Biomedicine & Pharmacotherapy 107 (2018) 475–483

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

# Identification of *N*-Hydroxycinnamamide analogues and their bio-evaluation against breast cancer cell lines

Akhilesh Kumar Shukla[a], Hamidullah[b,c,d], Manoj Kumar Shrivash[a], Vishwa Deepak Tripathi[e], Rituraj Konwar[c,d], Jyoti Pandey[a,*]

[a] Department of Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, UP, India
[b] Department of Dermatology, University of Wisconsin Madison, WI 53706, USA
[c] Endocrinology Division, CSIR-Central Drug Research Institute (CDRI), Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India
[d] Academy of Scientific and Innovative Research (AcSIR), CSIR-Central Drug Research Institute Campus, Lucknow 226031, India
[e] Department of Chemistry, Maharani Kalyani College Laheriasarai, Lalit Narayan Mithila University, Darbhanga 846001, Bihar, India

ARTICLE INFO

*Keywords:*
Breast cancer
MDA-MB-231
MCF-7
*N*-Hydroxycinnamamide
HEK-293

ABSTRACT

The present study demonstrates the identification of *N*-hydroxycinnamamide derivatives and their anticancer potential against human triple-negative breast cancer cell line MDA-MB-231, MCF-7 and non-malignant origin cell line, HEK-293 (human embryonic kidney). MTT assay was studied with HEK-293 cell line. Anticancer potential of the *N*-hydroxycinnamamide derivatives were compared with marked drug Tamoxifen through in vitro study. The compound numbers **3b** and **3h** exhibit most potent activity against antagonistic breast cancer cells (MDA-MB-231) with $IC_{50}$ **13μM** and **5μM** respectively. **Compound 3h** promotes DNA fragmentation and induction of apoptosis. Furthermore, loss of mitochondrial membrane potential induced by **compound 3h**. The major mechanism of **compound 3h** for anti-breast cancer activity was probably initiation of reactive oxygen species (ROS) in cancer cells thereby persuading apoptotic cell deaths in cancer cells.

## 1. Introduction

Cancer is one of the most severe public health issue around the globe according to the World Health Organization (WHO) [1]. Among various type of cancers breast cancer is also one of the major causes of cancer death among women worldwide. Due to its complex cancer biology, it is necessary to use multiple therapeutic modalities. So far, the conventional treatments for breast cancer are surgical intervention, hormonal therapy, radiotherapy and chemotherapy. It is merely responsible for 20–25% of all cancer cases and 15–18% of cancer deaths among women [2]. Although the emergence of drugs such as Tamoxifen and Toremifene makes chemotherapy a viable choice for breast cancer patients, the development of drug resistance and severe side effects are unresolved problems in clinical oncology [3]. Therefore, the search for novel anti-cancer compounds with improved features is needed. In recent oncology research, different breast cancer cell lines have been applied by investigators for drug discovery purposes and among these cells estrogen non-dependant MDA-MB-231 is one of the most extensively used model [4].

Hydroxamic acids or hydroxamates are carboxylic acids or aldehyde analogues where −COOH group or −CHO group has been replaced by −CONHOH or −CONHR [5]. Hydroxamic acids are well known as efficacious molecules in the field of cancer chemotherapy and as a mutagenic agent. Several hydroxamates based drugs are functioning very good in clinics for cancer chemotherapy such as SAHA [6,7], PXD-101 (Belinostat,Topotarget) [8] and LBH-589 (panobinostat) [9], which are approved by the U.S. Food and Drug Administration (FDA) in October 2006, July 2014 and February 2015, respectively (Fig. 1). There are some other hydroxamate based molecules are in clinical trials, such as m-carboxycinnamic acid bishydroximic acid (CBHA) [10], SB-939 (phase II) [11] and 4SC-201 (Resminostat, phase II) [12].

Among various derivatives of hydroxamic acid, SAHA (Suberoylanilide Hydroxamic Acid) is considered as a potent anticancer agent [13]. These molecules possess very good chelating ability [14]. This chelating property makes them very favourable for enzyme inhibition and therefore hydroxamates possess a special place in cancer drug discovery research. Due to these special properties hydroxamates are very interesting group for scientists from all over the world. Several research groups have synthesized different hydroxamic acid moieties with well-known inhibitors of matrix metalloproteinases (MMPs) [15], peptidyldeformylases [16], adenylyl cyclases (ACs) [17], inosine monophosphate dehydrogenase (IMPDH), histone deacetylase (HDAC)

* Corresponding author.
*E-mail address:* drjyotibbau@gmail.com (J. Pandey).

https://doi.org/10.1016/j.biopha.2018.08.015
Received 11 June 2018; Received in revised form 24 July 2018; Accepted 6 August 2018

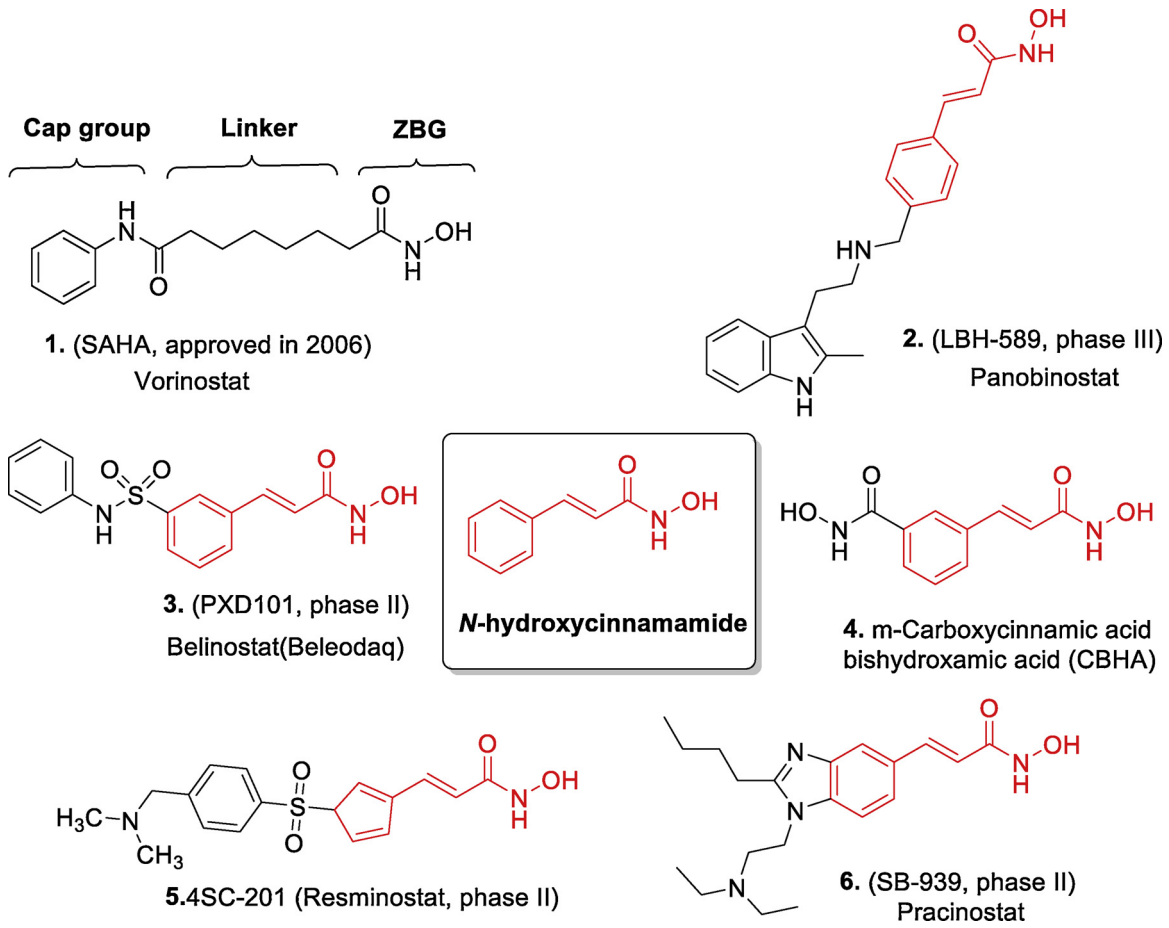


**Fig. 1.** Approved and clinical HDACLs with *N*-hydroxycinnamamide fragment highlighted in red.

[15], carbonic anhydrase [18], tumor necrosis factor converting enzyme (TACE, ADAM17) [19] and TNF-α–converting enzyme [20]. One research group reported antileukemic activity in hydroxamic acids [21,22]. Azaindolehydroxamic acid derivatives are known to possess potent anti-HIV activities [23] while sulfonamidohydroxamates are good anti-osteoarthritis agents [24–26]. In addition to these interesting properties this moiety is also present in many growth factors [27], food additives [28], antibiotics [29,30], antitumors [31], antifungals [32], cell division factors and enzyme inhibitors [33,34]. They have also shown inhibition against melanogenesis [18]. As many enzymes are inhibited by hydroxamates, several physiological processes are affected by this versatile class. Some molecules with hydroxamic acid functionality have also been reported as NO donors [35] and the acetylated hydroxamates derivatives can act as effective aspirin analogues by prostaglandin H2 synthase inhibition [36,37].

It is evident from literature survey that hydroxamic acid derivatives have attracted scientists for their potential as highly efficacious in combating various etiological factors associated with cancer [38]. They have also been used as acyl cation equivalents for the preparation of carbonyl compounds and *N*-methoxyamides as precursors of *N*-methoxy-*N*-acylnitrenium ions in electrophilic aromatic substitutions and as precursor for β-lactams synthesis [39,40]. Different *N*-benzoxyamides as precursors of hydroxamic acids, are known for different biological activities including anti-inflammatory [41] antiasthmatic [42] antimetastatic [43] antibiotic [44] psychotropic [33] insecticidal [45] acaricidal [46] and nematocidal activity [33]. In this article we present anticancer property of hydroxamic acid derivatives to show that this single functional moiety has not only fit in the receptor site but also create a diversified activity.

Present work demonstrates synthesis of simple hydroxamic acids by aldehyde using HWE reaction and synthesis of 2-*O*-alkyl benzhydroxamic acids.

## 2. Materials and methods

### 2.1. Materials

All chemicals, reagents and solvents were purchased from Sigma Aldrich and Merck. Thin layer chromatography was performed using silica gel 60 F254 plates with detecting agent iodine vapours, Merck Silica gel (60–120 mesh) was used for column chromatography. IR spectra were recorded as thin films or in chloroform soln with a Perkin–Elmer Spectrum RX-1 (4000–450 $cm^{-1}$) spectrophotometer. 1H and 13C NMR spectra were recorded on a Brucker DRX-400 and 101 MHz in DMSO-d6. Chemical shift values are reported in ppm relative to SiMe4 as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m(multiplet); J in hertz. FAB mass spectra were performed using a mass spectrometer Jeol SX-102 and ESI mass spectra with Quattro II (Micromass). Melting points were obtained manually by capillary methods and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer. The organic extracts were dried over anhydrous Na2SO4 and evaporation of the solvent was carried out on a rotary evaporator under reduced pressure.

### 2.2. Synthesis of α, β-unsaturated hydroxamic acids

The starting acrylate derivatives (**2a-k**) were prepared by the Horner-Wadsworth-Emmons (HWE) olefination of different aromatic aldehydes (**1a-k**) with triethylphosphonoacetate in the presence of LiOH in THF at ambient temperature. The reaction of acrylate