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4-Hydroxybenzoic acid derivatives as HDAC6-specific inhibitors modulating microtubular structure and HSP90α chaperone activity against prostate cancer

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ABSTRACT

Histone deacetylase (HDAC)6 is a unique isoenzyme targeting specific substrates including α-tubulin and heat shock protein (HSP)90. HDAC6 is involved in protein trafficking and degradation, cell shape and migration. Deregulation of HDAC6 activity is associated with a variety of diseases including cancer leading to a growing interest for developing HDAC6 inhibitors. Here, we identified two new structurally related 4-hydroxybenzoic acids as selective HDAC6 inhibitors reducing proliferation, colony and spheroid formation as well as viability of prostate cancer cells. Both compounds strongly enhanced α-tubulin acetylation leading to remodeling of microtubular organization. Furthermore, 4-hydroxybenzoic acids decreased HSP90α regulation of the human androgen receptor in prostate cancer cells by increasing HSP90α acetylation levels. Collectively, our data support the potential of 4-hydroxybenzoic acid derivatives as HDAC6-specific inhibitors with anti-cancer properties.

1. Introduction

Histone deacetylase (HDAC) activities are essential for the removal of acetyl groups from the ε-amino group of lysine residues leading to modulation of activity, cellular localization or stability of targeted proteins [1–3]. The HDAC family contains eighteen members divided in four classes: class I (HDAC1, 2, 3, 8), class II (IIa: HDAC4, 5, 7, 9; IIb: HDAC6, 10), class III (sirtuins 1–7) and class IV (HDAC11). Beside their implication in the regulation of chromatin structure and therefore in gene expression, HDACs target proteins involved in various biological processes [4].

Among the HDAC isoenzymes, HDAC6 is mainly localized in the cytoplasm where it deacetylates many non-histone proteins [5]. Deacetylation of α-tubulin and cortactin by HDAC6 is associated with microtubular depolymerization and actin filament polymerization, respectively [6,7]. This modulation of cytoskeletal dynamics is required for efficient cell cycle progression and cell motility [8].

Moreover, HDAC6 is able to deacetylate other proteins including peroxiredoxins 1 and 2, Ku70 or heat shock protein (HSP) 90α [9–12]. Among these proteins, HSP90 is involved in cellular homeostasis through its ability to regulate protein folding and proteasomal degradation. In addition, post-translational modifications of HSP90 regulate their chaperone activity; particularly the acetylation of several lysines, including lysine 294, in HSP90α was shown to be crucial for its binding capacity to co-chaperone as well as client proteins [13,14]. HDAC6-mediated deacetylation promotes the binding of client proteins to HSP90α including androgen receptor (AR) [15]. This interaction prevents AR degradation and alters its folding leading to improved ligand binding. Conversely, HSP90α hyperacetylation disrupts its interaction with client proteins inducing their proteasomal degradation [9].

Upon androgen binding, AR is activated through a conformational change that in turn causes its dissociation from HSPs and its translocation to the nucleus where it dimerizes and activates transcription of target genes including *KLK3* encoding the prostate specific antigen (PSA) or *TMPRSS2* encoding transmembrane protease serine 2, both known to be up-regulated in

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androgen-dependent prostate cancer cells [16]. Furthermore, castration-resistant prostate cancer depends on AR function for growth, and the progression from castration sensitive to castration-resistant prostate cancer involves reactivation of AR in a low androgen environment. Accordingly, AR is considered as a marker of prostate cancer development and targeting AR remains an important therapeutic approach [17].

Considering the dual role of HDAC6 in maintaining elevated AR protein levels as well as in favoring proliferation and migration by cytoskeletal modulations, the inhibition of HDAC6 activity may represent a novel strategy to reduce prostate cancer aggressiveness [5]. Indeed, HDAC6 silencing or inhibition by a pan-HDAC inhibitor in different castration-resistant prostate cancer cell lines was already shown to lead to decreased AR expression and subcellular localization [18]. Interference with AR activity results in down-regulation of PSA expression and reduced cell growth [15]. Nevertheless, only few HDAC6-specific inhibitors including tubacin were so far investigated for their anti-cancer properties [8].

Here we identified two new HDAC6-specific inhibitors, 4-hydroxybenzoic acids (4-HBAs) 13a and 7b, able to modulate microtubular structure in androgen-dependent (LNCaP) and independent (PC-3) prostate cancer cells. 4-HBAs decrease cancer cell viability as well as proliferation with a differential toxicity compared to non-cancerous prostate cells. 4-HBAs decrease cell cycle progression and migration in androgen-independent cells, together with HSP90α hyperacetylation leading to strongly decreased AR levels and target gene expression.

2. Materials and methods

2.1. Compounds

4-HBAs 13a and 7b were synthetized as previously described [19]. 4-HBAs, suberoylanilide hydroxamic acid (SAHA; Cayman Chemical Company, Bio-connect, Huissen, The Netherlands) and tubacin (Sigma–Aldrich, Bornem, Belgium) were dissolved in DMSO. Cisplatin (ONCO-Tain® vial, Hospira, USA), paclitaxel (Hospira) and vinblastine (Teva® Pharma, Israel) were in ready-to-use injectable solutions.

Table 1
IC_{50} values and specificities against different HDAC isoenzymes of compounds 13a and 7b.

	13a		7b	
Enzyme	IC_{50} (μM)	HDAC6 selectivity[a]	IC_{50} (μM)	HDAC6 selectivity
HDAC1	499	25	$>1\times10^{4}$	$>5\times10^{4}$
HDAC2	$>1\times10^{6}$	$>5\times10^{4}$	$>1\times10^{4}$	$>5\times10^{4}$
HDAC3	$>1\times10^{6}$	$>5\times10^{4}$	$>1\times10^{6}$	$>5\times10^{6}$
HDAC8	$>1\times10^{6}$	$>5\times10^{4}$	$>1\times10^{4}$	$>5\times10^{4}$
HDAC6	20	NA	0.2	NA
HDAC10	$>1\times10^{6}$	$>5\times10^{4}$	$>1\times10^{6}$	$>5\times10^{6}$
HDAC11	$>5\times10^{4}$	>2500	$>1\times10^{6}$	$>5\times10^{6}$
SIRT1	$>1\times10^{5}$	$>5\times10^{3}$	$>1\times10^{6}$	$>5\times10^{6}$
SIRT2	$>1\times10^{5}$	$>5\times10^{3}$	$>1\times10^{6}$	$>5\times10^{6}$
SIRT3	650	33	$>1\times10^{6}$	$>5\times10^{6}$

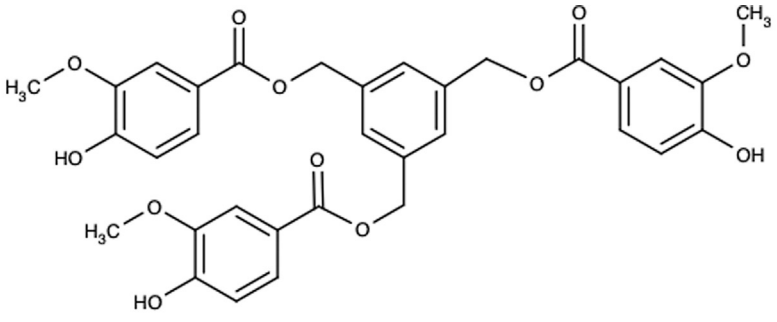
IC (inhibitory concentration)$_{50}$ values were determined by nonlinear regression with the GraphPad Prism 6.0 software.
NA: not applicable.
[a] The HDAC6 selectivity (as the HDAC or SIRT isoenzymes/HDAC6 IC_{50} ratio) for each compound is reported. Inhibition assays were performed in triplicate.

2.2. In vitro *HDAC activity assay*

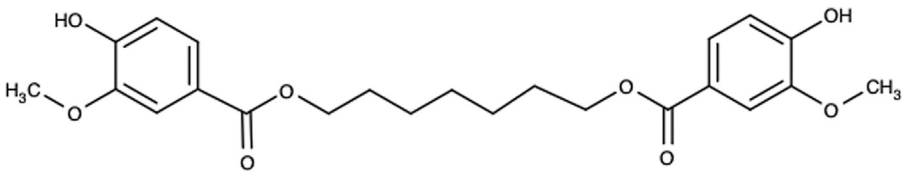
In vitro HDAC activities were measured as previously described [20].

2.3. Cell culture and transfection

Prostate cancer cell lines (PC-3 and LNCaP), breast cancer cell lines (MDA-MB-231 and MCF-7) and the non-tumorigenic mammary epithelial cell line MCF-10A were obtained from the American Type Culture Collection (ATCC). Benign prostatic



13a



7b

Fig. 1. Chemical structures of compounds 13a and 7b.