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Review article

Synthesis and Anti-cancer activity of new optically active 1, 3, 4-thiadiazole derivatives acting as histone deacetylase inhibitors by rational approach

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ABSTRACT

Histone deacetylase (HDACs) play a key role in homeostasis of protein acetylation in histones and other proteins and in regulating fundamental cellular activities such as transcription. A wide range of tumors are associated with imbalances in protein acetylation levels and transcriptional dysfunctions. Treatment with various HDAC inhibitors can correct these deficiencies and has emerged as a promising new strategy for therapeutic intervention in anticancer disease. 1,3,4- thiadiazoles represents an important heterocyclic system due to their pharmacological activity like anti-bacterial, anti-fungal, anti-epileptic, antiulcer, anti-mycobacterial, anti-inflammatory, anti-cancer. Here, we review and discuss intriguing recent developments in the use of HDAC inhibitors to combat anticancer conditions in cellular and disease models. We discuss the targets and mechanisms underlying these effects of HDAC inhibition and comment on the potential for some HDAC inhibitors to prove clinically effective in the treatment of anticancer activity. New optically active 1,3,4-thiadiazole derivatives containing heterocyclic are synthesized by using simple starting materials like optically active stereo center containing amino acid like L-alanine by protection of the amino group with the help of BOC anhydride to convert amino acid to lead to formation of thiadiazole by Cyclization using simple laboratory conditions and followed by attachment of different heterocycle including benzothiazole to form thiadiazole derivatives.

Keywords: Cancer, chiral, L-alanine, benzothiazole, histone deacetylase.

INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The Based on the GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008. The earlier sources of drugs were plants, animals and minerals, but due to the lack of potential action, definitive cure and sometimes more toxicity, the discovery of new drugs that are more potent and less toxic is essential. Today more than 60% drugs used in practice are synthesized derivatives and day-by-day the scope of synthetic medicinal chemistry is broadening.

Cancer (a malignant growth) is medically known as neoplasm which means a relatively autonomous growth of tissues and commonly defined as an uncontrolled growth of cells, with loss of differentiation & commonly with metastasis, spread of the cancer to other tissues and organs. Cell division is a genetic process in which a cell passes its genes onto two daughter cells, each of which is a clone or exact of itself. Sometimes, this orderly process goes wrong, the genes in a cell may suffer a mutation or some mistakes may occur in DNA replication and recombination during cell division [1].

Histonede Acetylase Inhibitors

Various disorders in human are caused by the dysfunction of enzyme as well as their hyper expression. Histone deacetylase (HDACs) is an important class of enzyme which plays an important role in transcriptional regulation [2].

Mechanism of action

To carry out gene expression, a cell must control the coiling and uncoiling of DNA around Histone. This is accomplished with the assistance of Histone acetylates (HAT), which acetylates the lysine residues in core Histone leading to a less compact and more transcriptional active chromatin and on the converse, the actions of Histone deacetylase (HDAC) which remove the acetyl groups from the lysine residues leading to the formation of a condensed and transcriptional silenced chromatin. it constitutes mechanism for remodeling chromatin structure and controlling gene expression. HDAC inhibitors (HDI) block this action and can result in hyper acetylation of Histone, thereby affecting gene expression [3].

The HDAC family is divided into the Zn- dependent (Class I and Class II) and NAD- dependent (Class III) enzymes. The Zn-dependent enzymes have been the focus of intense research, while the Sir2 family recently implicated in acetylation and regulation of key cell cycle proteins such as p53. Till date, eleven HDAC family members in Classes I and II have been characterized i.e. HDACs 1, 2, 3, 8 are Class

I and HDACs 4, to 7, 9 and 10 are Class II, a grouping based on sequence similarity. The most recently identified member of HDAC family is HDAC 11, most likely Class I, although its similarity to HDAC 1 is weak. Inhibitors of these enzymes are known to induce cell cycle arrest.

Independent induction of cyclin dependent kinase inhibitor p21, tumor selective apoptosis, and differentiation of normal and malignant cells. So, this direct and indirect effect of HDAC enzyme on tumor cells and metastasis makes histone deacetylase inhibitors (HDACi) as a potential class of anti-cancer agent. SAHA is the first HDACi to meet the FDA Approval for the treatment of cutaneous T cell lymphoma. Several other small molecules which inhibit the HDAC are under clinical trial for cancer.

Radiograph crystallographic studies seveal that the histone deacetylase inhibitors, suberoylanilide hydroxamic acid and trichostatin A, fit into the catalytic site of histone deacetylase, which has a tubular structure with a zinc atom at its base. Histone deacetylase inhibitors cause acetylated histones to accumulate in both tumor and peripheral circulating mono nuclear cells. Accumulation of acetylated histones has been used as a marker of the biologic activity. Hydroxamic acid-based histone deacetylase inhibitors limit tumor cell growth in animals with little or no toxicity. These compounds act selectively on genes, altering the transcription of only approximately 2% of expressed genes in cultured tumor cells. The role that these other targets play in histone deacetylase inducement of cell growth arrest, differentiation, or apoptotic cell death is not known [4].

Mechanism of Action of HDAC Inhibitors HDAC enzymes remove the acetyl group from histones using a charge-relay mechanism consisting of two adjacent histidine residues, two aspartate residues and one tyrosine residue, and crucial for this charge-relay system is a Zn2+ ion, which binds deep in the pocket of the enzyme. There are a large number of literatures which indicates that HDAC Inhibitors blocks the cell cycle and induce differentiation depending on the cell type and environmental factors. HDAC Inhibitors such as trichostatin A (TSA), SAHA and PXD101, acts by displacing the zinc atom. There are two major pathways of apoptosis, first extrinsic" or death-receptor pathway and second the intrinsic" or mitochondrial pathway. All HDAC inhibitors have been reported to follow either one or both of these cell death pathways in many cancer models. The proposed mechanisms of cancer cell death resulting from HDAC inhibitor treatment are as following.

Death receptor (extrinsic) pathway of apoptosis

Mitochondrial (intrinsic) pathway of apoptosis

Inhibition of angiogenesis

Generation of reactive oxygen species (ROS) Autophagy.

HDAC inhibitors have a multiple effects on cancer cells. HDAC inhibitors also induce apoptosis via both the extrinsic and intrinsic pathway.

FDA Approved and Under Clinical Trial HDACI

Vorinostat (SAHA, Zolinza®), a hydroxamate-based inhibitor was the first HDACi to be approved by the Food and Drug Administration (FDA) in 2006 for the treatment of CTCL for patients who had already received two or more prior systemic therapies. It has long been considered to inhibit all zinc-dependent HDACs in the nanomolar conc. Recent studies suggest that [5].

Hydrogen peroxide- scavenging activity Scavenging activity of hydrogen peroxide in Ascorbic acid (10µg and 500µg) and Garcinia cambogia (10µg and 500µg) as reference compounds was shown to be 62.12-94.67% and 36.06-78.69%.

The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant components presents in the extract are good electron donors, they may accelerate the conversion of H2O2 to H2O.

It has only weak inhibitory affect on class IIa enzymes. Romidepsin (Istodax®), a structurally different cyclic peptide (depsipeptide or FK-228), was second HDAC inhibitor that approved by the FDA at the end of 2009. Romidepsin was isolated from Chromobacterium violaceum and inhibits the activity of HDACs at nanomolar conc. This compound inhibits if at all possible class I HDACs and is therefore called a class-selective inhibitor in compare to Vorinostat which also acts robustly on HDAC6. Besides its approval for CTCL, Romidepsin is investigated as a treatment option in other cancer types as monotherapy as well as in combination

therapy. Panobinostat (LBH589) is in clinical trials for various cancers including a phase III trial for cutaneous T cell lymphoma (CTCL)

Valproic acid, as Mg valproate is in phase III trials for cervical cancer and ovarian cancer. Mocetinostat (MGCD0103) is a benzamide histone deacelytase inhibitor undergoing clinical trial in phase II for treatment of various cancers including submission planned. Entinostat (MS-275) is in phase II for Hodgkin lymphoma, lung cancer and breast cancer.

Rationale

It was found that several compounds with 6C-bridge linking benzothiazole moiety and hydroxamic functional groups showed good inhibition against HDAC3 and exhibited potent Cytotoxicity against five cancer cell lines with average IC50 values of low dose, almost equipotent to SAHA. This review summarizes the synthesis of a varieties of inhibitors of histone deacetylase having the chiral center and their effects on transformed cells in culture and tumor growth in animal models. Several structurally different histone deacetylase inhibitors are in phase I or II clinical trials in patients with cancers.

Anticancer activity

Cancer is a disease characterized by uncontrolled growth of normal body cells. No specific cause was known that might be causing cancer but different factors like environmental factors, genetics, hereditary etc may be termed to define the cause of cancer in humans. The in vitro cancer screening is carried out at NCI, USA. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10-5 M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program.

Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well micro titer plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addiction (Tz).

Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50µg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, with 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried.

Sulforhodamine B (SRB) solution (100 µL) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma (tris(hydroxymethyl) aminomethane) base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µL of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100] for concentrations for which Ti>/=Tz [(Ti- Tz)/Tz] x 100 for concentrations for which Ti<Tz. Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.]

Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested [6].

RESULTS AND DISSCUSION

This review focuses that HDAC inhibitors have a multiple effects on cancer cells.

The scheme for the synthesis of new optically active 1,3,4 thiadiazoles is successfully determined .The compounds are screened for anticancer activity against various cancer cell lines at National cancer Institute (NCI, USA). All the selected compounds submitted to National Cancer

Institute (NCI) for in vitro anticancer assay are evaluated for their anticancer activity. Primary in vitro one dose anticancer

assay is performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. Results for each compound are reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. Synthesized compounds are screened up to date for in-vitro anticancer activity against human tumor cell lines at 10-5M at the NCI, NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI. Compounds show growth inhibition against leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate and breast cancer cell lines [7].

CONCLUSION

In this review we summarize studies on classification, mechanism of action, chemistry and the use of HDACi. Vorinostat and Romidepsin are two potent HDACi that got FDA approval for the treatment of cutaneous T cell leukemia. Panobinostat, Mocetinostat, Givinostat, Belinostat and Entinostat are some other HDACi that are under clinical trial for cancer. In the conclusion, new class of optically active compounds are prepared by using eco friendly reagents, relatively cheap and safe compounds are prepared with numbers of derivatives containing thiadiazole and benzothiazole ring system and evaluated for their anticancer potency on different cell lines.

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