Therapeutic Potential of Hydroxamic Acids for Microbial Diseases

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Abstract Hydroxamic acid derivatives have recently been recommended for therapeutic treatment of several diseases, such as hypertension, cancer, as well as inflammations and infectious diseases due to their ability to chelate metals, especially in metalloenzymes. This chapter will focus on the role of metallopeptidases and their homologs in microbial diseases and the potential use of hydroxamates and their derivates for the treatment and control of such diseases. A general overview of the structure, synthesis, and inhibition mechanisms of hydroxamates as well as their potential use, including the advantages and relative problems, for medicinal chemistry will be discussed.

Keywords Hydroxamic acid · Metalloenzyme inhibitors · Zinc metalloproteinases · LpxC inhibitors · HDAC inhibitors

Abbreviations

AAT	Aminoglycoside acetyltransferases
AIDS	Acquired immunodeficiency syndrome
ANTs	Aminoglycoside nucleotidyltransferases
APH(3')s	Aminoglycoside 3′-phosphotransferases
BNZ	Benznidazole
cART	Combination antiretroviral therapy

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DC	Dendritic cells
ECM	Extracellular matrix
Glu	Glutamate
HDACs	Histone deacetylases
HDACi	Histone deacetylase inhibitors
HIV	Human immunodeficiency virus
*Lma*CP1	*Leishmania major* carboxypeptidase
LpxC	UDP-3-O-(R-3-hydroxymyristol)-*N*-acetylglucosamine deacetylase
LPS	Lipopolysaccharide
MCPs	Metallocarboxypeptidases
MMPs	Matrix metalloproteinases
MRSA	Gram-positive methicillin-resistant *Staphylococcus aureus*
MVEC	macrovascular endothelial cells
NFX	Nifurtimox
6-PGDH	6-Phosphogluconate dehydrogenase
*Tc*MCP-*t*	*Trypanosoma cruzi* metallocarboxypeptidase-*type* Ex. *Trypanosoma cruzi* metallocarboxypeptidase-1
TIMPs	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor-alpha
WHO	World Health Organization
ZBG	Zinc-binding group

Contents

1 Introduction

The involvement of matrix metallopeptidases (MMPs) and their homologs in microbial disease pathogenesis has been extensively investigated in recent years. Several studies demonstrated the multiple functions of these enzymes in viral,

protozoan, and bacterial infections. These findings point to the metallopeptidases as being a potential target for the treatment and control of these infections. Many researcher groups are involved in the search for new therapeutic agents focused on metallopeptidases. Based on such works, the hydroxamic acids and their derivatives have been highlighted as a new, effective class of compounds for the inhibition of the metallopeptidases (Hoekstra et al. 2001; Hou et al. 2012; Pepeljnjak et al. 2005).

Several articles in the literature report the involvement of these peptidases with the pathogenesis of microbial diseases including those caused by viruses, protozoa, fungi, and bacteria (Lindsey and Zamilpa 2012; Marson et al. 2012).

The dengue virus has been shown to infect dendritic cells (DC) and macrovascular endothelial cells (MVEC) with different effects. In dendritic cells, the infection increases the MMP-9 production triggering vascular leakage. In MVEC, the virus infection causes an overproduction of MMP-2 leading to an enhanced endothelial permeability (Luplertlop and Missé 2008).

Metallopeptidases have been described in a number of parasites. An increased production of tumor necrosis factor-alpha (TNF-α) by monocytes, macrophages, or lymphocytes is involved in symptoms of severe malaria such as hypoglycemia, hyperthermia and neurological manifestations, dyserythropoiesis, and immunodepression. Phagocytosed hemozoin (malarial pigment) stimulated the production of TNF-α and other proinflammatory cytokines in human monocytes and displayed increased gelatinaseB (MMP-9) activity showing higher matrix invasion ability (Prato et al. 2011). Studies using a mouse model for study of the *Plasmodium berghei* ANKA cerebral malaria infection showed an increase of MMP-9 expression in positive cells and $CD11b^{+}$cells in the brain (Van den Steen et al. 2006).

Toxoplasma gondii, an obligate intracellular protozoan that can be found worldwide, is the etiologic agent of toxoplasmosis, a zoonotic infection of humans and animals. Following oral infection, *T. gondii* crosses the intestinal epithelium, disseminates into the deep tissues and crosses many biological barriers such as the blood–brain barrier and placenta. However, the molecular mechanisms involved in migration of *T. gondii* remain poorly characterized. The study of Buache et al. (2007) demonstrated that the *T. gondii* RH strain invasion of THP-1 cells induced a decrease in latent gelatinase A (proMMP-2) and latent gelatinase B (proMMP-9) secretion and the author postulated that *T. gondii* may mediate its effects on gelatinase expression through the modulation of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation pathway. Toxoplasmic encephalitis is a marked astrocyte reaction to sites of inflammation and parasite replication. Using astroglia infected with the TS-4 strain of *T.gondii* tachyzoites, MMP-2, and MMP-9 increased significantly until 12 h post-infection in the cell homogenates, and they increased until 48 h post-infection in the cell-cultured supernatants. The author suggested that MMP-2 and MMP-9 cleave fibronectin and may contribute to the astroglial reaction and leukocyte migration to the sites of *T. gondii* replication during toxoplasmic encephalitis (Lu and Lai 2012).

In *Trypanosoma cruzi*, metallopeptidases, belonging to the MMP-9 family, were revealed after Western blotting as an 85 kDa polypeptide in both cellular and secreted parasite extracts. The surface location of homologs of MMP-9 in *T. cruzi* was also evidenced by means of flow cytometry analysis (Nogueira de Melo et al. 2010). Furthermore, in hepatocyte culture infected with *T. cruzi*, the active (85 kDa) and latent (100 kDa) forms of MMP-9 were detected using Western blotting and immunocytochemistry. MMP-9-like activity was detected in the cytoplasm of *T. cruzi* during in vitro infection of hepatocyte cells (Nogueira de Melo et al. 2010). Gutierrez et al. (2008) suggested an important role for MMPs in the induction of *T. cruzi*-induced acute myocarditis, since mice treated with an MMP inhibitor showed a significant decrease in heart inflammation, delayed peak in parasitemia, and improved survival rates compared with the control group.

On the other hand, studies have suggested a great important role for gp63 (63-kDa glycoprotein) in the pathogenesis of leishmaniasis. gp63 is a zinc-dependent metalloprotease found on the surface of the parasite whose expression enhances capacity of the parasite migration through extracellular matrix (McGwire et al. 2003). Other studies demonstrated the presence of metallopeptidases during the leishmanicidal activity in infected macrophages (Costa et al. 2008). An increase in TGF-β production in *Leishmania chagasi*-hepatocyte-macrophage co-culture supernatants was observed during the highest leishmanicidal activity by macrophages, coinciding with higher MMP-9 activity. The high levels of TGF-β can be related to the synthesis of metallopeptidases and the conversion of the latent form to the active form (Costa et al. 2008).

MMPs have also been studied in bacteria. In group A *streptococcus* (GAS), *Streptococcus pyogenes* causes a wide range of human diseases, including bacterial arthritis. Bacterial septic arthritis occurs by bacterial invasion into the joint cavities through blood circulation. Bacterial infection and IL-1 activate different signaling pathways and transcription factors involved in the expression of MMP-13. GAS infection induces MMP-13 expression in chondrocytes through the activation of the c-Jun *N*-terminal kinase (JNK) and the AP-1 transcription factor. MMP-13 plays an important role in the destruction of infected joints during the development of septic arthritis (Sakurai et al. 2008).

In keratomycosis, an experimental murine (BALB/c mice) model, the transcriptional and translational levels of MMP-8, -9, -13, and TIMP-1 increase during the early stages of *Candida albicans* keratitis indicating a possible role of these enzymes in the pathogenesis of the infection (Yuan et al. 2009).

The role of the metallopeptidases in the microbial infections cited above is an example of the participation of this class of peptidase in the pathogenesis process of microorganisms. This chapter discusses the current understanding of the role of metallopeptidases in microbial diseases and their inhibition by hydroxamates and derivates. The following section will be discussed in more detail about this potential target of hydroxamic acids.

2 Metalloenzymes

2.1 Peptidases

Peptidases are hydrolases that hydrolyze peptide bonds in proteins and peptides. They are classified using three criteria: the chemical mechanism of catalysis, the catalytic reaction and, the molecular structure and homology. The former classification is based on the presence of catalytic amino acids, any metal ion or any unknown catalytic thing at their active site. The amino acid may be Aspartic acid (A), Cysteine (C), Glutamic acid (G), Asparagine (N), Serine (S), and Threonine (T). The catalytic reaction depends on the selectivity for the bonds that the peptidases will hydrolyze. The molecular structure and homology is the next approach. The amino acid sequences and three-dimensional structures of the peptidases are analyzed and compared in order to classify and evaluate relationships. The MEROPS database integrated the three systems of peptidase classification and grouped them into protein species, which are in turn grouped into families, and then into clans (release 9.8). Metallopeptidases are currently grouped in 67 families in the MEROPS database (Rawlings et al. 2012).

2.2 Metallopeptidases: Endopeptidases

Metallopeptidases are produced by all species of plants, animals, and microorganisms. In mammals they are called matrix metallopeptidases (Rawlings et al. 2012). Based on the work of Schechter and Berger (1967), Gomis-Rüth et al. (2012) suggested a "standard orientation" for the overall description of MMPs as shown in Fig. 1. In the interaction mode, the active site of the enzyme have subsites named S1, S2, S3, etc., interacting with the side chains of the residues flanking the scissile bond. The substrate side chains on the non-primed side away from the scissile bond are termed P1, P2, P3, and their cognate enzyme subsites (S1, S2, S3) (Gomis-Rüth et al. 2012).

Metallopeptidases contain one or two metal ions in their active site. Most metallopeptidases contain Zn^{2+}, while a few contain Mg^{2+}, Ni^{2+}, or Cu^{2+}. The role of the catalytic metal ions in metallopeptidases is to activate a water molecule, which serves as a nucleophile in catalysis. Many metallopeptidase inhibitors developed against these enzymes included pseudopeptides, mimicking their substrates, as well as small molecules that bind with the catalytic zinc ion. Small non-peptidic molecules are mostly hydroxamate derivatives (Whittaker et al. 1999).

Some metallopeptidases function within the cell or on the membrane (Wu and Chen 2011). Other bacterial metallopeptidases are secreted to the periplasm or outside the cell and are called extracellular metallopeptidases. Microorganisms secrete peptidases in order to degrade environmental proteins for nutrition. However, in recent years due to their role in pathogenicity, metallopeptidases have

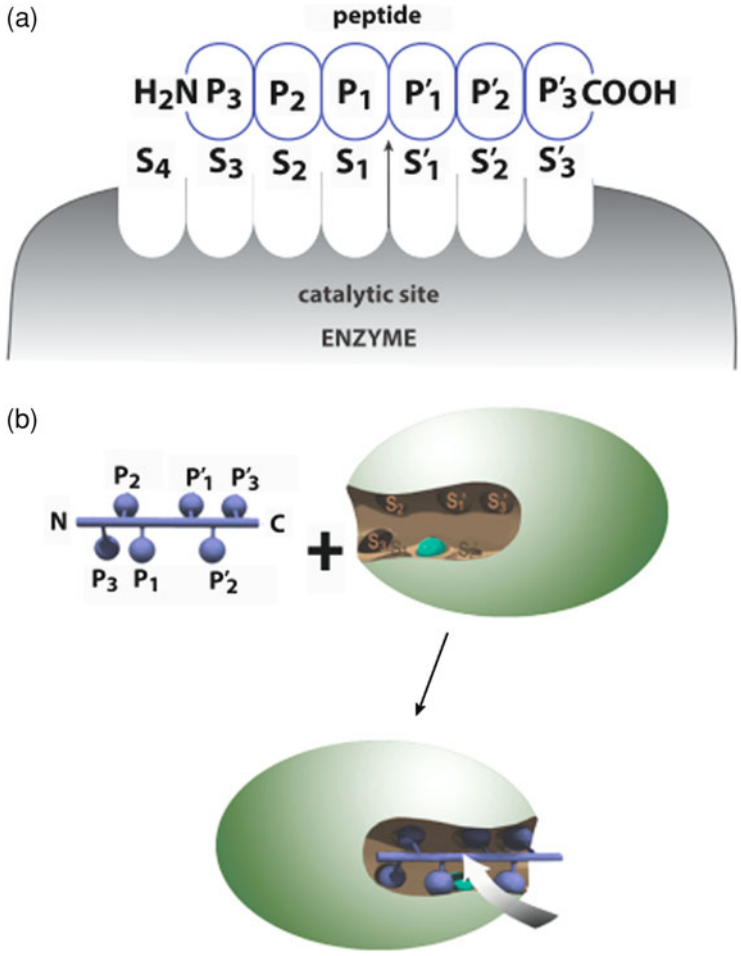
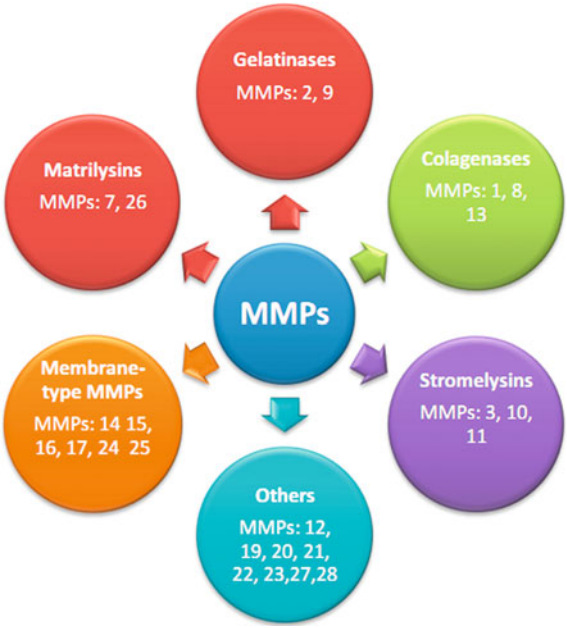


Fig. 1 **a** Model of enzyme-substrate interaction according to Schechter and Berger nomenclature (Schechter and Berger 1967) showing the active site of the peptidase (papain) with subsites (S) and the peptide substrate (P). **b** Three-dimensional model of a metallopeptidase. The metal ion in the active site is shown by *green* and the *arrow* points to the cleavage point. Diagram based on Gomis-Rüth et al. (2012)

been exploited as a target for drug development (Klemba and Goldberg 2002; McKerrow et al. 2008, Vermelho et al. 2010; Wu and Chen 2011).

An important subset of MMPs comprises a short zinc-binding consensus sequence, HEXXH—first reported by McKerrow et al. (1987) which includes two metal-binding histidines and the general-base/acid glutamate for catalysis. The most studied metallopeptidases are the MMPs or matrixins. MMPs represent an important family of metal-dependent endopeptidases that are responsible for the degradation of extracellular matrix (ECM) components. These enzymes can degrade all of the components of the extracellular matrix, including fibrillar and

Fig. 2 MMPs groups



non-fibrillar collagens, fibronectin, laminin, and basement membrane glycoproteins. Since the discovery of MMPs in 1962 (Gross and Lapiere 1962), the MMP family has grown to include six groups (Fig. 2) and at least 28 members. Table 1 shows the members of each group.

MMPs are secreted in a latent, proenzyme form and require activation by a proteolytic cleavage of a propeptide domain at the *N*-terminus of the MMP molecule (Wojtowicz-Praga et al. 1997). They are regulated at multiple levels including gene transcription and by oxidative stress (Castro et al. 2009). Under physiological conditions the proteins are selectively regulated by inhibitors called tissue inhibitors of metalloproteinases (TIMPS) (Murphy 2011). MMP activity has been related to a number of important diseases such as rheumatoid arthritis, osteoarthritis, abdominal aortic aneurysm, acute myocardial infarction, end-stage kidney disease, and cancer. Although MMPs are crucial for a normal inflammatory response, uncontrolled activity of these enzymes after infection could lead to tissue damage, microbial dissemination, and immunopathology in the host that might lead to death. Besides inducing MMP secretion by host cells, pathogens themselves may also produce MMPs which are required for virulence (Geurts 2012; Vargová et al. 2012).

2.3 Metallocarboxypeptidases: Exopeptidases

Another class of metallopeptidases includes the metallocarboxypeptidases (MCPs). They are exopeptidases that catalyze the hydrolysis of peptide bonds at

Table 1 Members of MMPs

MMPs	Enzyme
MMP-2	Gelatinase A
MMP-9	Gelatinase B
MMP–3	Stromelysin–1
MMP-10	(Progelatinase)
MMP-11	Stromelysin–2
	Stromelysin–3
MMP-1	Collagenase–1
MMP-8	Neutrophil collagenase;
MMP-13	Collagenase–3
MMP-7	Matrilysin–1
MMP-26	Matrilysin–2
MMP-14	MT1–MMP
MMP-15	MT2–MMP
MMP-16	MT3–MMP
Mmp-24	MT5–MMP
MMP-25	MT6–MMP
MMP-12	Macrophage elastase
MMP-19	RASI–1
MMP-20	Enamelysin
MMP-21	XMMP (Xenopus)/Cy–MMP
MMP-22	Femalysin
MMP-23	CA–MMP
MMP-27	CMMP (Gallus)
MMP-28	Epilysin

the C-terminus of peptides and proteins. These enzymes possess a tightly bound Zn^{2+} ion directly involved in catalysis (Vendrell et al. 2000). The most studied MCPs are those that belong to the family M14 which contains four subfamilies. The M14 family includes enzymes which participate in diverse processes such as blood coagulation and fibrinolysis, inflammation, innate immunity response, food digestion and pro-hormone, and neuropeptide processing. The family M32 with the *carboxypeptidase Taq* (*Thermus aquaticus*) as the enzyme type has also been subject of study. This group contains two zinc-binding histidines and a catalytic glutamate in an HEXXH zinc-binding motif. The peptidases and homologs of this family include peptidases from trypanosomatids, such as the *Tc*MCP1 and *Tc*MCP2 (*T. cruzi* carboxypeptidase 1 and 2) and *Leishmania major* carboxypeptidase 1 (*Lma*CP1), and recently a M32 metallocarboxypeptidase of *Trypanosoma brucei* was described (Frasch et al. 2012). Peptidase members of M32 family have been detected in several bacteria, archaea, plants, and animals.

The following section describes the recent studies on structure–activity relationship, synthesis, inhibition mechanisms, and clinical trials of hydroxamic acids developed against microbial diseases.

3 Hydroxamic Acids

Hydroxamic acids were discovered by Lossen more than 100 years ago. Since then these acids have been extensively studied due to their multiple uses. Hydroxamic acid derivatives have shown numerous applications from insecticides to the treatment of many diseases, such as cancer, cardiovascular diseases, HIV, Alzheimer's, malaria, allergic diseases, tuberculosis, and antimicrobials. It is mainly because of their ability to coordinate with metal ions in metal-containing enzymes such as metalloproteins, urease, MMPs, carbonic anhydrase, and many others (Codd 2008; Muri et al. 2002).

The most frequent and economical methodology employed to obtain hydroxamic acids (**1**, Fig. 3) is from the reaction between a carboxylic acid derivative and hydroxylamine. Usually the carboxylic acid is converted to the ester and sequentially undergoes a reaction with the hydroxylamine (Codd 2008).

Other methodologies for the synthesis of hydroxamic acids have been reported that include the use of carboxylic acids and *N*-protected amino acids with cyanuric chloride (Giacomelli 2003) and the treatment of *N*-acyloxazolidinones with hydroxylamines using samarium triflate as a Lewis acid (Sibi 2002). In addition, the solid-phase synthesis of hydroxamic acids has been widely described which is becoming an important method for obtaining this acid (Floyd 1996; Grigg 1999; Nandurkar 2011; Zhai 2012).

An important strategy for the design of these drugs is predicting how they will reach their site of action in a concentration which produces a pharmacological effect. Their absorption through a membrane into solution in the blood is affected by physical–chemical factors. For drugs which are weak acids or bases, their dissociation constant (pKa) and the pH of the gastrointestinal tract fluid and blood stream will control their solubility. Ionized drugs will not be able to get through the lipid membrane. However, they can do so in the non-ionized form when they have increased lipid solubility (Fazary 2005).

Hydroxamic acids are basically acids but also behave as weak bases because of the NC=O moiety. In aqueous solvents, they have their pKa values in the range of 8.5–9.4. Studies have shown that their dissociation constant is a function of temperature, and usually, it has a minimum pKa value near room temperature, which it could be decreased as the temperature is raised (Fazary 2005). In addition, their acidity has received particular attention since two structures of the anion are possible. This equilibrium depends on both the structure of the hydroxamic acid and the reaction conditions. *N*-deprotonation yields the hydroxamate anion (**3**, Fig. 4) and it could be generated in the gas phase. In non-protic solvents, it is strengthened by an electron attracting substituent. In this case, the generated anion

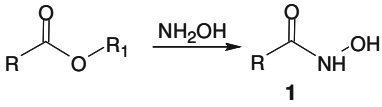


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Fig. 3 Conventional synthesis of hydroxamic acids

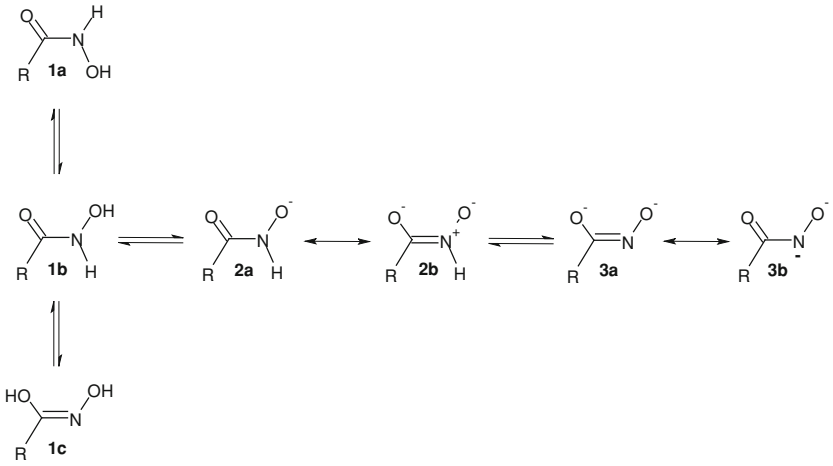


Fig. 4 Structures of hydroxamic acids (**1**), hydroxamates (**2**) and hydroximates (**3**) (based on Codd 2008)

may be stabilized by resonance. In water, the *O*-deprotonation yields the hydroxamate anion (**2**, Fig. 4) and it may be found in a comparable amount or even prevailing. Nevertheless, some studies have suggested that the acidity of hydroxamic acids is due to the destabilizing inductive effect of the hydroxyl group in the acid molecule and not due to any effect of the anion (Böhma 2003). The proven anions have some resonance contributing structures. On the other hand, hydroxamic acids may also be present in the tautomeric form (Fig. 41b, c).

In addition, in consequent to the free rotation about the C–N bond, hydroxamic acid and, the hydroxamate and hydroxamate groups can exhibit cis/trans (or Z, E) isomerism (Fig. 41a, b). Among other factors, the preferential configuration could be related to the steric effects of the N- and C-substituents since studies have shown that for *N*-methyl-substituted hydroxamic acids, the Z/E ratio increases with the bulk of the C-substituent in DMSO-*d*6, $CDCl_3$, or C_6D_6.

However, for the metal ion coordination, a change to the cis-geometry must occur. Studies have shown that increasing the negative charge on the coordinating oxygen atom with the electron donation to C or N atoms form more stable metal-hydroxamato/imato complexes. The coordination compounds of hydroxamic acids have been intensively studied (Riedel and Kaupp 2009). This is because negatively charged species, such as hydroxamates (Fig. 42a, 3a), act as good ligands with positively charged metal ions present in metalloenzymes (Goyer 2004).

The hydroxamic acids are one of the most important families of organic bioligands, as they constitute as one of the major classes of naturally occurring metal complexing agents and have been thoroughly studied as ligands. Complexes of metals or metalloids with hydroxamic acids are spread widely throughout and are well characterized by X-ray crystallography (Farkas et al. 2000).

The formation of complex of the hydroxamate group with metals can occur in different ways but the most common mode of binding is bidentate coordination. Coordination is through the deprotonated hydroxamate oxygen and carbonyl oxygen forming a very stable five-membered ring. Monodentate binding through the deprotonated nitrogen or oxygen atoms have also been reported but this requires specially designed coordination environments to provide additional stabilization (Farkas et al. 2000).

The formation of simple complexes with primary hydroxamic acid ligands in aqueous solution depends on the pH. The hydroxamato (charge -1) type mode arises from the first deprotonation step and involves the coordination of the NHO^- moiety (**4**, Fig. 5). The hydroximato form (charge -2) of the ligand is produced by further metal-induced deprotonation of NHO^- (**5**, Fig. 5) (Farkas et al. 2000).

Mononuclear homoleptic complexes with bidentate *O*,*O*-hydroxamato/imato coordination from monohydroxamic acids have been characterized with Fe(III), Cr(III), Co(III), Ga(III), In(III), Si(IV) or Ge(IV) (octahedral geometry), Cu(II) (square planar geometry), B(III) (tetrahedral geometry), or Hf(IV) or Th(IV) (distorted dodecahedral geometry). Mononuclear heteroleptic complexes with bidentate *O*,*O*-hydroxamato/imato coordination from monohydroxamic acids have been characterized with Si(IV), V(V), Co(II), Co(III), Ni(II), Zn(II), Mo(VI), Ru(III), Rh(III), Sn(IV), W(VI), Os(III), Pt(II), and U(IV) (Codd 2008).

Zinc is the second most prominent trace metal in the human body after iron. In the adult human there are 2–3 g of zinc, as compared to 4–6 g of iron and only 25 mg of copper. Zinc deficiency may cause growth defects, although unlike copper and iron, few harmful effects due to an excess of zinc have been observed. Zinc is known to have an important role in a wide range of cellular processes, including cell proliferation, reproduction, immune function, and the defense against free radicals. The biochemical functions of zinc can be classified as catalytic, structural, and regulatory. At neutral pH, all acid–base catalyzes by metal ions in biological systems are catalyzed by Zn^{2+}. At acid pH, manganese and iron adopt the role. Zn^{2+}, a d^{10} electron system, is a good Lewis acid which is unable to undergo redox reactions. In acidic media, Zn^{2+} complexes are unstable; therefore, Zn^{2+} is a good Lewis acid only under neutral or basic pH conditions. Zn^{2+} as a catalyst in metallobiomolecules is in low symmetry sites bound to amino acid side chains containing N, S, or O donor ligands. In addition, H_2O/OH^- is an excellent ligand in biological systems (Goyer 2004).

Metal has an essential role in metalloenzymes. For example, all known matrix MMPs use a zinc ion during hydrolysis of the substrate. This catalytic mechanism is described in Fig. 6. The active site zinc (II) ion is generally bound by three protein ligands and a water molecule suggestive of an open coordination site which

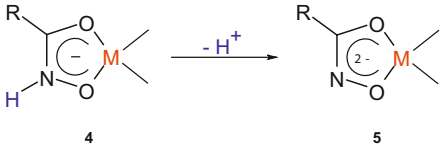


Fig. 5 Deprotonation of hydroxamic acid (based on Farkas et al. 2000)

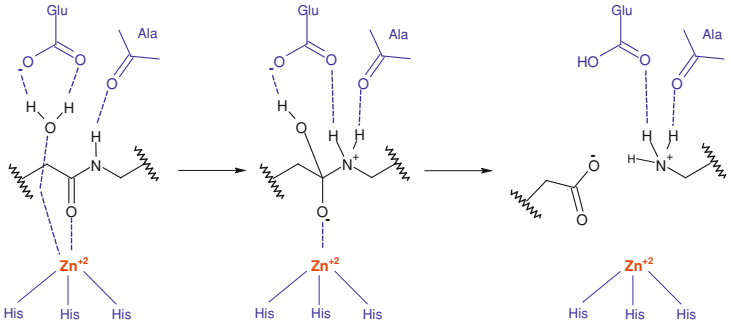


Fig. 6 Role of zinc ion in metalloproteins: reaction mechanism for MMPs (based on: Muri et al. 2002)

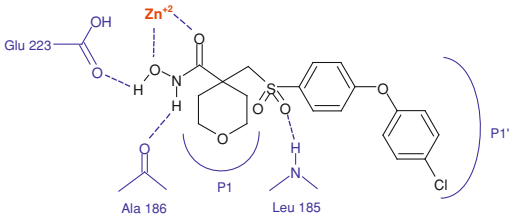


Fig. 7 Inhibition mechanism of hydroxamic acid derivative (RS 130830) in MMP (based on Zhang et al. 2000)

is considered essential for the function of zinc during catalysis. Thereby, the substrate amide carbonyl group also coordinates to zinc. This carbonyl group undergoes nucleophilic attack by a water molecule that are both hydrogen bonded to a conserved glutamic acid and coordinated to the zinc ion.

The water donates a proton to the active site glutamate (Glu) residue which transfers it to the nitrogen of the amide group. Transferring the proton from the Glu to the amide nitrogen is followed by the breaking of the N–C amide between Glu and the free amine of the cleaved substrate (Muri et al. 2002).

The molecular design of effective MMPs inhibitors has as a prerequisite to insert a group capable of chelating the zinc ion. The hydroxamic acid derivatives are, so far, the most extensively studied class of MMP inhibitors. This is due to the ability of the hydroxamate group to efficiently combine with the catalytic zinc ion besides its ability to form hydrogen bonds to glutamic acid and alanine residue MMPs (Fig. 7).

4 Bacterial Diseases

Bacterial resistance to multiple antibiotics is a global public health problem, especially for seriously ill, hospitalized patients. Frequently, these infections fail to respond to usual treatment and this results in prolonged illness and greater risk of

death (WHO 2012a–d). Unfortunately, while resistance to recent therapies continues to increase, there are inadequate numbers of new clinical drugs to treat these infections (Brown et al. 2012).

A strategy to combat multiresistant bacteria could be to focus on new targets involved in the biochemical processes that are essential for bacterial growth and thus develop new antibacterial agents. Highlighted among the multiresistant pathogens could be the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) (Gould et al. 2012) and the Gram-negative bacteria resistant to multiple antibiotics that are responsible for several difficult-to-treat infections in humans, such as *Escherichia coli* and *Pseudomonas aeruginosa* (Elhani et al. 2012).

The most frequent resistance mechanism used by bacteria is to modify their structural enzymes so that they are able to make the antibacterial agents inactive. Some families of resistant enzymes are well-known, such as those in aminoglycoside antibiotics. In this case, specifically three enzymes - aminoglycoside 3′-phosphotransferases [APH(3′)s], aminoglycoside nucleotidyltransferases (ANTs), and aminoglycoside acetyltransferases (AAT) have been extensively studied and among these, APH(3′) is most studied. Their mechanism of resistance is based on the enzyme's ability to transfer the α-phosphoryl group of ATP to the 3′-hydroxyl of aminoglycosides, such as kanamycin A (**6**, Fig. 8), thus making them inactive and clinically obsolete (**7**, Fig. 8) (Haddad et al. 1999).

Infectious diseases caused by Gram-negative bacteria are clinically relevant mainly as nosocomial pathogens. In these infections, there is an increased risk due to their endotoxin that may become a complicating factor in many serious diseases. The syndromes most frequently associated with bacterial endotoxin are systemic complications such as septicemias. Systemic infections or septicemias caused by invasive Gram-negative bacteria are a well-known effect of endotoxin exposure and *E. coli* is the most frequently isolated Gram-negative organism. Approximately 600,000 cases of septicemia are diagnosed annually in the US, leading to about 100,000 deaths per year (Shin et al. 2007).

Gram-negative bacteria septicemia results from the systemic response to infection, generally in the overexpression of cytokines and inflammatory mediators in response to macrophage activation by lipopolysaccharide (LPS). LPS, the primary component of the outer monolayer of the outer membrane of Gram-negative bacteria, and an essential protective barrier against such agents as detergents and

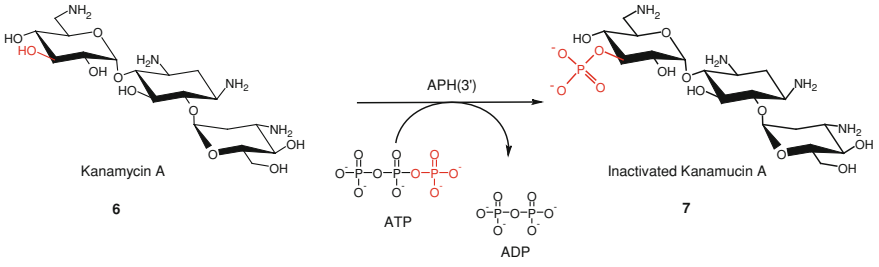


Fig. 8 Kanamycin A inactivation by APH(3′) (based on Haddad et al. 1999)

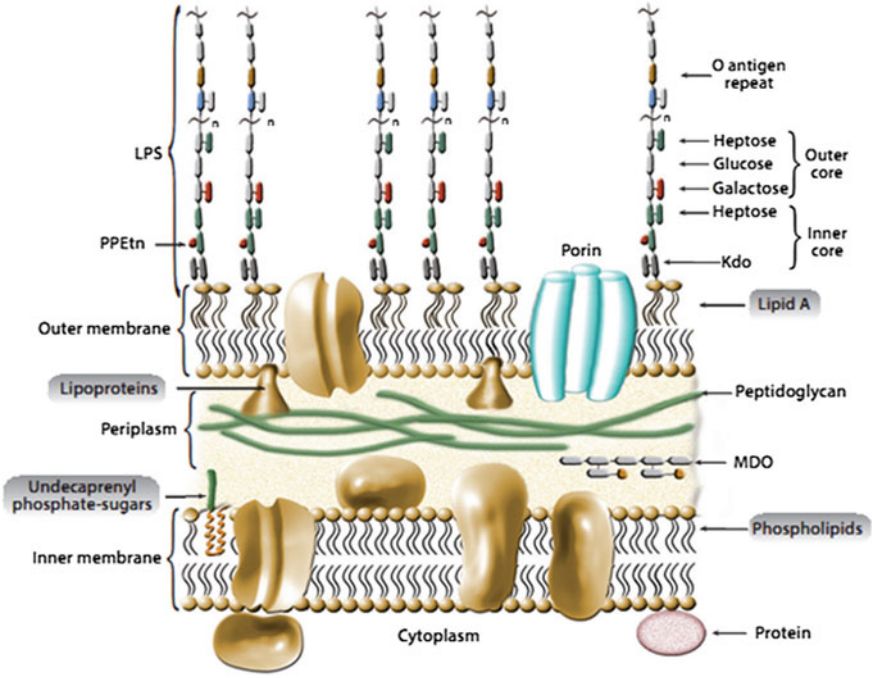


Fig. 9 Model of the inner and outer membranes of *E. coli* (based on Raetz and Whitfield 2002)

antibiotics, is anchored to a hydrophobic domain known as lipid A (Fig. 9) (Brown et al. 2012).

The hexaacylated disaccharide lipid A, also known as endotoxin, is a glucosamine-based phospholipid required for bacterial growth (Raetz and Whitfield 2002; Shin et al. 2007) and it is estimated that there are 10^6 lipid A residues and 10^7 glycerophospholipids in a single cell of *E. coli* (Raetz and Whitfield 2002). When there is a lack of lipid A, the bacteria either become not viable or increase their susceptibility to anti-bacterial agents (Brown et al. 2012).

Lipid A biosynthesis is catalyzed by nine enzymes, however, the UDP-3-O-(R-3-hydroxymyristol)-*N*-acetylglucosamine deacetylase (LpxC) has been widely studied due to the lack of homology to mammalian metalloamidases. Therefore, LpxC has been considered an attractive chemotherapy target for the treatment of the gram-negative bacteria infections. LpxC is a cytosolic zinc-metalloamidase enzyme that uses a Zn^{2+} ion as cofactor, which is essential for cell viability. This enzyme is present in all Gram-negative bacteria and plays a crucial role in the pathway leading to the construction of Lipid A (**11**, Fig. 10) which specifically participates in the first biosynthetic step (Brown et al. 2012). In this step the metal-dependent deacetylase, LpxC, hydrolyzes UDP-3-O-(R-3-hydroxymyristoyl)-*N*-acetylglucosamine (**8**, Fig. 10) to form acetate and UDP-3-O-(R-3-hydroxymyristoyl) glucosamine (**9**, Fig. 10) (Gennadios et al. 2006).

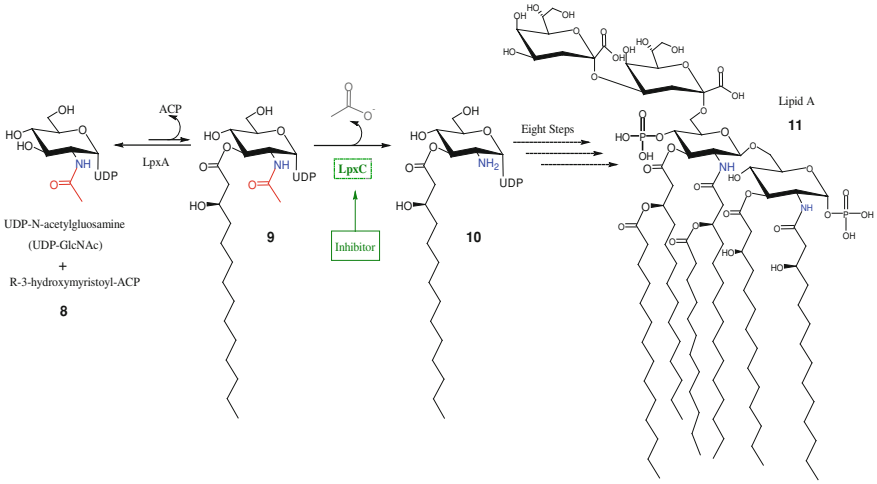


Fig. 10 Lipid A biosynthesis (based on Barb et al. 2007 and Zhang et al. 2012)

Studies have shown that the inhibition of enzymes in the lipid A biosynthetic pathway kills Gram-negative bacteria and reduces toxic lipid A concentrations shed by dying bacteria. Specific studies with LpxC have proven that the LpxC gene inactivation was able to suppress bacterial growth (Mdluli et al. 2006). Consequently, this is considered an attractive target for the development of a new class of antibiotics. Thus the research for novel chemical substances with potential inhibitory action of LpxC has been stimulated (Shin et al. 2007). Since the discovery of this enzyme target, several LpxC inhibitors have been reported, especially those focused on the hydroxamate class (Warmus et al. 2012). However, none of these inhibitors have as yet effectively advanced through clinical trials (Brown 2012).

The first hydroxamic acid-based potent LpxC inhibitor discovered was L-161,240 (**12**, Fig. 11) (Onishi et al. 1996). This compound contains an aryloxazoline moiety. Thus, the discovery of this compound opened up a new pathway in the design and development of novel aryloxazoline-based hydroxamate LpxC inhibitors. However, this oxazoline derivative does not have a broad spectrum against gram-negative bacteria; it has been found to be potent particularly against *E. coli* but weak against *Pseudomonas aeruginosa* (Warmus et al. 2012; Zhang et al. 2012).

In order to search for potent LpxC inhibitors against broad spectrum gram-negative bacteria the substrate-based hydroxamates, such as TU-517, were designed (**13**, Fig. 11). In these new structures, the hydroxamate group was introduced into the tetrahydropyran ring of the natural LpxC substrate and appended at the position analogous to the location of the *N*-acetyl group. However, these compounds, although demonstrated a considerable potency against the wild-type strain of the *E. coli* LpxC, were not able to inhibit the growth of a mutant

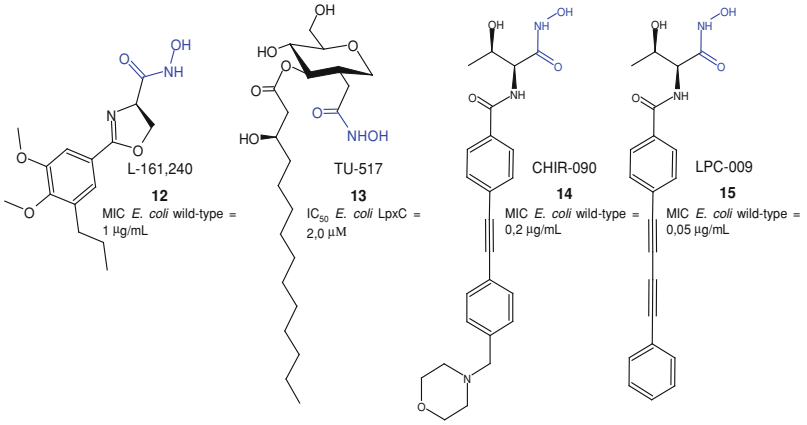


Fig. 11 LpxC inhibitors (based on Warmus et al. 2012)

strain of the *E.coli* (Jackman et al. 2000; Zhang et al. 2012). Afterward, in an attempt to obtain the effective interaction with the hydrophobic tunnel in the active site of LpxC, the CHIR-090 (**14**, Fig. 11), which is based on the diphenyl-acetylene scaffold, was developed.

Thus CHIR-090 is the first reported compound that in fact kills both *E. coli* and *Pseudomonas aeruginosa* in bacterial disk diffusion assays (Liang et al. 2011). Nonetheless, CHIR-090 was about 600-fold less effective against LpxC orthologs from the Rhizobiaceae family than against *E. coli* LpxC. In this way, studies have shown that the removal of morpholine ring (LPC-009, **15**, Fig. 11), based on a chemical scaffold of reduced radius, was able to increase affinity by 20-fold for LpxC enzymes from the Rhizobiaceae family of bacteria and enhance the antibiotic activity against *E. coli* and *P. aeruginosa* by 2–4 fold over CHIR-090 (**14**, Fig. 11).

5 Protozoal Diseases

Today, more than a billion people, around one-sixth of the world population, are infected with one or more of the neglected tropical diseases (NTDs). However, since these diseases do not cause sudden outbreaks and are mainly in countries with limited resources for investment in public health, they do not attract the necessary attention of the competent authorities, and thus become neglected diseases (WHO 2012a–d). Prominent among the existing NTDs could be leishmaniasis, sleeping sickness, Chagas disease, and malaria, which together account for nearly 50 % of all deaths caused by NTDs every year (WHO 2012a–d). Such NTDs are caused by protozoa, which are among the most prevalent pathogens throughout the world.

More than 225 million individuals are infected with malaria alone and this NTD causes about 700 thousand deaths each year. This disease is caused by the Plasmodium species, of which there are four main types able to infect humans: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium falciparum* (Andrews et al. 2009). However, *P. falciparum* is the most common causative agent. Nowadays, some strains of *P. falciparum* have been showing resistance against chloroquine, the most accessible antimalarial drug available. The most recent antimalarial treatment is artemisinin-based combination therapy (Andrews et al. 2012; Aguiar et al. 2012). However, due to treatment failure and growing parasite resistance to current antimalarial drugs new research has been focusing on novel therapeutic targets and new classes of chemical substances. A better understanding of the biology of these parasites is contributing to the discovery and development of novel drugs and vaccines (Aguiar et al. 2012).

In this context, histone deacetylases (HDACs) have been identified as an important chemotherapeutic target since they play essential roles in regulating gene transcription in both eukaryotes and prokaryotes. HDACs regulate chromatin status and gene expression, and their inhibition is of significant therapeutic interest. This enzyme affects the post-translational modification of proteins by altering the acetylation state of the lysine residues. Thus, the acetylated form induces the gene expression while the deacetylated form silences genes. Consequently, this enzyme has also been considered a potential target for the treatment of several diseases, such as cancer, neurodegeneration, metabolic, inflammatory and autoimmune disorders, as well as infectious and cardiovascular diseases (Carafa et al. 2013). The HDACs have also been investigated as novel prospective drug targets for malaria. HDACs are grouped into two families, Class I and II, which differ significantly in size and structural organization, but share a similar catalytic core that uses zinc as the essential co-factor for deacetylase activity (Andrews et al. 2012).

There are several HDAC inhibitors under development and most of them are hydroxamate-based inhibitors such as Trichostatin A (TSA, **16**, Fig. 12),

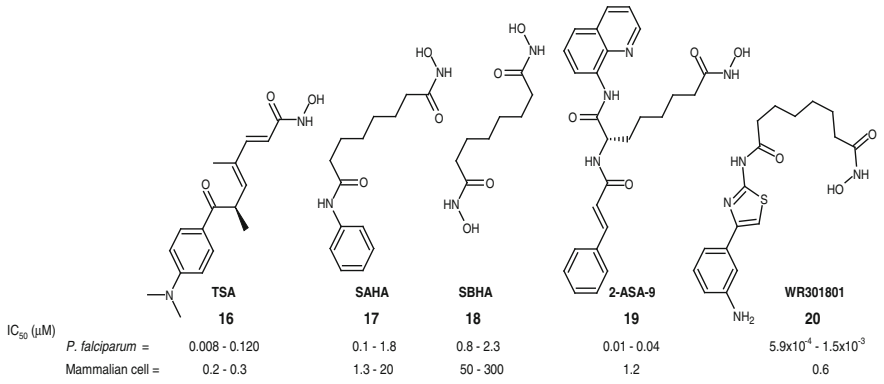


Fig. 12 Histone deacetylase inhibitors (based on Andrews et al. 2012)

suberoylanilide hydroxamic acid (SAHA or Vorinostat®, **17**, Fig. 12), suberic bishydroxamate (SBHA, **18**, Fig. 12), 2-aminosuberic acid-based hydroxamate (2-ASA-9, **19**, Fig. 12), and WR301801 (**20**, Fig. 12) (Andrews et al. 2012).

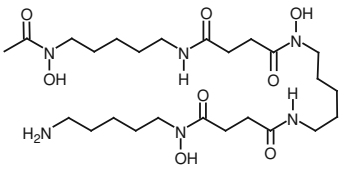
Recently, hydroxamic acid-based compounds, selective for *P. falciparum* have been described. The selectivity has been identified by screening compounds with variations in the basic structure of HDAC inhibitors. This includes a small zinc-binding group (ZBG) that accesses the active zinc ion site, a linker region capable of fitting the narrow, hydrophobic, tubular cavity emanating from the ZBG to the HDAC surface, and a capping group that blocks the entrance to the active site cavity (Andrews et al. 2012).

Other neglected tropical diseases such as Leishmaniasis, caused by the parasitic protozoa of the genus Leishmania, are fatal if not treated, and the majority of deaths go unrecognized, even with access to treatment. According to World Health Organizations (WHO) there are four main forms of leishmaniasis: the cutaneous, diffused cutaneous, mucocutaneous, and visceral forms, which affect approximately 12 million people worldwide. Furthermore, there are 350 million people in 88 countries at risk of contracting it (WHO 2012a–d). Vaccination remains the best hope for control of all forms of this disease. Despite great advances in research, no vaccine is yet available (Palatnik-de-Sousa 2012).

Studies of new drugs for the treatment of this parasitic disease have shown that iron deficiency induced by an iron chelator such as desferrioxamine (**21**, Fig. 13), which is a hydroxamic acid derivative, could favor the host in Leishmaniosis. This is because metal chelating groups form complexes with iron in hemoglobin, and thus with no iron available to the parasite its multiplication would be reduced and infection attenuated (Malafaia et al. 2011).

The most important diseases caused by protozoan parasites of the genus *Trypanosoma* are African trypanosomiasis or sleeping sickness and the American disease or Chagas disease. According to the World Health Organization (WHO), African trypanosomiasis affects at least 70,000 people per year and there are about 500,000 people infected with this disease. Furthermore, 60 million people in 36 countries are at risk of contracting it (WHO 2012a–d; Lopes et al. 2010). The treatment available for the advanced stage of disease is the nifurtimox-eflornithine combination therapy (NECT). It consists of a simplified co-administration of nifurtimox, which is given orally, and eflornithine, which is given intravenously (DND*i* 2012).

Fig. 13 Desferrioxamine



Desferrioxamine

21

Researchers have identified a hydroxamic acid derivative as a potential therapeutic agent for the treatment of the disease. This is because studies have shown the importance of 6-phosphogluconate dehydrogenase (6-PGDH), the third enzyme of the pentose phosphate pathway, for the parasite survival, especially in *Trypanosoma brucei*. 6-PGDH of the *Trypanosoma brucei* has been shown to be inhibited by 2,3-O-Isopropylidene-4-erythrono hydroxamate. However, this compound has reduced membrane permeability and it does not have trypanocidal activity. Therefore, the attempt is being made to improve the antiparasitic activity of this inhibitor by converting the phosphate group into a lower charged phosphate prodrug. Based on this new compound, a small library of phosphoramidates was synthesized, where some of the compounds showed high trypanocidal activity (Ruda et al. 2010).

In Chagas disease, there are about 10,000 deaths per year caused by *Trypanosoma cruzi*, and it is estimated that 10 million people are infected worldwide, mostly in Latin America, and that 75–90 million are exposed to infection. There is an urgent need for safer and more efficient drugs against Chagas disease given that there are only two compounds in clinical use, which were introduced in the 1960s and 1970s. They are Benznidazole (BNZ) {*N*-benzyl-2-nitroimidazole-1-acetamide} (Rochagan®, Roche) and Nifurtimox (NFX) {4[(5-nitrofurfuryl-idene)amino]-3-methylthio-morpholine-1,1-dioxide} (Lampit®, Bayer) (Capaci-Rodrigues et al. 2010). Several studies have been made and new chemical substances and new therapeutic targets investigated. However, none of the chemicals investigated are in the advanced stage of clinical trials.

Studies of MMPs in *T. cruzi* infections have shown that the expression and activity of two of them, MMP-2 and MMP-9, were up regulated in cardiac tissue during the acute phase of the disease. This study detected an association with inflammatory cells infiltrating the myocardium. Additionally, in order to establish the role of MMPs in vivo, *T. cruzi*-infected mice were treated with doxycycline, a potent inhibitor of MMP activity. It was found that mice treated with doxycycline, a member of the tetracycline antibiotic group able to chelate the zinc metallopeptidases, showed a significantly decreased inflammation of the heart, delayed peak in parasitemia, and improved survival rates, compared with the control group. These results suggest an important role of MMPs in *T. cruzi*-induced acute myocarditis (Gutierrez et al. 2008).

Metallopeptidase activities have been expressed at high density at the surface of Leishmania promastigotes such as gp63 and in multiple isoforms in *T. cruzi.* An MMP-9-like activity was detected in the cytoplasm of the *T.cruzi* during in vitro infection of embryonic hepatocyte cells (Nogueira de Melo et al. 2004). Subsequent studies have also demonstrated the presence of MMP-9 homologs in *T. cruzi* cells and spent culture medium (Nogueira de Melo et al. 2010). There are no efficient non-toxic inhibitors developed for these potential targets, since this proteolytic activity could possibly contribute to an ECM protein degradation, facilitating invasion of host cells, an activity that is probably highly relevant in vivo during the navigation of interstitial tissue spaces by trypomastigote forms.

Peptidase-dependent ECM remodeling is one of the key events for the regulation of *T. cruzi* infection and pathogenesis of Chagas disease (Capaci-Rodrigues et al. 2010).

6 Viral Disease

The human immunodeficiency virus (HIV) affects 34 million people worldwide, of which 16.7 million are women, 14 million men, and 3.3 million children below 15. Only in 2011, 2.5 million individuals became infected with the virus. HIV is a retrovirus that infects cells of the immune system, destroying or impairing their function. As the infection progresses, the immune system becomes weaker, and the person becomes more susceptible to infections. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS). It can take 10–15 years for an HIV-infected person to develop AIDS; antiretroviral drugs can slow down the process even further (WHO 2012a–d).

Now 25 years on, HIV therapy, previously focused on developing and optimizing drugs based on inhibiting active HIV replication, is about to enter a new phase. Recently, researchers have demonstrated the need for new therapeutic strategies able to present an effective cure for latent HIV. This is because the therapeutic strategy adopted at the moment has limitations since HIV persists even when viral replication is suppressed. HIV persists during effective therapy, in part because its genome becomes stably integrated in certain white blood cells known as resting memory $CD4^+$ T cells. In this way, these infected cells remain invisible to the immune system as they do not express viral proteins. Supposedly, drugs that reverse latency might lead consecutively to HIV RNA synthesis, viral protein production, and release of HIV particles. It could lead to the death of the infected cell and to the elimination of the viral reservoir by the virus or by the patient's immune system. Histone deacetylase (HDAC) plays an important role in latency related to the removal of acetyl groups from the DNA-bound histone proteins and, in so doing, affects gene expression. Thereby, it could reverse latency (Deeks 2012).

Some histone deacetylase inhibitors (HDACIs) have been tested. Vorinostat® or SAHA (**17**, Fig. 12) that is licensed for the treatment of cutaneous T cell lymphoma, has been tested in vitro and was able to activate HIV production in latently infected cell lines from 0.25 to 100 mm. Thus, this drug was shown to be a potent inducer to activate HIV production of latently infected monocytic cell lines (U1), T cell lines (J89 and ACH2), primary $CD4^+$ T cells and of $CD4^+$ T cells from HIV-infected patients on suppressive combination antiretroviral therapy (cART). In view of these results, several other HDACIs have been tested which can also activate HIV production from latently infected cells such as givinostat, belinostat, entinostat, and panobinostat (Wightman et al. 2012).

On the other hand, although HDACIs present an exciting new approach to activate HIV production from latently infected cells and so possibly enhance

elimination of these cells and achieve a cure, there are no answers for some questions such as how much of the viral reservoir might be eliminated by HDAC inhibition. Thus, there is a need for new studies to evaluate the toxicity of this treatment (Deeks 2012; Wightman et al. 2012).

7 Conclusion

Hydroxamic acids involve a simple synthesis and possess a substantial versatile pharmacological usefulness. Innumerous new hydroxamic acid-based compounds have been developed for the treatment of various diseases. This is mainly due to their ability to form complexes with various metals, particularly iron, zinc and calcium, which are most abundant in the human body. In this chapter, the importance of the formation of this complex with enzymes of microorganisms has been discussed. In this context, the metallopeptidases were shown to be extremely significant since they have been discovered in several pathogens with a key role in their survival. Also other metalloenzymes of microorganisms that are inhibited by hydroxamate derivatives are shown to act as potential therapeutic targets.

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References

Aguiar AC, Rocha EM, Souza NB, França TC, Krettli AU (2012) New approaches in antimalarial drug discovery and development: a review. Mem Inst Oswaldo Cruz 107:831–845

Andrews KT, Tran TN, Wheatley NC, Fairlie DP (2009) Targeting histone deacetylase inhibitors for anti-malarial therapy. Curr Top Med Chem 9:292–308

Andrews KT, Tran TN, Fairlie DP (2012) Towards histone deacetylase inhibitors as new antimalarial drugs. Curr Pharm Des 18:3467–3479

Barb AW, Jiang L, Raetz CR, Zhou P (2007) Structure of the deacetylase LpxC bound to the antibiotic CHIR-090: Time-dependent inhibition and specificity in ligand binding. Proc Natl Acad Sci U S A 104:18433–18438

Böhma S, Exner O (2003) Acidity of hydroxamic acids and amides. Org Biomol Chem 1:1176–1180

Brown MF, Reilly U, Abramite JA, Arcari JT, Oliver R, Barham RA, Che Y, Chen JM, Collantes EM, Chung SW, Desbonnet C, Doty J, Doroski M, Engtrakul JJ, Harris TM, Huband M, Knafels JD, Leach KL, Liu S, Marfat A, Marra A, McElroy E, Melnick M, Menard CA, Montgomery JI, Mullins L, Noe MC, O'Donnell J, Penzien J, Plummer MS, Price LM, Shanmugasundaram V, Thoma C, Uccello DP, Warmus JS, Wishka DG (2012) Potent inhibitors of LpxC for the treatment of Gram-negative infections. J Med Chem 55:914–923

Buache E, Garnotel R, Aubert D, Gillery P, Villena I (2007) Reduced secretion and expression of gelatinase profile in Toxoplasma gondii-infected human monocytic cells. Biochem Biophys Res Commun 359:298–303

Capaci-Rodrigues G, Aguair AP, Vianez-Júnior JLSG, Macrae A, Nogueira de Melo AC, Vermelho AB (2010) Peptidase Inhibitors as a possible therapeutic strategy for chagas disease. Curr Enz Inhib 6:183–194

Carafa V, Miceli M, Altucci L, Nebbioso A (2013) Histone deacetylase inhibitors: a patent review (2009–2011). Expert Opin Ther Pat 23:1–17

Castro MM, Rizzi E, Rodrigues GJ, Ceron CS, Bendhack LM, Gerlach RF, Tanus-Santos JE (2009) Antioxidant treatment reduces matrix metalloproteinase-2-induced vascular changes in renovascular hypertension. Free Radic Biol Med. 46:1298–1307

Codd R (2008) Traversing the coordination chemistry and chemical biology of hydroxamic acids. Coord Chem Rev 252:1387–1408

Costa JD, Nogueira de Melo AC, Vermelho AB, Meirelles Mde N, Porrozzi R (2008) In vitro evidence for metallopeptidase participation in hepatocyte damage induced by *Leishmania chagasi*-infected macrophages. Acta Trop 106:175–183

Deeks SG (2012) HIV: Shock and kill. Nature 487:439–440

Drugs for Neglected Diseases initiative (DNDi) - http://www.dndi.org/treatments/nect-c-treatments.html. Accessed in 29.11.2012***

Elhani D, Elhani I, Aouni M (2012) Resistance in gram negative bacteria: what is the current situation? Tunis Med 90:680–685

Farkas E, Enyedy EA, Micera G, Garribba E (2000) Coordination modes of hydroxamic acids in copper(II), nickel(II) and zinc(II) mixed-ligand complexes in aqueous solution. Polyhedron 19:1727–1736

Fazary AE (2005) Thermodynamic studies on the protonation equilibria of some hydroxamic acids in $NaNO_3$ solutions in water and in mixtures of water and dioxane. J Chem Eng. 50:888–895

Floyd CD, Lewis CN, Patel SR, Whittaker M (1996) A method for the synthesis of hydroxamic acids on solid phase. Tetrahedron Lett 37:8045–8048

Frasch AP, Carmona AK, Juliano L, Cazzulo JJ, Niemirowicz GT (2012) Characterization of the M32 metallocarboxypeptidase of Trypanosoma brucei: differences and similarities with its orthologue in *Trypanosoma cruzi*. Mol Biochem Parasitol 184:63–70

Gennadios HA, Whittington DA, Li X, Fierke CA, Christianson DW (2006) Mechanistic inferences from the binding of ligands to LpxC, a metal-dependent deacetylase. Biochemistry 45:7940–7948

Geurts N, Opdenakker G, Van den Steen PE (2012) Matrix metalloproteinases as therapeutic targets in protozoan parasitic infections. Pharmacol Ther 133(3):257–279

Giacomelli G, Porcheddu A, Salaris M (2003) Simple one-flask method for the preparation of hydroxamic acids. Org Lett 5:2715–2717

Gomis-Rüth FX, Botelho TO, Bode W (2012) A standard orientation for metallopeptidases. Biochim Biophys Acta 1824:157–163

Gould IM, David MZ, Esposito S, Garau J, Lina G, Mazzei T, Peters G (2012) New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. Int J Antimicrob Agents 39:96–104

Goyer R, Golub M, Choudhury H, Hughes M, Kenyon E, Stifelman M (2004) U.S. Environmental Protection Agency. Issue paper on the human health effects of metals. pp 1–22

Grigg R, Major JP, Martin FM, Whittaker M (1999) Solution and solid-phase synthesis of hydroxamic acids via palladium catalysed cascade reactions Original Research Article. Tetrahedron Lett 40:7709–7711

Gross J, Lapiere CM (1962) Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc Natl Acad Sci U S A 48:1014–1022

Gutierrez FR, Lalu MM, Mariano FS, Milanezi CM, Cena J, Gerlach RF, Santos JE, Torres-Dueñas D, Cunha FQ, Schulz R, Silva JS (2008) Increased activities of cardiac matrix metalloproteinases matrix metalloproteinase (MMP)-2 and MMP-9 are associated with mortality during the acute phase of experimental *Trypanosoma cruzi* infection. J Infect Dis 197:1468–1476

Haddad J, Vakulenko S, Mobashery S (1999) An antibiotic cloaked by its own resistance enzyme. J Am Chem Soc 121:11922–11923

Hoekstra R, Eskens FA, Verweij J (2001) Matrix metalloproteinase inhibitors: current developments and future perspectives. Oncologist 6:415–427

Hou T, Zhang W, Xu X (2012) Molecular docking studies of a group of hydroxamate inhibitors with gelatinase-A by molecular dynamics. J Comput Aided Mol Des 16:27–41

Jackman JE, Fierke CA, Tumey LN, Pirrung M, Uchiyama T, Tahir SH, Hindsgaul O, Raetz CR (2000) Antibacterial agents that target lipid A biosynthesis in gram-negative bacteria. Inhibition of diverse UDP-3-O-(r-3-hydroxymyristoyl)-n-acetylglucosamine deacetylases by substrate analogs containing zinc binding motifs. J Biol Chem 275:11002–11009

Klemba M, Goldberg DE (2002) Biological roles of proteases in parasitic protozoa. Annu Rev Biochem 71:275–305

Liang X, Lee CJ, Chen X, Chung HS, Zeng D, Raetz CR, Li Y, Zhou P, Toone EJ (2011) Syntheses, structures and antibiotic activities of LpxC inhibitors based on the diacetylene scaffold. Bioorg Med Chem 19:852–860

Lindsey ML, Zamilpa R (2012) Temporal and spatial expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases following myocardial infarction. Cardiovasc Ther 30:31–41

Lopes AH, Souto-Padrón T, Dias FA, Gomes MT, Capaci-Rodrigues G, Zimmermann LT, Silva TLA, Vermelho AB (2010) Trypanosomatids: odd organisms, devastating diseases. Open Parasit J 4:30–59

Lu CY, Lai SC. (2012) Matrix metalloproteinase-2 and -9 lead to fibronectin degradation in astroglia infected with *Toxoplasma gondii*. Acta Trop (in Press)

Luplertlop N, Missé D (2008) MMP cellular responses to dengue virus infection-induced vascular leakage. Jpn J Infect Dis 61:298–301

Malafaia G, Marcon LN, Pereira LF, Pedrosa ML, Rezende AS (2011) *Leishmania chagasi*: effect of the iron deficiency on the infection in BALB/c mice. Exp Parasitol 127:719–723

Marson BP, Poli de Figueiredo CE, Tanus-Santos JE (2012) Imbalanced matrix metalloproteinases in cardiovascular complications of end-stage kidney disease: a potential pharmacological target. Basic Clin Pharmacol Toxicol 110:409–415

McGwire BS, Chang KP, Engman DM (2003) Migration through the extracellular matrix by the parasitic protozoan Leishmania is enhanced by surface metalloprotease gp63. Infect Immun 71:1008–1010

McKerrow JH (1987) Human fibroblast collagenase contains an amino acid sequence homologous to the zinc-binding site of Serratia protease. J Biol Chem 262:5943

McKerrow JH, Rosenthal PJ, Swenerton R, Doyle P (2008) Development of protease inhibitors for protozoan infections. Curr Opin Infect Dis 21:668–672

Mdluli KE, Witte PR, Kline T, Barb AW, Erwin AL, Mansfield BE, McClerren AL, Pirrung MC, Tumey LN, Warrener P, Raetz CR, Stover CK (2006) Molecular validation of LpxC as an antibacterial drug target in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 50:2178–2184

Muri EM, Nieto MJ, Sindelar RD, Williamson JS (2002) Hydroxamic acids as pharmacological agents. Curr Med Chem 9:1631–1653

Murphy G (2011) Tissue inhibitors of metalloproteinases. Genome Biol 12:233

Nandurkar NS, Petersen R, Qvortrup K, Komnatnyy VV, Taveras KM, Le Quement ST, Frauenlob R, Givskov M, Nielsen TE (2011) A convenient procedure for the solid-phase synthesis of hydroxamic acids on PEGA resins. Tetrahedron Lett 52:7121–7124

Nogueira de Melo AC, Meirelles MNL, Porrozzi R, Costa JD, Branquinha MH, Vermelho AB (2004) Reduced activity of matrix metalloproteinase-9 in *Trypanosoma cruzi*-infected mouse embryo hepatocyte cell. Hepatol Res 28:49–56

Nogueira de Melo AC, de Souza EP, Elias CG, dos Santos AL, Branquinha MH, d'Avila-Levy CM, dos Reis FC, Costa TF, Lima AP, de Souza Pereira MC, Meirelles MN, Vermelho AB (2010) Detection of matrix metallopeptidase-9-like proteins in *Trypanosoma cruzi*. Exp Parasitol 125:256–263

Onishi HR, Pelak BA, Gerckens LS, Silver LL, Kahan FM, Chen MH, Patchett AA, Galloway SM, Hyland SA, Anderson MS, Raetz CRH (1996) Antibacterial agents that inhibit Lipid A biosynthesis. Science 274:980–982

Palatnik-de-Sousa CB (2012) Vaccines for canine leishmaniasis. Front Immunol 3:69

Pepeljnjak S, Zorc B, Butula I (2005) Antimicrobial activity of some hydroxamic acids. Acta Pharm 55:401–408

Prato M, Giribaldi G (2011) Matrix Metalloproteinase-9 and Haemozoin: Wedding Rings for Human Host and *Plasmodium falciparum* Parasite in Complicated Malaria. J Trop Med 2011:628435

Raetz CR, Whitfield C (2002) Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635–700

Rawlings ND, Barrett AJ, Bateman A (2012) MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res 40:D343–D350

Riedel S, Kaupp M (2009) The highest oxidation states of the transition metal elements. Coord Chem Rev 253:606–624

Ruda GF, Wong PE, Alibu VP, Norval S, Read KD, Barrett MP, Gilbert IH (2010) Aryl phosphoramidates of 5-phospho erythronohydroxamic acid, a new class of potent trypanocidal compounds. J Med Chem 53:6071–6078

Sakurai A, Okahashi N, Maruyama F, Ooshima T, Hamada S, Nakagawa I (2008) *Streptococcus pyogenes* degrades extracellular matrix in chondrocytes via MMP-13. Biochem Biophys Res Commun 373:450–454

Schechter I, Berger A (1967) On the size of the active site in proteases. I. Papain. Biochem Biophys Res Commun 27:157–162

Shin H, Gennadios HA, Whittington DA, Christianson DW (2007) Amphipathic benzoic acid derivatives: synthesis and binding in the hydrophobic tunnel of the zinc deacetylase LpxC. Bioorg Med Chem 15:2617–2623

Sibi MP, Hasegawa H, Ghorpade SR (2002) A convenient method for the conversion of *N*-acyloxazolidinones to hydroxamic acids. Org Lett 4:3343–3346

Van den Steen PE, Van Aelst I, Starckx S, Maskos K, Opdenakker G, Pagenstecher A (2006) Matrix metalloproteinases, tissue inhibitors of MMPs and TACE in experimental cerebral malaria. Lab Invest 86:873–888

Vargová V, Pytliak M, Mechírová V (2012) Matrix metalloproteinases. EXS 103:1–33

Vendrell J, Querol E, Avilés FX (2000) Metallocarboxypeptidases and their protein inhibitors. Structure, function and biomedical properties. Biochim Biophys Acta 1477:284–298

Vermelho AB, Branquinha MH, d'Ávila-Levy CM, Santos ALS, Dias EPS, Noguera de Melo AM (2010) Biological roles of peptidases in trypanosomatids. The Open Parasitol J 4:5–23

Warmus JS, Quinn CL, Taylor C, Murphy ST, Johnson TA, Limberakis C, Ortwine D, Bronstein J, Pagano P, Knafels JD, Lightle S, Mochalkin I, Brideau R, Podoll T (2012) Structure based design of an in vivo active hydroxamic acid inhibitor of *P. aeruginosa* LpxC. Bioorg Med Chem Lett 22:2536–2543

Whittaker M, Floyd CD, Brown P, Gearing AJ (1999) Design and therapeutic application of matrix metalloproteinase inhibitors. Chem Rev 99:2735–2776

Wightman F, Ellenberg P, Churchill M, Lewin SR (2012) HDAC inhibitors in HIV. Immunol Cell Biol 90:47–54

Wojtowicz-Praga SM, Dickson RB, Hawkins MJ (1997) Matrix metalloproteinase inhibitors. Invest New Drugs 15:61–75

World Health Organization–WHO (2012a) http://www.who.int/hiv/data/2012_epi_core_en.png. Accessed 29 Nov 2012

World Health Organization–WHO (2012b) http://www.who.int/leishmaniasis/resources/leishmaniasis_epidemiology_access_to_medicine/en/index.html. Accessed 29 Nov 2012

World Health Organization–WHO (2012c) http://www.who.int/neglected_diseases/diseases/chagas/en/index.html. Accessed in 30.11.2012

World Health Organization–WHO (2012d) http://www.who.int/tdr/research/ntd/en/ Accessed 29 Nov 2012

Wu JW, Chen XL (2011) Extracellular metalloproteases from bacteria. Appl Microbiol Biotechnol 92:253–262

Yuan X, Mitchell BM, Wilhelmus KR (2009) Expression of matrix metalloproteinases during experimental *Candida albicans* keratitis. Invest Ophthalmol Vis Sci 50:737–742

Zhai W, Gerritz SW, Sofia MJ (2012) Solid phase synthesis of 1,5-disubstituted pyrazole-4-hydroxamic acids and pyrazole-4-carboxamides via direct amidation of β-ketoesters. Tetrahedron Lett 53:267–270

Zhang X, Gonnella NC, Koehn J, Pathak N, Ganu V, Melton R, Parker D, Hu SI, Nam KY (2000) Solution structure of the catalytic domain of human collagenase-3 (MMP-13) complexed to a potent non-peptidic sulfonamide inhibitor: binding comparison with stromelysin-1 and collagenase-1. J Mol Biol 301:513–524

Zhang J, Zhang L, Li X, Xu W (2012) UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) inhibitors: a new class of antibacterial agents. Curr Med Chem 19:2038–2050