Commentary

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HDAC1/3 dual selective inhibitors - New therapeutic agents for the potential treatment of cancer

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Summary **Histone deacetylases (HDACs) have attracted a great deal of interest as anticancer drug targets, and many HDAC inhibitors (HDACIs) have displayed clinical efficacy in treating specific tumors. However, all of these agents have significant toxicity, including fatigue, nausea, vomiting, thrombocytopenia, and neutropenia. Thus, increased effort is being directed toward developing selective HDACIs that are tolerated better and cause fewer adverse reactions. This article focuses mainly on the *N*-hydroxycinnamamide-based HDAC 1/3 dual inhibitors, and this article outlines the anticancer potential of these inhibitors. Since selective HDAC1/3 inhibitors may cause fewer adverse reactions than selective pan-HDACIs and selective Class I inhibitors in clinical settings, further study of their mechanism of anticancer activity and optimization of their structure is warranted.**

Keywords: Epigenetic, HDACs, selective HDACIs, HDAC1/3 selective inhibitors, anti-cancer agent

Histone deacetylases (HDACs) are a class of zinc-dependent metalloproteinases that catalyze the removal of acetyl groups from lysine residues on histones and non-histone proteins. This action results in a "closed" chromatin configuration, thereby regulating the expression of genes, which include tumor suppressor genes (*1,2*). HDAC inhibitors (HDACIs) have attracted a great deal of interest as anticancer drug agents. Over the past 10 years, over 490 clinical trials of more than 20 HDACI candidates as anticancer agents have been conducted. Three HAACIs, vorinostat (SAHA, Zolinza®), romidepsin (FK-228, Istodax®), and belinostat (PXD101, Beleodaq®) have been approved for the treatment of hematologic tumors. In clinical use as anti-cancer agents (such as vorinostat, panobinostat, belinostat, and abexinostat), many HDACIs inhibit a broad spectrum of HDACs, including Class I, II, and IV isoforms. Although these HDACIs have promising efficacy in treating specific tumors, they all exhibit significant toxicity, including fatigue, nausea, vomiting, thrombocytopenia, and neutropenia (*3*). Thus, increased effort is being directed toward developing HDACIs that selectively inhibit certain classes or a single isoform. This should result in agents that are tolerated better and cause fewer adverse reactions. Several selective Class I, class IIa, and HDAC6 inhibitors (Figure 1) have been reported, but only a few selective Class I inhibitors are used clinically (*4-7*). Selective HDAC6 inhibitors are expected to be beneficial since they may cause fewer adverse reactions. However, the literature indicates that such small molecules have not played a prominent role in cancer therapy, with the exception of their combination with other chemotherapeutics (*8*). Selective Class I HDACIs such as MS-275 and MGCD0103 (HDAC1, 2, and 3-selective) are the most studied selective HDACIs in clinical use or in development. However, these therapeutic have been found to have similar toxicity profiles and overall tolerability in comparison to pan-HDACIs (*9*). A reasonable explanation for this is that selective Class I HDACIs in clinical use may not be selective enough to offer a superior therapeutic benefit over pan-inhibitors. Given this fact, several improved selective inhibitors have been described (Figure 2). These inhibitors have better potency and selectivity for HDAC1 and 2 versus HDAC3 (*10,11*). Medicinal chemists have worked to develop more selective HDACIs, such as inhibitors targeting individual isoforms.

Recently, a series of *N*-hydroxycinnamamide-based HDAC 1/3 dual inhibitors were described by the current

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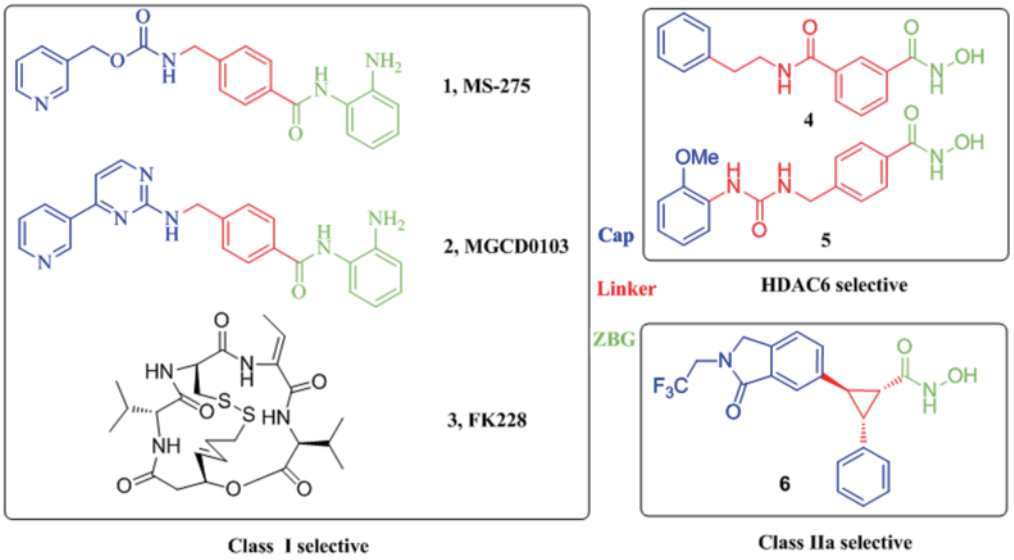

Figure 1. Examples of selective Class I and Class IIa HDACIs and a selective HDAC6 inhibitor

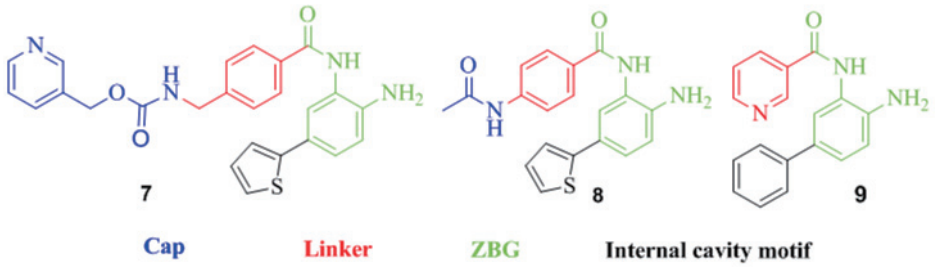

Figure 2. Example of a selective HDAC1/2 inhibitor

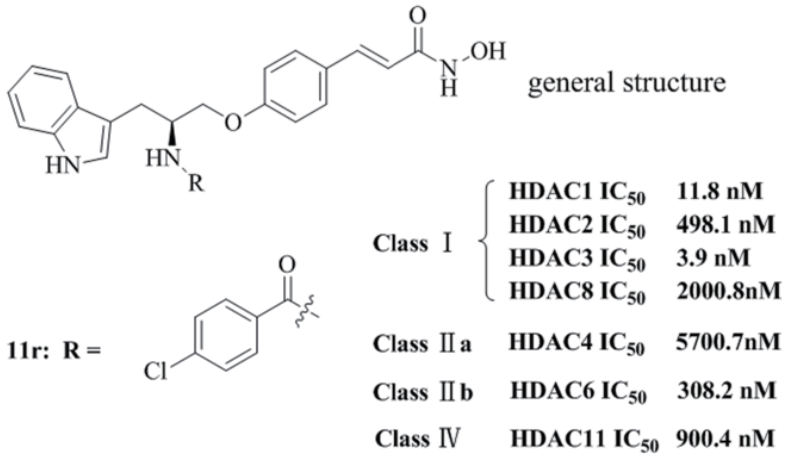

Figure 3. Selective HDAC1/3 inhibitors (12)

authors' laboratory. This work represents the first report of such selective inhibitors with oral activity. The representative compound **11r** had low nanomolar IC_{50} values in response to HDAC1 (11.8 nM) and HDAC3 (3.9 nM) and micromolar or submicromolar IC_{50} values in response to other HDACs such as HDAC2, HDAC4, HDAC6, HDA8, and HDAC11 (Figure 3). Both *in vitro* and *in vivo* studies demonstrated that these HDAC1/3 dual inhibitors could help treat cancer. *In vitro*, some of the selective inhibitors block the proliferation of cancer cell lines, including those of solid and hematologic tumor cells, better than pan-HDACI vorinostat (Table 1). Western blot analysis of procaspase 3 and flow cytometry analysis revealed that the potent HDAC1/3 dual selective inhibitors significantly induce cancer cell apoptosis in a time-dependent and dose-dependent

Table 1. *In vitro* antiproliferative activity of representative compound 11r and positive control SAHA (*12*)

Compound	IC_{50} (μM)									
	U937	K562	HEL	KG1	HL60	MDA-MB-231	PC-3	MCF-7	HCT116	A549
11r	0.16	0.51	0.19	0.22	1.69	0.22	0.46	2.68	0.52	2.74
SAHA	1.45	3.24	0.49	1.59	4.26	1.72	3.57	3.78	2.81	3.90

Table 2. *In vivo* antitumor activity of representative compound 11r and positive control SAHA* (*12*)

Compound	Tumor growth inhibition (TGI)	Relative increment ratio (T/C)
11r	55.1%	37%
SAHA	32.1%	47%

* An *in vivo* study was conducted using a subcutaneous U937 xenograft model. Treatment groups were given compound **11r** (100 mg/kg/d) or SAHA (100 mg/kg/d) orally for 16 days.

manner. An *In vivo* study in a subcutaneous U937 xenograft model revealed that the most potent and selective compound was **11r**, which inhibited tumor growth 55.1% (Table 2). Moreover, mice treated with **11r** had no significant weight loss and no signs of liver or spleen toxicity (*12*). More detailed study of their mechanism of anticancer activity and optimization of their structure to improve transcellular permeability and isoform selectivity are underway in this laboratory.

Selective HDAC1/3 inhibitors are selective for HDAC1 and 3 versus HDAC2, so this type of selective inhibitor may offer a better therapeutic approach over pan-HDACIs. In addition to their activity against cancer, HDACIs have been studied in the treatment of neurodegenerative disorders, including Huntington's disease and Friedreich's ataxia. Thus far, a phase I clinical study of selective Class IHDACI RG2833 for the treatment of Friedreich's ataxia has begun, and phase II clinical trials of selective SIRT 1 inhibitors to treat Huntington's disease (HD) have been conducted. Moreover, HDACIs, and particularly hydroxamate-based inhibitors, have surprisingly been found to show synergistic activity with antifungal agents against fungal species at concentrations that are not toxic to the host. One example is HDACI MGCD290, which was discovered by MethyGene. This inhibitor has been found to have activity against fungal pathogens (including azole-resistant yeasts) especially when used in combination with azole antifungals (*13*). Human HDACs are related to yeast transcriptional regulators and have similar sequences to yeast *Rpd3*, *Hda1*, and *Sir2*, so an interesting question is whether selective HDACIs have the potential to exhibit such antifungal activity. The anticancer activity of selective HDAC1/3 inhibitors has been verified, but their potential use in other ways, such as treatment of neurodegenerative disorders and fungal infection, has yet to be explored. Thus, HDACIs need to be studied a great deal more.

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