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Antiproliferative and proapoptotic activities of 4-hydroxybenzoic acid-based inhibitors of histone deacetylases

Carole Seidel [a], Michael Schnekenburger [a], Mario Dicato [a], Marc Diederich [a,b,*]

[a] Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9, rue Edward Steichen, L-2540 Luxembourg, Luxembourg
[b] Department of Pharmacy, College of Pharmacy, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea

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ABSTRACT

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate cellular processes by modifying the acetylation status of many proteins. Pathologically altered HDAC activity contributes to cancer development and thus characterization of novel acetylation modulators is important for future anti-cancer therapies.

In this study, we identified three novel 4-hydroxybenzoic acid derivatives as pan-HDAC inhibitors that increased protein acetylation levels, arrested cell cycle progression and triggered apoptotic cell death, without affecting viability of normal cells. Our data support the potential of 4-hydroxybenzoic acid derivatives as pan-HDAC inhibitors with anticancer properties.

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1. Introduction

Lysine acetylation is a major post-translational modification regulating function, activity, subcellular localization and protein-protein interaction. Histone acetylation controls gene expression together with other histone modifications and epigenetic mechanisms including DNA methylation. Enzymes responsible for addition and removal of histone acetylation were identified as histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [6].

As altered acetylation profiles were associated to tumor development, HDACs became an interesting target for anticancer therapy [8,22,27]. HDAC family comprises 18 members, subdivided in four classes (Class I: HDACs 1, 2, 3 and 8; class II: HDACs 4, 5, 6, 7, 9 and 10; class IV: HDAC11; class III, also called sirtuins (SIRT): SIRT1 to 7) based on activity, sequence similarity and cellular localization.

To date, numerous proteins were reported as HDAC targets: Class I HDACs maintain proliferation and survival of cancer cells by inhibiting p53 or p21 expression [33]. HDAC5 and 9 repress transcriptional activity of myocyte enhancer factor 2 (MEF2) and Yin Yang 1 (YY1) that prevent cardiac hypertrophy [4,30]. HDAC6 deacetylates α-tubulin and cortactin, which contribute to the polymerization of microtubule and actin filaments, thus affecting cellular mobility and division [12,35].

Inhibition of HDAC activity became an interesting therapeutic target for patients relapsing or refractory to classical chemotherapy [8,22,27]. HDAC inhibitors (HDACi) lead to hyper-acetylation of histone and non-histone proteins inducing differentiation, inhibition of cell cycle progression and/or apoptotic cell death. HDACi target many cancer cell models with moderate effects on normal cells [9,28]. HDACi inhibit aberrant cell cycle progression by inducing cyclin-dependent kinase inhibitor 1A (CDKN1A, p21) and by inhibiting cyclin E [7,11,20]. In addition, HDACi induce cell death by inducing or inhibiting transcription of pro-apoptotic (*e.g.* Bim) or anti-apoptotic (*e.g.* survivin) proteins [3,34].

Accordingly, over the last decades, numerous natural and synthetic HDACi were identified. So far, Food and Drug Administration approved Vorinostat (suberoylanilide hydroxamic acid, SAHA) against zinc-dependent HDACs and Romidepsin (FK228, FR901228) against class I HDACs for the treatment of cutaneous T-cell lymphoma [10,21]. However, improved HDACi are required for increased specificity and reduced side effects.

Here, we describe novel 4-hydroxybenzoic acid (4-HBA)-based compounds with HDAC inhibitory properties. Biological activities on proliferation, cell cycle progression and apoptotic cell death were characterized in both normal and cancer cells.

* Corresponding author. Address: Department of Pharmacy, College of Pharmacy, Seoul National University, Building 20, Room 303, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 8919.
E-mail address: marcdiederich@snu.ac.kr (M. Diederich).

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Table 1
In vitro screening of 4-hydroxybenzoic acid derivatives on total HDAC activity. Values represent percentage of residual activity at 100 μM. Inactive means that 100 μM of compounds is not enough to inhibit 10% of HDAC activity.

Compounds	Residual activity at 100 μM (%)
1b	90
3b	60
4b	90
4f	85
4g	Inactive
5b	65
6a	Inactive
6b	85
6c	85
6d	80
6e	80
7a	85
7b	85
7c	Inactive
7e	Inactive
8b	80
9b	80
9d	70
10b	90
11b	80
12a	60
13a	75
13b	90
13c	65
17b	75
22a	Inactive

2. Materials and methods

2.1. Compounds

Derivatives of 4-HBA: 1b, 3b, 4b, 4f, 4g, 5b, 6a, 6b, 6c, 6d, 6e, 7a, 7b, 7c, 7e, 8b, 9b, 9d, 10b, 11b, 12a, 13a, 13b, 13c, 17b, 22a, were synthetized as previously described [15]. 4-HBA derivatives, SAHA (Cayman, Bio-connect, Huissen, The Netherlands) and etoposide (VP16; Sigma, Bornem, Belgium) were dissolved in DMSO.

2.2. In vitro HDAC activity assay

In vitro total HDAC activity (classes I, II and IV) was measured according to manufacturers instructions using 25 μg of total protein extract from K-562 cells incubated for 45 min at 37 °C (Fluor-de-Lys®, Enzo® Life Sciences, Antwerpen, Belgium). Reaction was stopped by addition of a developing solution (Fluor-de-Lys® developer I concentrate, Enzo® Life Sciences) containing SAHA (2 μM). After 15 min of incubation at room temperature, fluorescence signal was measured on a fluorescence microplate reader (Spectra MAX Gemini, Molecular devices, Inc. Sunnyvale, CA, USA) using the Soft Max Pro software with an exiting wavelength of 360 nm and emission wavelength of 460 nm. All dilutions were prepared in a buffer containing 50 mM Tris–HCl, pH 8; 137 mM NaCl; 2.7 mM KCl and 1 mM $MgCl_2$.

In vitro HDAC isoenzyme activities were tested using recombinants proteins (Enzo® Life Sciences) incubated with the substrate Fluor-de-Lys®-SIRT1 (Enzo® Life Sciences), except for HDAC8 requiring Fluor-de-Lys®-HDAC8 substrate. Quantity of enzymes per well, concentration of substrates and cofactor, and incubation times are presented in supplementary data (Table SI). Reactions were stopped by adding a developer II solution (Fluor-de-Lys® developer II concentrate, Enzo® Life Sciences) containing 2 μM SAHA for HDACs from classes I, II and IV, or 1 mM nicotinamide for sirtuins. After 10 min of incubation at room temperature, fluorescence is measured as for total HDAC activity in 50 mM Tris–HCl pH 8; 137 mM NaCl; 2.7 mM KCl; 1 mM $MgCl_2$ and 1 mg/mL BSA, except for HDAC8 requiring in addition 10% of polyethylene glycol.

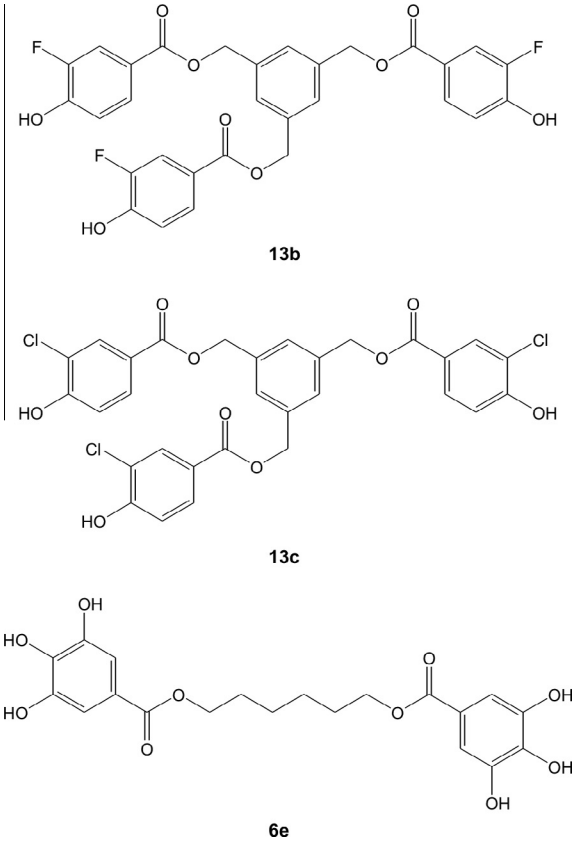

Fig. 1. Chemical structure of the 4-hydroxybenzoic acid derivatives 13b, 13c and 6e.

2.3. Cell culture and treatment

Chronic myelogenous leukemia K-562, histiocytic lymphoma U-937, breast cancer MCF-7, prostate cancer PC-3, erythroleukemia TF-1, Burkitt's lymphoma RAJI, neuroblastoma SK-N-AS and the non-tumorigenic mammary epithelial MCF-10A cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). Peripheral blood mononuclear cells (PBMCs) were isolated as previously reported [23]. All cells, except MCF-10A, were cultured in RPMI 1640 (BioWhittaker®, Lonza, Verviers, Belgium) supplemented with 10% heat-inactivated fetal calf serum (BioWhittaker®) and 1% antibiotic-antimycotic (BioWhittaker®) at 37 °C in humid atmosphere and 5% CO_2. MCF-10A cells were cultured in Dulbecco's Modified Eagle Medium/F-12 supplemented with 5% heat-inactivated horse serum (BioWhittaker®), 100 ng/ml cholera toxin (Sigma), 0.5 mg/ml hydrocortisone (Sigma), 10 mg/ml insulin (Sigma) and 20 ng/ml recombinant human epidermal growth factor (Sigma).

Table 2
In vitro inhibition of selected HDAC isoenzymes by 4-hydroxybenzoic acid derivatives. Inactive means that 100 μM of compounds is not enough to inhibit 50% of HDAC activity. Compounds 3b, 5b, 6d, 7b, 8b, 12a and 13a are inactive against HDAC1, 2, 3, 8, 10 and 11.

Compounds	IC_{50} (μM)									
	Class I				Class IIb		Class III			Class IV
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC6	HDAC10	SIRT1	SIRT2	SIRT3	HDAC11
6e	11	61	Inactive	14	69	100	Inactive	Inactive	Inactive	Inactive
9d	82	Inactive	Inactive	Inactive	Inactive	Inactive	ND	ND	ND	100
13b	5	40	75	Inactive	Inactive	Inactive	12	56	96	4
13c	12	8	Inactive	Inactive	14	Inactive	29	Inactive	Inactive	0.6
17b	Inactive	Inactive	Inactive	77	Inactive	Inactive	ND	ND	ND	Inactive
SAHA	0.1	0.1	0.1	0.3	0.1	0.1	NA	NA	NA	0.1

ND: not determined, NA: not applicable.

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