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Parallel medicinal chemistry approaches to selective HDAC1/HDAC2 inhibitor (SHI-1:2) optimization

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ABSTRACT

The successful application of both solid and solution phase library synthesis, combined with tight integration into the medicinal chemistry effort, resulted in the efficient optimization of a novel structural series of selective HDAC1/HDAC2 inhibitors by the MRL-Boston Parallel Medicinal Chemistry group. An initial lead from a small parallel library was found to be potent and selective in biochemical assays. Advanced compounds were the culmination of iterative library design and possess excellent biochemical and cellular potency, as well as acceptable PK and efficacy in animal models.

Histone deacetylases (HDACs) are a family of enzymes that catalyze the deacetylation of lysine residues located on the N-terminal tails of histone proteins.[1] HDACs are divided into four distinct classes.[2] The chosen targets, HDAC1 and HDAC2, are class 1 enzymes involved in chromatin-modifying activities, that are thought to play a role in carcinogenesis.[3,5–9] As shown in Figure 1, the structural characteristics of HDAC inhibitors contain three pharmacophores: a surface recognition domain, a linker region, and metal binding region.[4,5,7,10–13]

Zolinza™, is Merck's first-in-class HDAC 1, 2, 3, and 6 inhibitor for the treatment of cutaneous manifestations of T-cell lymphoma.[5] Following the success of Zolinza™, efforts were initiated to improve the efficacy and tolerability of this clinical agent. [16,17,21]

The Parallel Medicinal Chemistry Group at MRL-Boston is charged with assisting project teams using parallel/technology enabled chemistry wherever applicable in order to solve issues of potency, selectivity, pharmacokinetics, etc. Towards this end, members of the group are 'embedded' within a project team with a mission of identifying areas where library synthesis is both applicable and useful. Use of automated systems and databases allows us to generate SAR quickly in order to push projects forward in a useful and rapid manner. Embedded within the HDAC1/HDAC2 project team, we made small focused libraries to quickly generate information in order to advance the project.

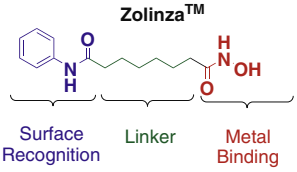

Figure 1. Common pharmacophore of HDAC inhibitors, as exemplified by Zolinza™.

At the start of our efforts in the HDAC1/HDAC2 program, the team's goal was to identify new lead series, which would show improved tolerability and selectivity over HDAC3 as compared to Zolinza™.

Keeping the metal binding domain constant, we initially varied both the surface recognition and linker domains in a library format to identify novel active lead series and establish SAR for these regions. Bi-functional linker scaffolds were coupled with a diverse amine surface recognition set to generate 16 libraries totaling approximately 500 compounds. Nine of these diverse linker scaffolds exhibited an IC_{50} of <1 μM in the HDAC1 assay (see Fig. 2).[16]

Due to its low molecular weight, chemical tractability, and activity in the HDAC1 biochemical assay (see Table 1), we chose

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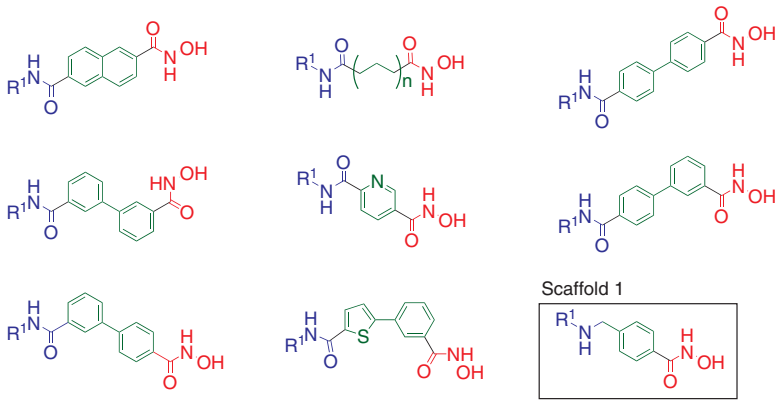
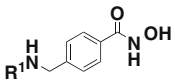


Figure 2. Summary of various linkers used in the initial library synthesis.

Table 1
HDAC1 inhibition from Library A



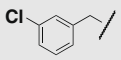
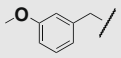
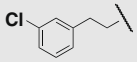
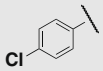
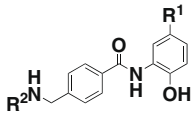
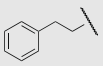
Compound	R^1-NH_2	HDAC1 inhibition IC_{50} (nM)
1		348
2		477
3		1006
4		1620
5		24,060

Table 2
Aminophenol SAR



Compound	R^1	R^2-NH_2	HDAC1 inhibition IC_{50} (nM)	HCT-116-96 h (nM)
6	Ph		972	n/a
7	Ph		112	n/a
8	Ph		32	1357
9	Ph		13	1825
10	Ph		10	1735
11	M_e		20,570	n/a
12	Cl		5032	n/a
13	F		2013	n/a
14	Br		5223	n/a
15	H		1568	n/a
16	OM_e		7964	n/a

to follow-up on Scaffold 1.[18] Our first follow-up library of 48 compounds around Scaffold 1 was synthesized in two steps using commercially available methyl 4-formylbenzoate as the linker scaffold (see Fig. 3).

SAR for this initial series indicated that benzyl amines were favored over their phenethyl or aniline counterparts. It was also shown that aromatically substituted amines were more potent than allylic building blocks. See Table 2.

In addition to gaining potency, replacement of the hydroxamate moiety became a priority. Based on an HTS hit, 2-aminophenols were chosen as a potential replacement for the hydroxamate groups used in the previous library (see Fig. 4). For our next iteration, we devised a rapid solid phase synthetic route to incorporate this new Zn binding motif.

Preliminary SAR suggested that having an aromatic group at R^1 is optimal for binding (this theory was further substantiated by compounds **22–26** (see Table 4). Based on the hypothesis that the phenol group limited cellular activity, our next library replaced the hydroxyl group of the aminophenol moiety with the primary

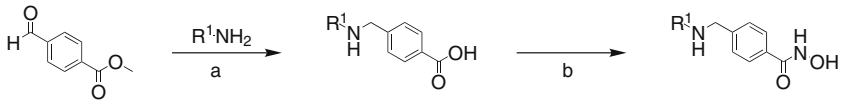


Figure 3. Synthetic scheme for Library A derived from Scaffold 1. (a) PS-$NaBH(OAc)_3$, DMF; (b) NH_2OH (aq), DMF.

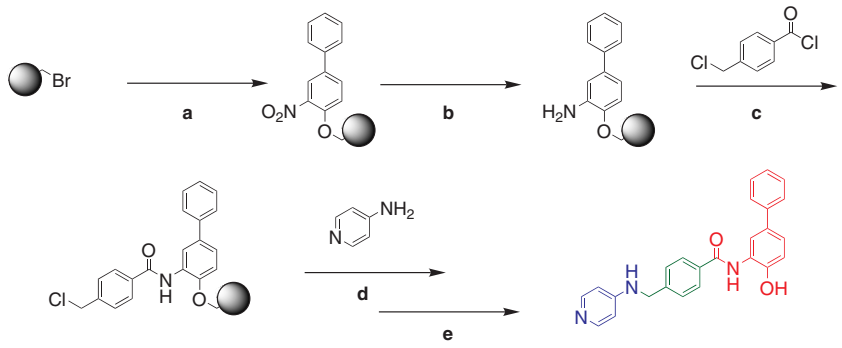


Figure 4. Synthetic scheme of 2-aminophenol derived libraries. (a) Cs_2CO_3, DMF, microwave at 40 °C; (b) $SnCl_2$, DMF; (c) DIPEA, DMAP, DCM; (d) NaI, proton sponge, DMF; (e) 1:1 TFA:DCM.

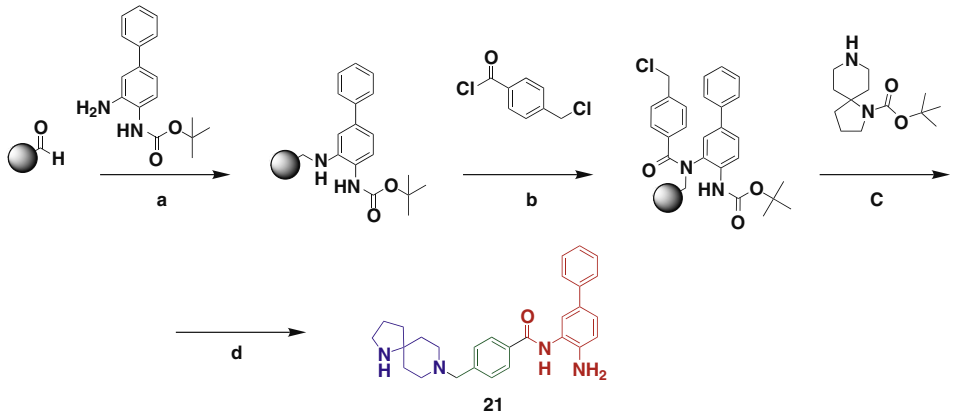
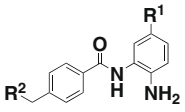
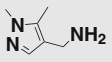
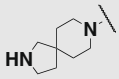
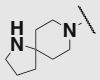


Figure 5. Synthetic scheme of libraries derived from diamino-based zinc binders. (a) $NaBH(OAc)_3$, 5% AcOH/DCE; (b) DIPEA, DCM; (c) NaI, proton sponge, DMF; (d) TFA:DCM.

Table 3
Biphenyl diamine SAR

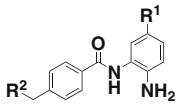


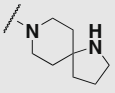
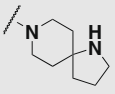
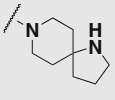
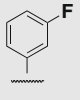
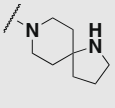
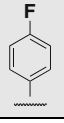
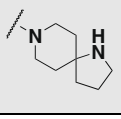
Compound	R^1	R^2-NH_2	HDAC1 inhibition IC_{50} (nM)	HDAC3 inhibition IC_{50} (nM)	HCT-116-72 h[a] (nM)
17	Ph		10	6390	1745
18	Ph		10	5384	3609
19	Ph		13	19,320	670
20	Ph		33	6928	309
21	Ph		8	16,480	118

[a] HCT-116-96 cellular assay was changed to a 72 h assay.

amine (Fig. 5).[14,15] It was only after transitioning to this group that we began to see smaller shifts in the biochemical to cell assay potency (Table 3).[19]

Table 4
Biphenyl diamine SAR

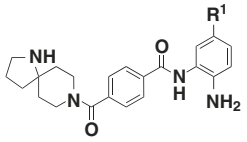


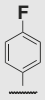
Compound	R^1	R^2-NH_2	HDAC1 inhibition IC_{50} (nM)	HDAC3 inhibition IC_{50} (nM)	HCT-116-72 h IC_{50} (nM)	hERG activity IC_{50} (nM)
22			4	9617	82	1597
23			8	8706	73	1492
24			16	17,390	470	183
25			47	5727	1067	167
26			13	4803	276	237

SAR in the R2 portion of the scaffold was consistent from the aminophenol to the diamine based libraries (see Table 3). Amino spirocyclic building blocks like those in compounds **20**–**21** were identified as potent inhibitors of HDAC1. Inhibitor **21** showed excellent enzymatic and cellular potency, with approximately 2000-fold selectivity against HDAC3 (Table 3). Pharmacokinetic properties of compound **21** in rat plasma include excellent bioavailability (70%), a half life of 8 h, but a moderately high clearance and volume of distribution of 45 mL/min/kg and 22 L/kg, respectively.

Introduction of various other Zn binding elements such as 4-(2-thienyl)benzene-1,2-diamine and 4-(3-thienyl)benzene-1,2-diamine) improved selectivity against HDAC3, however, these compounds exhibited IC_{50}'s of 1.6 μM and 1.5 μM, respectively, in the biochemical hERG binding assays, indicating the potential for cardiovascular toxicity.

Table 5
Terephthalamide SAR



Compound	R^1	HDAC1 inhibition IC_{50} (nM)	HDAC3 inhibition IC_{50} (nM)	HCT-116-72 h IC_{50} (nM)	hERG activity IC_{50} (nM)
27		11	4330	70	25,090
28		11	>50,000	290	–
29		12	3202	138	–
30		8	5102	103	12,390

Incorporation of terephthalamide linkers (Table 5) proved to be a successful way of mitigating hERG liabilities. A library of 26 members was synthesized using the synthetic route outlined in Figure 6.

Enzymatic and cellular potencies, as well as the low serum shift of 3 and selectivity were maintained in this set of compounds. Compound **30** was identified as a potent and selective inhibitor of HDAC1. Ancillary pharmacology showed it to be inactive, at <10 μM, against hERG and Ca^+ ion channels as well as the Cytochrome P450 isozymes (CYP's). Pharmacokinetic studies in rat and dog revealed good drug-like properties, but with low oral bioavailability (Table 6).

In spite of having low bioavailability values of 4% and 17% in dog and rat, respectively, the molecule was advanced into in vivo pharmacodynamic (PD) studies. Compound **30** proved efficacious, as the 25 mg/kg dose IP gave a threefold increase in Ac H2B/Total H2B in the acute PD HCT-116 xenograft model study.[20]

Figure 7 shows how linear library synthesis with embedded parallel chemists rapidly improved chemical properties and aided in the development of an HDAC1 inhibitor.

Through tight integration with the HDAC1/HDAC2 project chemists and the synthesis of parallel libraries, a Parallel Medicinal Chemistry group was able to identify and explore a novel class of HDAC-selective inhibitors. Compound **30** possessed excellent biochemical and cellular potency, acceptable PK profile and off-target activity profiles that exhibit selectivity distinct from that of Zolinza™, which has been shown to be a broad spectrum HDAC inhibitor; equipotent against HDAC's 1, 2, 3 and 6. Compound **30** also proved to be efficacious in the acute pharmacodynamic (PD) HCT-116 xenograft model study.

Table 6
Phamacokinetic properties of Compound **30**

	Rat	Dog
IV dose (mg/kg)	2.0	0.5
Cl (ml/min/kg)	26	8.2
V_{dss} (L/kg)	14	5.1
$t_{1/2}$ (h)	7.0	8.5
PO dose (mg/kg)	4.0	1.0
AUCN (μM h kg/mg)	0.25	0.17
F (%)	17	4

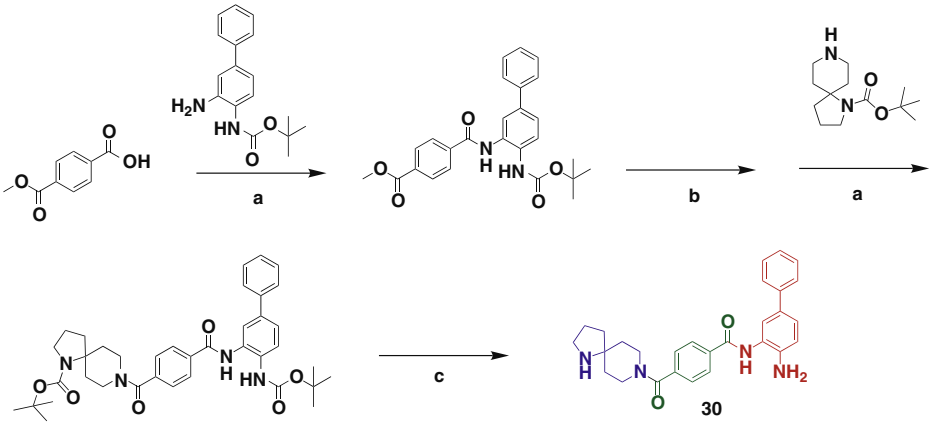


Figure 6. Synthetic scheme of terephthalamide based library. (a) PS-CDI, HOBt, DMF; (b) NaOH, MeOH; (c) 1:1 TFA:DCM.

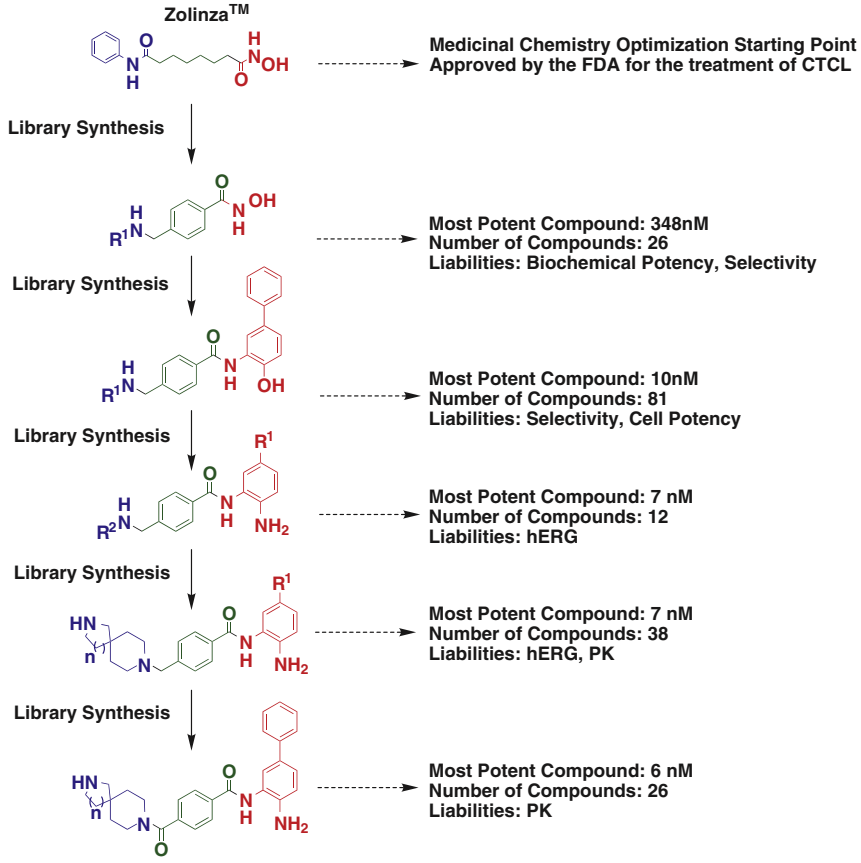


Figure 7. Schematic of the progression of the parallel medicinal chemistry library series.

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19. For cell viability assay procedure, please see Ref. 18.
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