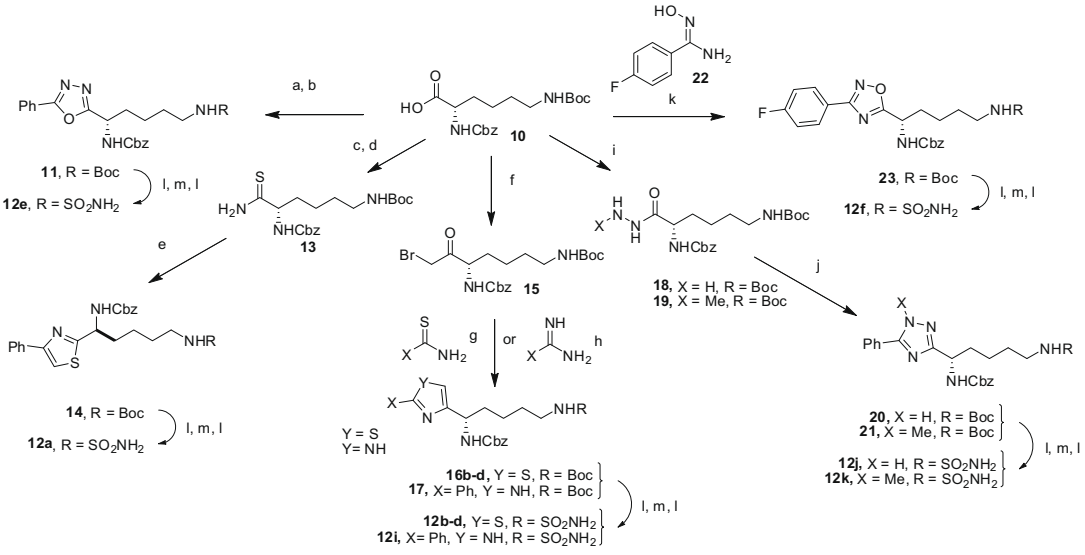
Scheme 2. Reagents and conditions: (a) $ClSO_2NCO$, *t*BuOH, NEt_3, DCM; (b) H_2, Pd/C, MeOH; (c) ethyl chloroformate, PS-NMM, MP-isocyanate, DCM; (d) phenylisocyanate, DMAP, THF; (e) LiOH, THF, MeOH, H_2O; (f) 4-phenylthiazol-2-amine and PS-CDI, DMF/DCM or $POCl_3$, pyr; (g) TFA/DCM.

with its ethyl ketone unit as the zinc-binding group and its cyclic tetrapeptide as the surface recognition domain, also shows mainly class I selectivity.[2a]

Previously,[8] we have reported on an alternative class of HDAC inhibitors featuring a sulfamide moiety as a novel replacement for the more common hydroxamic acid, benzamide or ethyl ketone zinc-binding groups. Furthermore, it was found that the nature of the surface recognition domain that was paired with the sulfamide led to changes in isotype selectivity. For instance, compound **1** with a linear 5-carbon linker was selective against HDAC6 (IC_{50} 0.9 μM) while compound **2a** based on the *N*-carbobenzyloxy (Cbz) derived lysine scaffold was active against both HDAC1 and HDAC6 (IC_{50}'s 0.16 μM and 0.18 μM). In addition to its enzyme potency, sulfamide **2a** also showed whole cell HDAC inhibition in 293T cells (IC_{50} 2.3 μM) and displayed histone (H3Ac, EC_{50} 0.7 μM) and α-tubulin (TubAc, EC_{50} 0.6 μM) acetylations in T24 cancer cells. Given the compound's activity profile and its relative ease of synthesis, it was chosen for further optimization.

Diversification and replacement of the phenylthiazolylamide of **2a** were achieved using the synthetic procedures described previously[8b] while functionalization of the α-amino group on the lysine scaffold was performed using different orthogonally protected lysine derivatives. Starting from commercially available lysine derivative **3**, the protected sulfamide **4** was obtained via coupling with 4-phenylthiazol-2-amine using $POCl_3$ to give the amide, followed by removal of the Boc group from the ε-nitrogen, installation of the protected sulfamide using $ClSO_2NCO$ and *t*-butanol, and then deprotection of the α-nitrogen from the Fmoc group (Scheme 1). The free amine on **4** was further functionalized to the amide, carbamate, urea and the sulfonamide derivatives (**6b–d**, **6f**, and **6h–j**). The removal of the Boc group from the sulfamide group was easily achieved by exposure to TFA to give the final compounds. For the synthesis of ethyl carbamate **5e** and phenyl urea **5g**, lysine derivative **7** was utilized by first installing the protected sulfamide, as described above, cleavage of the Cbz group from the α-amino group to allow for subsequent N-functionalization, followed by ester hydrolysis, then amide formation and finally deprotection of the sulfamide (Scheme 2).

Most replacements of the amide unit of **2a** with various heterocycles were achieved from the commercially available lysine derivative **10** and by introducing the sulfamide unit at the end of each synthetic sequence (Scheme 3). The 1,3,4-oxadiazole **12e** was obtained by the coupling of **10** with benzhydrazide followed by dehydrative cyclization. The 2-phenylthiazole containing sulfamide **12a** was obtained via conversion of **10** to the thioamide **13** followed by treatment with 2-bromo-1-phenylethanone, while the 4-phenylthiazole isomers, **12b–d**, were formed via the addition and condensation of the α-bromoketone intermediate (**15**) with various thioamides. The 2-phenylimidazole analog **12j** was accessible through the reaction of benzimidamide with α-bromoketone **15**.

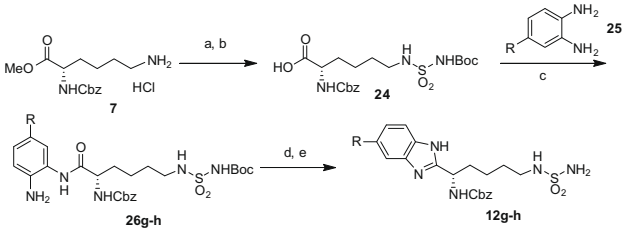


Scheme 3. Reagents and conditions: (a) benzhydrazide, EDCI, DMAP, DCM; (b) (i) $SOCl_2$, Pyr, THF 0 °C; (ii) toluene, reflux; (c) EDCI, HOBt, NH_4OH, DCM; (d) Lawesson's reagent, THF, reflux; (e) 2-bromo-1-phenylethanone, MeOH; (f) (i) ethyl chloroformate, Et_3N, THF; (ii) CH_2N_2 Et_2O; (iii) 48% HBr (aq), Et_2O; (g) thioamide, ethanol; (h) benzimidamide, $KHCO_3$, THF/water, reflux; (i) EDCI, HOBt, hydrazine or methylhydrazine, DCM; (j) methyl benzimidate hydrochloride, Et_3N, THF then xylenes 155 °C; (k) **22**, DCC, DCM then pyr, reflux; (l) TFA/DCM; (m) $ClSO_2NCO$, *t*BuOH, Et_3N, DCM.

The 1,2,4-triazoles **12j** and **12k** were obtained by heating the hydrazide and methylhydrazide analogs of lysine **10** in the presence of methyl benzimidate. Finally, coupling of **10** with hydroxyamidine **22** followed by cyclization gave the 1,2,4-oxadiazole **12f** after installation of the sulfamide unit. For the synthesis of benzimidazoles **12g–h**, the lysine derivative **7** was used and the Boc-protected sulfamide unit was attached prior to the formation of the benzimidazole ring. Cyclization was achieved by heating benzamide intermediates, **26g–h** in acetic acid (Scheme 4).

All synthesized compounds were screened against a panel of recombinant human HDAC's 1–8.[8c] The IC_{50}'s of the inhibition of HDAC1 (representative of class I HDAC's) and HDAC6 (representative of class IIb) are summarized in the tables below, while the results for the other HDAC's were omitted for clarity. These compounds were tested for their ability to inhibit the cellular HDAC activity in 293T cells.[8d]

Table 1 depicts the HDAC inhibitory activities of different thiazole amides compared to the parent compound **2a**. The unsubstituted thiazolylamide (**2b**) had almost negligible activity against both HDAC1 and HDAC6, while the benzothiazole (**2c**) retained its potency on HDAC6 at the dramatic cost of HDAC1 leading to an isotype selectivity of 24 to 1. A phenyl substituent off the thiazole ring of **2a** was more favored than the bulky *t*-butyl group (**2d**), which lost significant activity against both enzymes. The ethyl ester substitution (**2e**) abrogated HDAC1 activity but retained some of it against HDAC6. The 4,5-diphenyl substituted thiazole amide (**2f**) was five and threefold less active than **2a** against HDAC1 and HDAC6, respectively, implicating that a mono-phenyl substituent is superior to disubstitution. Compounds **2g–j** were prepared to investigate the effect of substitution off the phenyl ring of **2a**.



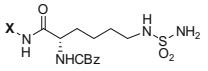
Scheme 4. Reagents and conditions: (a) $ClSO_2NCO$, *t*BuOH, Et_3N, DCM; (b) LiOH, THF/MeOH/H_2O; (c) dianiline, PS-CDI, HOBt, DCM; (d) AcOH, 90 °C; (e) HCl/dioxane.

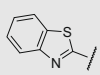
As can be seen from Table 1, substitution on the phenyl group was well tolerated as demonstrated by compounds with electron donating (**2g** and **2h**) and electron withdrawing groups (**2i**), all showing submicromolar activities. Bulkier *para*-aliphatic substituents on the phenyl ring such as an *iso*-propyl (**2j**) were partially tolerated against HDAC1 but less so against HDAC6. Overall, monoaryl thiazoles provided the best activity, but within this series no particular compound exhibited a significant improvement with the exception of compound **2g** which had four times the cellular activity of **2a** in 293T cells.

Modifications of the α-amino substituent on **2a** were also performed to further explore the tolerance of substitution at this position (see Table 2). Complete removal of the benzyl carbamate to leave the free amine (**5a**) resulted in a significant reduction in the activity against both enzymes, thus indicating the importance of functionalization at this position. Substitution on the phenyl unit of the benzyl carbamate of **2a**, as illustrated by compounds **5b–d**, gave an overall decrease in both enzyme and whole cell HDAC activities. Aliphatic carbamates, such as the ethyl carbamate **5e**, showed a noticeable loss in HDAC1 activity in both the enzyme and cell, but remained significantly potent on HDAC6. The removal of the oxygen from the benzyl carbamate to give the benzyl amide analog (**5f**) resulted in a drop of potency against the HDAC1 enzyme. Likewise, the phenyl urea (**5g**), lost most of its activity especially on HDAC1, yet the benzyl urea derivative (**5h**) regained some of it at the enzyme level thus suggesting the importance of the extra atom on the inhibitors' side chain. Further sensitivity on HDAC1 towards amino-substitution was illustrated by the sulfonamide on compound **5i** which was poorly tolerated by the enzyme. Finally, the use of the 2-methyl-5-methoxyindole (**5j**) resulted in exceptional HDAC1 activities (0.064 μM and 0.59 μM on HDAC1 and whole cell, respectively). Overall, marginal improvements were observed by replacing the benzyl carbamate of **2a** and therefore, for further studies we retained this group given the ease of obtaining commercially available Cbz-protected lysine derivatives for synthesis.

During our investigation, it was discovered that compound **2a** had a poor pharmacokinetic (PK) profile in rats (see below). Also, preliminary evaluation of the metabolic stability of **2a** indicated that only 3% of the compound remained intact over 1 h in rat plasma at 37 °C. This instability was determined to be mostly due to cleavage of its amide bond to give the acid and 4-phenylthiazol-2-amine. These observations lead us to explore isosteric heterocy-

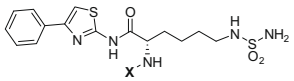
Table 1
SAR on the thiazole amide capping unit[a]

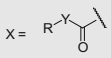
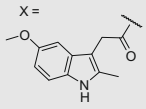


Compound	X =	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (μM)	293T cells IC_{50} (μM)
2a	R^1 = H, R^2 = Ph	0.16	0.18	2.3
2b	R^1 = H, R^2 = H	>10	4.1	>50
2c		4.5	0.19	19
2d	R^1 = H, R^2 = *t*Bu	3.6	0.84	9.1
2e	R^1 = H, R^2 = CO_2Et	>10	0.58	>50
2f	R^1 = Ph, R^2 = Ph	0.85	0.45	7.2
2g	R^1 = H, R^2 = *p*-$MeOC_6H_4$	0.12	0.20	0.53
2h	R^1 = H, R^2 = *m*-$MeOC_6H_4$	0.46	0.42	3.2
2i	R^1 = H, R^2 = *p*-ClC_6H_4	0.15	0.25	4.9
2j	R^1 = H, R^2 = 4-*i*-PrC_6H_4	0.52	1.4	3.2

[a] Values are means of at least two experiments.

Table 2
SAR of the α-amino substituent[a]



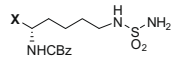
Compound	X = 	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (μM)	293T cells IC_{50} (μM)
2a	R = $PhCH_2$, Y = O	0.16	0.18	2.3
5a	X = H	>10	1.1	>50
5b	R = *p*-$ClC_6H_4CH_2$, Y = O	0.57	0.53	6.8
5c	R = *m*-$ClC_6H_4CH_2$, Y = O	0.35	0.28	3.6
5d	R = 3-pyridinyl-CH_2, Y = O	1.5	0.60	18
5e	R = Et, Y = O	2.5	0.20	17
5f	R = Ph, Y = CH_2	0.70	0.15	6.1
5g	R = Ph, Y = NH	2.3	0.56	>50
5h	R = p-$FC_6H_4CH_2$, Y = NH	0.35	0.37	29
5i	X = $PhCH_2SO_2$	1.4	0.52	38
5j	X = 	0.064	1.2	0.59

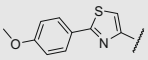
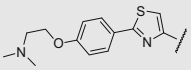
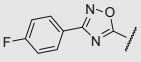
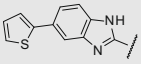
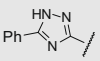
[a] Values are means of at least two experiments.

cles as replacements for the amide unit.[9] For instance; compound **12a** incorporates the same phenylthiazole as **2a**, yet excludes the amide group connecting it to the rest of the lysine scaffold. Unfortunately, the activity of **12a** suffered significantly as a result of this change (Table 3). However, by moving the sulfur atom on the thiazole next to the phenyl group to give its regioisomer **12b**, the enzyme activity returned to submicromolar levels (IC_{50} of 0.79 μM and 0.40 μM on HDACs 1 and 6, respectively). Substitution on the phenyl ring of **12b** improved the cellular potency from 6.7 μM for **12b**, down to an impressive 0.12 μM for the more basic **12d**. Both oxadiazoles **12e** and **12f** were poor isosteres of the amide unit and had an overall negative effect on the enzymatic activities, while the benzimidazoles (**12g–h**) and the imidazole containing compound (**12i**) displayed very potent profiles. Among the benzimidazole series, the unsubstituted benzimidazole (**12g**) was almost 30-fold more selective for HDAC6 over HDAC1, while aryl substitution at the heterocycle's 5-position (**12h**) resulted in compounds with equal potency against both HDAC1 and HDAC6. Particularly noteworthy is the submicromolar activity in 293T cells displayed by benzimidazole **12h** at 0.62 μM which is in line with that of vorinostat (0.6 μM).[8a] The triazole analogue **12j** also revealed partial selectivity for HDAC6, whereas the *N*-methyltriazole **12k** was almost devoid of any significant activity against both enzymes.

Given their good combinations of enzyme and cellular activities, the thiazole **12d** and benzimidazole **12h** were chosen for further pharmacokinetic and phasma stability studies. The metabolic stability of benzimidazole **12h** was an improvement upon **2a**, being stable in both rat and human plasma (data not shown). The PK profiles of **12d** and **12h** in rats revealed that both compounds had lower clearances and increased half lives compared to **2a** (Table 4). However, they still suffered from poor oral absorption as indicated by the low AUC values and dismal bioavailability. Furthermore, the poor PK profile was also observed across different species. Despite the acceptable clearance and half-life of **12h** in dog (0.51 (L/h)/kg and 5.7 h, respectively), the AUC was still poor at 0.2 μM h/(mg/kg), and the bioavailability was a mere 6%. The poor bioavailability displayed by this class of compounds could be attributed to the sulfamide unit. Middleton et al.,[10a] and Beaumont et al.,[10b] encountered a similar problem with their

Table 3
SAR of amide excluded HDAC inhibitors.[a]



Compound	X	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (μM)	293T cells IC_{50} (μM)
12a		4.9	1.2	12
12b		0.79	0.40	5.8
12c		0.50	0.48	n.a.[b]
12d		0.39	0.89	0.18
12e		3.2	1.8	9.9
12f		>10	1.7	>50
12g		2.9	0.1	9.5
12h		0.20	0.48	0.62
12i		0.29	0.41	2.2
12j		2.4	0.32	6.4
12k		4.3	2.0	>50

[a] Values are means of at least two experiments.
[b] Not available.

Table 4
PK profile in rat of selected compounds

Compound	AUC (PO) μM h/(mg/kg)	Cl (IV) (L/h)/kg	V_{ss} L/kg	$t_{1/2}$ (h)	%F
2a	0.063	2.5	0.02	0.025	4.9
12d	0.005	2.3	0.78	1.6	0.5
12h	0.023	0.41	0.03	1.2	0.5

NK2 antagonists bearing a sulfamide group, and have ascribed their compounds' poor oral absorption to weak intrinsic permeability and transporter-mediated efflux from the GI tract.[10]

In conclusion, a structure–activity investigation around the originally disclosed lysine-based sulfamide, **2a** has revealed that subtle modifications around the periphery of the molecule are tolerated when tested against HDAC 1 and 6 isotypes and in 293Tcells, but more significant modifications resulted in considerable losses in HDAC1 activity or, in some cases, both HDAC1 and HDAC6. However, replacement of the core amide linkage on **2a** with different heterocycles led to compounds of equal potency with improved pharmacokinetic profiles. In particular, benzimidazole **12h** showed promising potency and metabolic stability, however, further optimization of its pharmacokinetic properties is needed.

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