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MINI REVIEW

Hydroxyurea and hydroxamic acid derivatives as antitumor drugs

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Abstract Hydroxyurea has been used for decades and it is still valuable for the treatment of some types of cancer. It inhibits ribonucleotide reductase (RNR) enzyme known to be crucial in the conversion of ribonucleotides into deoxyribonucleotides. However, nowadays the main focus has shifted to structurally similar hydroxamic acid derivatives that target specific enzymes involved in cancer progression such as histone deacetylases, matrix metalloproteinases and also RNR.

Keywords Hydroxyurea · Hydroxamic acid derivatives · Cancer

Introduction

Hydroxyurea (HU) was synthesized by Dresler and Stein [1] (Fig. 1). It is a compound soluble in water that spreads equally throughout the body fluids [2, 3]. At first, it was shown that HU inhibits the leukocyte cell growth [4]. However, its use as an antitumor agent began only in the 1960s as it was found that HU blocks DNA synthesis through inhibition of the ribonucleotide reductase (RNR) enzyme. This enzyme is known to be crucial in conversion of ribonucleotides into deoxyribonucleotides and its inhibition by HU does not change the rate of RNA and protein synthesis [5–8]. Nowadays, it is used to treat leukemia and other malignances [9], sickle-cell anemia [10–12], HIV infection [13], thrombocythemia [14], psoriasis [15] and polycythemia vera [16]. HU is structurally related to hydroxamic acids, known as iron chelators and microbial siderophores that bear diverse biological activities such as antibacterial, antifungal, antitumor and anti-inflammatory properties [17–20].

The mechanism of action of HU is based on the inhibition of the iron-dependent enzyme RNR that converts ribonucleotides into deoxyribonucleotides by catalyzing the substitution of the 2′OH-group of a ribonucleotide with a hydrogen by a mechanism involving protein radicals [21]. HU is, therefore, sometimes called a "free radical quencher" because it quenches tyrosyl radical that is buried deeply inside the protein in a hydrophobic environment, located close to the iron center that is used in the stabilization of a tyrosyl radical [22]. In vivo, HU is converted to a free radical nitric oxide species along with other metabolic byproducts. However, 30–50% of the drug remains unchanged [23–25]. Although HU can directly quench the catalytically active tyrosyl radical in RNR, its conversion to the nitric oxide radical (·NO), generated upon the 3-electron oxidation of the drug, may also be responsible for the inhibition of the RNR [24, 26]. Structural activity studies [27] showed that the –NOH group is required to obtain inhibition of DNA synthesis in HeLa cells without affecting RNA or protein synthesis. In addition, it was shown that the substituents, which make the hydroxy group pKa resemble that of the alcohol, increase inhibitory action. This observation was used in the synthesis of many HU derivatives in order to achieve improved antiproliferative properties. Elford et al. [28] showed that amino group is not essential for inhibitory action and proposed it as possible attachment site for additional substituents. That group of HU derivatives, namely *N*-hydroxyureas, is inhibitors of various metal-containing enzymes including carboxypeptidase A, urease, carbonic anhydrase and redox enzymes such as lipo-oxygenase [29, 30].

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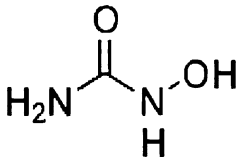

Fig. 1 Chemical structure of hydroxyurea. The free amino group of HU is in hydroxamic acid replaced with the organic residue

The main disadvantage of HU is the need for administration of rather large doses in order to maintain an effective concentration required for its activity. Precisely, it is a relatively weak inhibitor of the enzyme in vitro [31, 32], requiring 500 μM to inhibit 50% of the enzyme. HU may be given orally, intravenously where both types of administration have essentially the same kinetics [33], except for a 19.5% greater maximum plasma concentration after intravenous application. HU toxicity is dependent upon given concentration and duration of exposure to the drug. The terminal half-life is approximately 3.4 h with drug eliminated by the kidney [33]. A major dose-limiting effect is bone marrow depression (leucopenia, anemia and occasionally thrombocytopenia) [34, 35]. Therefore, leucopenia occurs approximately 10 days after administration along with anemia and thrombocytopenia symptoms that occur more rarely than leucopenia [36]. Gastrointestinal symptoms including nausea, vomiting, diarrhea and less common constipation and stomatitis are other side effects but are usually tolerable [36]. Dermatological reactions such as maculopapular rash, skin ulceration [37, 38], dermatomyositis-like skin changes, peripheral and facial erythema are also possible [39, 40]. Hyperpigmentation [41, 42], atrophy of skin and nails, scaling and violet papules have been observed in some patients after several years of long-term daily maintenance therapy with HU. Less common consequences of HU treatment that also require professional intervention include azotemia, hallucinations and convulsions. Temporary allopecia, hepatic dysfunction, allergic reaction and gonadal damage have also been reported in some patients [36].

Hydroxyurea and cancer

HU is an anticancer drug that belongs to the family of antimetabolites. In 1967, the Food and Drug Administration (FDA) approved its use. Beside its use in treatment of sickle-cell anemia, it is used in the treatment of many neoplastic diseases [34] such as chronic, resistant, myelocytic leukemia [43–45], ovary carcinoma (recurrent, inoperable, or metastatic), cervical carcinoma [46, 47], melanoma, meningioma [48, 49] and finally in combination with irradiation in treatment of primary squamous cell carcinoma of the head and neck [50–53]. Such a broad range of action on different malignances is mainly due to the capability of HU to enter cells via passive diffusion, including the brain and cerebrospinal fluid [23, 54].

Hydroxamic acid derivatives and cancer

For the purpose of creating more effective antitumor drugs many derivatives that possess the hydroxamic acid functional group were synthesized [55, 56]. Several hydroxamic acid derivatives that exert antitumor effects by targeting various enzymes are shown in Table 1. Many of those drugs act on histone deacetylase (HDAC) and matrix metalloproteinase (MMP), two enzymes known to be important in tumor development.

Especially, histone acetylation as one of the main mechanisms involved in the regulation of gene expression through the activity of histone acetyltransferases (HATs) and HDACs that activate and repress gene expression, respectively, might be aberrant during carcinogenesis. Therefore, tumor-suppressor genes could be silenced by aberrant histone deacetylation. This epigenetic modification has become an important target for tumor therapy. Histone deacetylation of tumor-suppressor genes occur in a variety of human tumors [57, 58] and studies have shown HDAC inhibition to result in cell growth arrest, differentiation, apoptosis and alterations in gene expression in cancer cell lines [59, 60]. HDAC inhibitors prevent cell proliferation and survival of tumor cells with very low toxicity towards normal cells. Many HDAC inhibitors of different structural types have been described so far, hydroxamate vorinostat (suberoylanilide hydroxamic acid) (Fig. 2) being the first FDA-approved HDAC inhibitor for the treatment of cutaneous T cell lymphoma (CTCL) in 2006. Vorinostat was active against solid tumors and hematologic malignancies if as intravenously or orally in the phase I development [61]. In two phase II trials, vorinostat 400 mg/day was safe and effective with an overall response rate of 24–30% in refractory advanced patients with CTCL including large cell transformation and Sézary syndrome [61].

On the other side, MMPs are zinc-dependent proteolytic endopeptidases that play an important role in the penetration and remodeling of extracellular structures. They have been implicated in the processes of tumor growth, invasion and metastasis [62]. Inhibitors of MMP regulate the malignancy and invasiveness of cancer cells. These enzymes have the zinc-ion coordinated by three imidazole side chains from histidine residues and water is the fourth ligand. Hydroxamic acid inhibitors usually replace the water molecule [63]. Inhibitors that act on the MMPs fall into two distinct generations. First-generation MMP inhibitors include hydroxamic acid peptidomimetics where a peptide backbone with a hydroxamate moiety mimics naturally occurring substrates for MMPs. Peptide backbone interacts with enzyme subsites and hydroxamic functional group is capable to coordinate with zinc. The hydroxamic acid group is, therefore, a very potent 1,4-bidenate zinc ligand that binds as an anion with two contacts to the cation and

Table 1 Hydroxamic acid derivatives as anticancer drugs and their targets: matrix metalloproteinase (MMP), histone deacetylase (HDAC), ribonucleotide reductase (RNR), peptide deformylase (PDF), lipoxygenase (LOX)

Hydroxamic acid derivative	Target				
	MMP	HDAC	RNR	PDF	LOX
Trichostatin A		*			
Suberoylanilide hydroxamic acid		*			
LAQ824		*			
LBH589		*			
3,4-Dihydroxybenzohydroxamic acid			*		
Actinonin				*	
CGS 27023A	*				
KB R7785	*				
PXD101		*			
Oxamflatin		*			
ITF 2357		*			
BL1521		*			
Azelaic bishydroxamic acid		*			
BB 3103	*				
Suberic bishydroxamate		*			
3,4,5-Trihydroxybenzohydroxamic acid			*		
N-Benzyl-*N*-hydroxy-5-phenylpentanamide					*
Pyroxamide		*			
Ro-31-9790	*				
TAPI-2	*				
Tubacin		*			
5-(4-Dimethylaminobenzoyl)-aminovaleric acid hydroxamate		*			
BB 3644	*				
CRA 024781		*			
CRA 026440		*			
FYK 1388	*				
KB R8301	*				
1-Benzyl-4-(4-(4-chlorophenoxy)benzenesulfonyl) piperidine-4-carboxylic acid	*				
3-(1-Methyl-4-phenylacetyl-1H-2-pyrrolyl)-*N*-hydroxypropenamide		*			
3-(3-(Benzofuran-2-carbonyl)phenyl)-*N*-hydroxyacrylamide		*			
Marimastat	*				
Batimastat	*				
Prinomastat	*				

* Marks the target enzymes of the derivatives

creates a distorted trigonal bipyramidal geometry around the metal [64]. These peptide-like compounds with hydroxamic acid portion are among the most potent inhibitors of the MMPs, with potencies in the nanomolar range [63]. Batimastat (BB-94) (Fig. 2), a hydroxamic acid derivative that has a collagen-like backbone, was the first MMP inhibitor to enter clinical testing [65, 66]. Batimastat is a nonorally bioavailable low-molecular weight hydroxamate. This compound is potent, but relatively nonselective, with IC_{50} values of <10 ng/mL for MMP-1, -2, -3, -7 and -9 inhibition. In vitro batimastat had cytostatic effects against a variety of cancer cell lines and was not cytotoxic [67]. Because of its poor solubility, batimastat was administered intraperitoneally and intrapleurally for the evaluation in clinical trials in cancer patients [68–70]. However, clinical trials with batimastat did not show any significant responses and it was replaced by marimastat (BB-2516) (Fig. 2), another peptidomimetic MMP inhibitor that could be administered orally. Marimastat is a broad-spectrum inhibitor for the MMP family with low nanomolar IC_{50} s

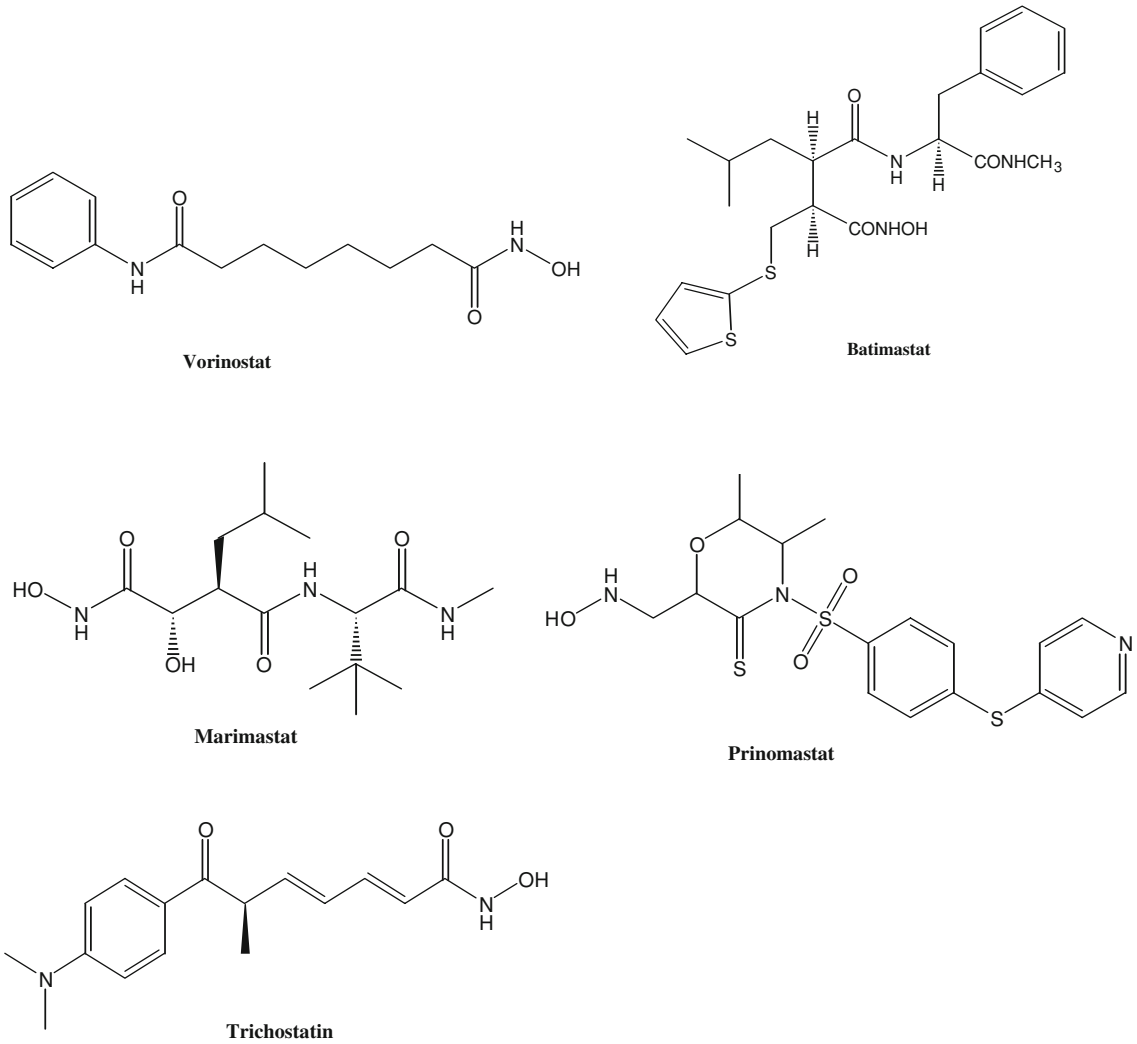

Fig. 2 Chemical structures of vorinostat, batimastat, marimastat, prinomastat and trichostatin

against all the MMPs except MMP-3. Patients experiencing a reduction in tumor markers after the administration of marimastat tended to survive for longer periods than those who did not receive the drug [71].

Second-generation MMP inhibitors are non-peptidic and more specific, probably because they have been designed on the basis of structural studies of the MMP active site by NMR and X-ray crystallography [63]. One of them, prinomastat (AG-3340) (Fig. 2) also bears hydroxamic acid functional group and was synthesized by the use of protein structure drug design program. The drug inhibits MMP-2, -9, -3 and -13, with IC_{50} s (concentration that causes 50% enzyme inhibition) of below 0.13 ng/mL [72].

Another less common target of hydroxamic acid derivatives is RNR. Benzohydroxamic acid and other six-member aromatic ring hydroxamic acids were found to be as inhibitory as was HU but further addition of hydroxy groups to the benzene ring of the benzohydroxamic acid made these potential drugs even more effective in the inhibition of RNR and the life span of L1210 leukemia-bearing mice was prolonged [28]. It was shown that important factor was the proximity of the added hydroxy groups and 2,3,4-trihydroxybenzohydroxamic acid was considered as the most potent enzyme inhibitor because it was 160 times more effective than HU and it increased life span of L1210-leukemic mice at a lower dosage [28]. It required only 1/20 of the dose of HU to achieve antitumor activity. 3,4-dihydroxybenzohydroxamic acid, very active antitumor compound, was most effective in prolonging the life span as it increased the survival time of L1210-leukemic mice over 100% at one-third of the HU dosage [28].

Molecular aspects of some hydroxamic acid derivatives effects in tumor cells

Histone deacetylase inhibitors can induce cancer cell death, whereas normal cells are relatively resistant to them [73].

Mechanism of these inhibitors action is the direct interaction with the active zinc site at the base of the catalytic pocket, which blocks substrate approach active zinc-ion of enzymes [74]. Molecular aspects of HDAC inhibitors effects in tumor cells are complex and not completely elucidated. HDAC inhibitors have been reported to cause differentiation and cell cycle arrest and to induce apoptosis by activating both the death receptor and intrinsic apoptotic pathway, although mitochondria play a central role during HDAC inhibitors-mediated apoptotic response [75–77]. These inhibitors are expected to suppress the cell cycle progression of human tumor cells and cause apoptosis by inducing the expression of cell cycle-arresting genes such as $p21^{WAF1/CIP1}$ [78] and GADD45 [79] as well as many pro-apoptotic genes. However, they repress the expression of several anti-apoptotic and cell cycle-related proteins such as bcl-2 and cyclin A [80]. Another protein, E-cadherin, which is involved in the cell–cell adhesion and whose loss of function has been associated with enhanced metastatic growth of tumor cells, is upregulated by HDAC inhibitors, suggesting a gain of tumor suppressor function in response to HDAC inhibition [80]. HDAC inhibitors have been found to modulate the activity of other cellular key regulators such as the NF-κB transcription factor. Indeed, in precancerous cells activated NF-κB suppresses apoptosis by increasing the expression of cellular survival genes, which results in the enhancement of the premalignant potential. Inhibition of its transactivation by HDAC inhibitors leads to the reversion of the malignant phenotype and this has a beneficial therapeutic effect [81].

Other important therapeutic targets of hydroxamic acid derivatives are already mentioned MMPs. MMPs are involved in tumor growth, invasion, metastasis and angiogenesis [71]. Many human tumors are characterized by locally increased concentrations of MMPs and as the inverse relationship between MMPs activities and clinical outcome in cancer became more and more obvious, inhibition of the function of the MMP cascade became a target for the development of new anticancer drugs [82]. Hydroxymates are particularly potent inhibitors of MMPs, due to their bidentate chelation of the zinc atom. Cancer treatment with MMP inhibitors alone or in combination with standard cytotoxic therapy represents a possible approach in control of tumor progression [83].

Clinical applications of some hydroxamic acid derivatives

Generally, HDAC inhibitors have been successfully introduced in clinical trials as antitumour agents. Recently, there has been a dramatic expansion of HDAC inhibitors in clinical investigation [84]. Among HDAC inhibitors, the most potent are the hydroxamic acid derivatives, like suberoylanilide hydroxamic acid (SAHA), which has been recently approved for therapy of CTCL and is being tested for other malignancies [85]. SAHA, LAQ824, LBH589A, ITF 2357 and PXD-101 (Table 1) are some of the hydroxamate HDAC inhibitors that were moved forward in clinical trials [86–90]. In the preclinical setting, SAHA inhibited MCF-7, MDA-MB 231, MDA-MB-435 and SKBr-3 breast cancer cell lines by inducing G_1 and G_2-M arrest and apoptosis [91] but phase II clinical trial suggested that SAHA and its class should be further evaluated in the treatment of breast cancer as part of a combination therapy [92]. SAHA showed modest antitumor activity in patients with advanced multiple myeloma [93]. It also had limited activity against relapsed diffuse large B cell lymphoma [94] and recurrent platinum-refractory ovarian or primary peritoneal carcinoma [95]. It showed lack of efficacy in patients with squamous cell carcinoma of the head and neck [96]. However, the regimen of SAHA, carboplatin and paclitaxel represents a novel strategy for the treatment of solid tumors as the promising anticancer activity was noted in these patients [97].

Another group of hydroxamic acid derivatives that showed positive preliminary results are inhibitors of MMP. The inability to control metastasis is the leading cause of death in patients with cancer. Control of metastasis, therefore, represents an important therapeutic target. Since MMPs also play an important role in tumor angiogenesis [98] MMP inhibitors may have a dual role in the treatment of cancer. Preclinical studies testing the efficacy of MMP suppression in tumor models were so compelling that synthetic metalloproteinase inhibitors were rapidly developed and routed into human clinical trials but the results of these trials have been disappointing [99]. Nevertheless, in a study with hormone refractory prostate cancer patients treatment with marimastat yielded in a 55% PSA reduction [100]. A significant PSA decrease is generally accepted as a sign of tumor growth inhibition in prostate cancer patients. In the most encouraging clinical trial, patients with unresectable gastric cancer who were treated with marimastat were reported to show a modest increase in survival, although this interpretation has been disputed on the basis of a P value of 0.07 [101]. MMP inhibitors are cytostatic rather than cytotoxic and tumor shrinkage is not a likely event with cytostatic agents so a new means of defining an objective response in phase II trials is required. There is a pressing need to develop and validate markers of tumor progression in this case. Preclinical studies of another MMP inhibitor, namely prinomastat have demonstrated reduction in the rate of primary tumor growth and in the number and size of distant metastases in animal tumor models [102]. Prinomastat indeed has antitumor activity against broad array of rodent tumor models after intraperitoneal and oral administration

[103]. Phase II clinical studies of prinomastat with early stage cancers are currently in progress, although Phase III trials for advanced prostate and lung cancer were stopped because they did not show beneficial effects [104].

The future of HU and hydroxamic acid derivatives

Although HU has been used for decades, it is still valuable for the treatment of some types of cancer. Nowadays, the challenge remains how to develop a novel HU derivative with low toxicity and improved cytostatic action. Few attempts have been made recently to find such compounds. For example, novel L- and D-amino acid derivatives of HU were tested for their effects on the proliferation of different human tumor cell lines [105] and they exerted a strong inhibitory action while showing low toxicity on normal human fibroblasts. Similarly, Perkovic et al. [106] evaluated the cytostatic activity of novel lipophilic HU derivatives of L- and D-amino acid amides against malignant tumor cell lines showing most of them to have strong antiproliferative effects against tumor cell lines and should be included in further evaluation as antitumor drugs.

Nowadays, the spotlight also turns on different hydroxamic acid derivatives, which are structurally related to HU. These derivatives have been proved to act on specific targets involved in cancer progression. One of the major group, the HDAC inhibitors such as trichostatin and SAHA, have thus been reported to inhibit cell growth, induce terminal differentiation in tumor cells [107, 108], prevent formation of malignant tumors in mice [109] and show antitumor activity on certain types of cancer. Further on, hydroxamate vorinostat (SAHA) is the first FDA-approved HDAC inhibitor for the treatment of CTCL. Another important cancer-related enzyme group, namely the MMPs, is targeted by a variety of hydroxamic acid derivatives. However, the first-generation MMP inhibitors were hampered by poor bioavailability and were rapidly replaced by second-generation orally active drugs such as prinomastat. Unfortunately, the results from phase III trials have been disappointing and gave rise to a general conclusion that MMP inhibitors have no therapeutic benefit in human cancer [99]. In addition, many other hydroxamic acids derivatives have been associated with problems such as poor pharmacokinetics and severe toxicity.

Despite all the above-mentioned problems, the synthesis of HU and hydroxamic acid derivatives remains quite appealing due to a generally high potency against malignant cells. In conclusion, those derivatives hold great potential and further attempts to synthesize novel compounds might result with the discovery of new and effective anticancer drugs.

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Conflict of interest statement None.

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